Contents

CHAPTER 1.1

How Ozone Acts and how it Exerts Therapeutic Effects

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Introduction

During the last three decades, on the basis of Wolff's suggestion (l974), ozone therapy has been used by practitioners in Europe in an empirical fashion. Unfortunately, even today, most ozone therapists have either a misconception or do not know how ozone acts on blood and biological fluids. This problem, associated with the lack of controlled clinical studies, has hindered a real progress and ozone therapy remains an obscure complementary practice. Worst of all, in some countries (USA, Canada, Mexico, Poland, Kenya, India, etc.) quite a few quacks, often without any medical qualification, continue to inject ozone intravenously, a procedure prohibited since 1984 in Germany because of the risk of pulmonary embolization and death. This is a good reason for the FDA to prohibit the use of ozone in most states of USA. Moreover, distinguished American scientists have affirmed the dogma that "ozone is toxic any way you deal with it", reinforcing the concept that ozone should never be used in medicine. This situation has generated scepticism and great antagonism towards ozone therapy also in the European medical establishment, and highly qualified journals assign a zero priority to papers dealing with this topic perpetuating the trend to isolate it in limbo. This happens in spite of the fact that ozone is considered one of the best drinking water disinfectants capable of preventing the outbreaks of infections.

Table 1 summarises several good reasons for refusing ozone therapy by orthodox medicine.

During the last fourteen years we have made a great effort to examine ozone therapy in a scientific fashion both at basic and clinical level, and we now have some ideas how ozone acts, how and why its toxicity can be controlled and how therapeutic effects can be exerted (Bocci et al, 1993a,b; 1998a).

Table 1: Why oxygen ozone therapy has it not yet been accepted by orthodox medicine?

- Excessive empiricism
- Lack of standardization
- No precise ozone generator
- Lack of solid scientific biological and clinical data
- Ozone toxicity
- The problem of charlatans
- Lack of regulation and disinterest of Health Authorities
- Lack of financial support
- Sceptical scientists

This chapter aims to give the reader the essential information and the frame of mind to operate as a real physician. An extensive description is available in a recent book (Bocci, 2002).

What is ozone and how can we use it?

Ozone is normally present as a gas made of three atoms of oxygen with a cyclic structure. The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (5–13 Megavolts) according to the reaction:

$$3\ O_2 + 68{,}400\ \text{cal} \text{ ------ } 2\ O_3$$

Consequently we always collect a gas mixture comprising no less than 95% oxygen and no more than 5%

ozone. Air must be excluded because toxic nitrogen dioxide (N_2 O) will be formed as well as ozone and it is imperative that generators are made of high quality, ozone resistant materials.

Ozone is 1.6-fold denser and 10-fold more soluble in water (49.0 ml in 100 ml water at 0° C) than oxygen. Although ozone is not a radical molecule, it is the third most potent oxidant (E°=+2.076 V) after fluorine and persulphate. Ozone is an unstable gas that cannot be stored and that should be used at once. It has a half-life of 40 min. at 20 °C, that means that its concentration measured in ug/ml of the gas mixture is halved every 40 min.

Ozone is a controversial gas because, while it is very useful in the stratosphere by absorbing dangerous B and C ultraviolet radiations, it is toxic for the pulmonary tract in the troposphere, particularly mixed with carbon monoxide (CO), N_2O and traces of acids as it occurs in smog. It must be clear that if we want to use ozone in medicine, we must avoid its toxicity that can be controlled only if we operate cautiously by:

Firstly, using a precise ozone generator equipped with a well-standardised photometer;

secondly, by collecting a precise gas volume with a defined ozone concentration; and

thirdly, by knowing the optimal dose for achieving a therapeutic effect. At variance with blood, the eyes and the respiratory mucosa are extremely sensitive to ozone because they have a minimal antioxidant and neutralizing capabilities. Thus ozone should never be inhaled and the medical clinic must be equipped with an appropriate monitor, ozone destructors and an emergency air depurator. Normally the appropriate facial masks should be worn.

What is the behaviour and fate of ozone after coming in contact with body fluids?

The essential concepts to bear in mind are the following:

a) As any other gas, ozone dissolves physically in pure water according Henry's law in relation to the temperature, pressure and ozone concentration. Only in this situation ozone does not react and in a tightly closed glass bottle ozonated water (useful as a disinfectant) remains active for a couple of days.

b) On the other hand, at variance with oxygen, ozone reacts IMMEDIATELY as soon as it is solubilised in biological water (plasma, lymph, urine):

$$O_3 + \text{Biomolecules} \text{ -------- } O + O_2$$

where atomic oxygen is very reactive. Contrary to the incorrect belief that ozone penetrates through the mucosae or enters into the cells, it is emphasised that, after the mentioned reaction, ozone does not exist any longer. In order of preference, ozone reacts with polyunsaturated fatty acids (PUFA), antioxidants such as ascorbic and uric acids, thiol compounds with –SH groups such as cysteine, glutathione (GSH) and albumin. Depending upon the ozone dose, carbohydrates, enzymes and RNA can also be affected. All of these compounds act as electron donor and undergo oxidation.

c) The main reaction:

$$\text{R-CH=R1-CH} + O_3 \text{ ------ R-CHO} + \text{R1-CHO} + H_2O_2$$

shows the simultaneous formation of reactive oxygen species (ROS) and of lipid oxidation products (LOP).

The fundamental ROS molecule is hydrogen peroxide (H_2O_2) that is a non radical oxidant able to act as an ozone messenger responsible of eliciting several biological and therapeutic effects. The transitory formation of O_2 (anion superoxide) and $^1O_2$ (singlet oxygen) is possible but we haven't demonstrated it. Presence of traces of Fe^{2+} should be avoided because they will catalyse the formation of the most reactive OH (hydroxyl radical).

$$Fe^{2+} + H_2O \text{ ------ } Fe^{3+} + OH. + OH^- \text{ (hydroxyl anion)}$$
$$Fe^{3+} + O_2^{-}. \text{ ------ } Fe^{2+} + O_2$$

Interestingly, we (Valacchi and Bocci, 2000) have also found formation of nitrogen monoxide (NO). Attention should be paid to the fact that an excess of ROS can lead to the formation of other toxic compounds such as peroxynitrite ($O=NOO^-$) and hypocloride anion (ClO^-). Although ROS have a lifetime of less than a second, they can damage crucial cell components and therefore their generation must be precisely calibrated to achieve a biological effect without any damage. This can be achieved by regulating the ozone dose (ozone concentration as

ug/ml of gas per ml of blood in 1:1 ratio) against the antioxidant capacity of blood that can be measured and, if necessary, strengthened by administration of antioxidants before and during ozone therapy.

d) LOP production follows peroxidation of PUFA present in the plasma: they are heterogenous and can be classified as lipoperoxides (LOO), alkoxyl radicals (LO), lipohydroperoxides (LOOH), isoprostanes and alkenals among which 4-hydroxy-2,3 transnonenal (4 HNE) and malonyldialdehyde (MDA). Radicals and aldehydes are intrinsically toxic and must be generated in very low concentrations. In such a case, upon blood reinfusion, they undergo dilution in body fluids, excretion, and metabolism by GSH-tranferase and aldehyde dehydrogenases. Thus only submicromolar concentrations should reach all organs, particularly bone marrow, liver, etc., where they act as signalling molecules of an ongoing acute oxidative stress. If the stage of disease is not too far advanced, these molecules can elicit the upregulation of antioxidant enzymes (superoxide-dismutase, SOD; GSH- peroxidases, catalase, etc.) and oxidative stress proteins, one of which is heme-oxygenase I (HSP-32) which, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin (Snyder and Baranano, 2001). It is emphasised that very low LOP levels can be stimulatory and beneficial, while high levels may be toxic. This conclusion, based on many experimental data, reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result: too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue) so that ideally they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity.

Which are the biological effects elicited by ROS and LOP?

Owing to space constraint, these are summarized in Tables 2 and 3. The plasma is the critical phase where ozone dissolves and reacts: hydrophilic and lipophilic antioxidants quench a considerable amount of the ozone dose but, if its concentration is correct, they allow formation of ROS and LOP.

H_2O_2 diffuses easily from the plasma into the cells and its sudden appearance represents the triggering stimulus: depending upon the cell type, different biochemical pathways can be concurrently activated in erythrocytes, leukocytes and platelets resulting in numerous biological effects (Table 2 and 3). One of the most interesting is the activation of the nuclear factor (NFKB) in lymphocytes (Fig. 1) with the consequent release of newly synthesised cytokines (Bocci and Paulesu, 1990). Once the ozonated leukocytes return into the circulation, they home in lymphoid microenvironments and successively release cytokines acting in a paracrine fashion on neighbouring and transit cells. This means that the initial stimulation can be transferred and progressively amplified *in vivo* (Fig. 2) with a possible reactivation of a depressed immune system. Figure 3 shows the variable release of interleukin-8 from thirteen normal blood donors and it is interesting because the process is influenced by the variable antioxidant capacity

Table 2: How ozone acts

Effector molecules	Cell targets	Biological effects	Therapeutic results
INITIAL PHASE ROS (H_2O_2, etc)	Plasma	Transient reduction of TAS	ROS and LOPs act as messengers
	Erythrocyte	Glycolysis (ATP, NADPH) 2,3, DPG ?	Oxygen delivery to hypoxic tissues
	Leukocytes	NFKB activation Cytokine synthesis	Activation of the immune system
	Platelets	Activation with release of growth factors and autacoids	Stimulation of healing
	Endothelial cells	Release of NO	Improved vasodilation Neoangiogenesis?

Table 3: How ozone acts

Effector molecules	Cell targets	Biological effects	Therapeutic results
Hepatocytes	Improved metabolism	Slight increase of fibrinogen and prothrombin Virucidal effect (?)	
LATE PHASE Lipid signals (LOPs): Hydroperoxides, lipoperoxides, malonyldialdehyde, 4-hydroxynonenal	Reticulo-endothelial system	Upregulation of heme-oxygenase I (HSP-32)	Increased release of CO+bilirubin
Erythroblasts	Upregulation of antioxidant enzymes	Improved transport and delivery of O_2	
Other cells	Upregulation of antioxidant enzymes	Adaptation to COS	

and the genetic make up of different individuals (Bocci et al, 1998b).

During the reinfusion of the ozonated blood into the donor, the vast expanse of the endothelial bed will be activated by LOP resulting in a enhanced release of NO and nitrosothiols with obvious relevance in ischemic diseases. Moreover, on the basis of the phenomenon of ozone tolerance that says the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response, we have postulated that LOP, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress. This interpretation is credible because it is supported by our findings of an increased level of antioxidant enzymes and HO-1 during ozonetherapy. The ability of the body to react to low-level stimuli like

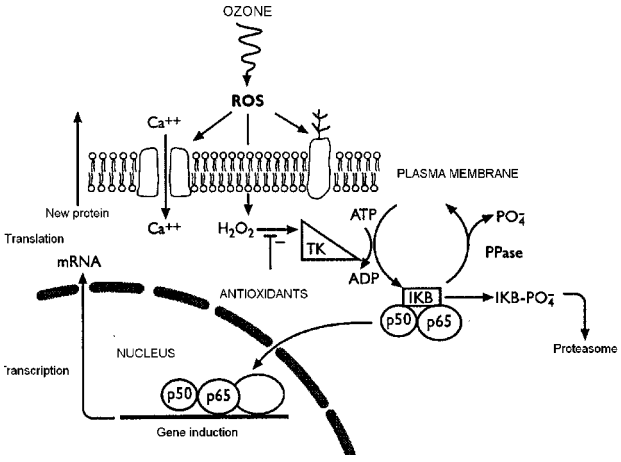

Figure 1: A schematic view of signal transduction in lymphocytes due to oxidative stress. The nuclear transcription factor NF-KB is a heterodimer composed of two subunits (p50 and p65). In resting T lymphocytes, it exists in an inactive form complexed with the inhibitor IKB. Ozone decomposes in plasma and generates ROS. These may act on lectins situated in the plasma membrane, possibly opening Ca^{++} channels, and/or by activating protein kinases. H_2O_2 activates a tyrosine kinase which phosphorylates IKB and causes its detachment from the inactive complex. While IKB-PO_4 is being degraded in the proteasome, the heterodimer moves promptly from the cytosol into the nucleus, where it regulates gene expression. Activation of a phosphatase (PPase) or an excess of intracytoplasmic antioxidants (GSH, NAC, CAT, thioredoxin, α-lipoic acid, etc.) inhibits the process.

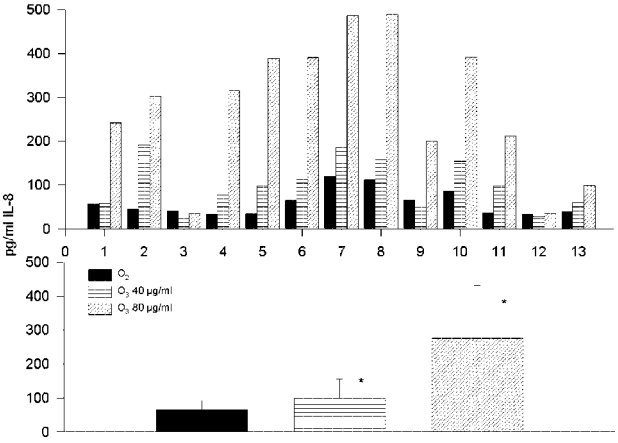

Figure 2: Effect of 1 min exposure of either O_2 or O_3 (40 and 80 µg/ml) on the production of IL-8 after 8 hr incubation of 13 blood samples. Average values are reported in the lower panel after subtraction of control values.
*Significant difference ($p<0.01$) compared with samples treated with O_2. The variable production of IL-8 among donors is noteworthy, particularly the lack of production of donors no. 3 and 12 due to a high antioxidant capacity.

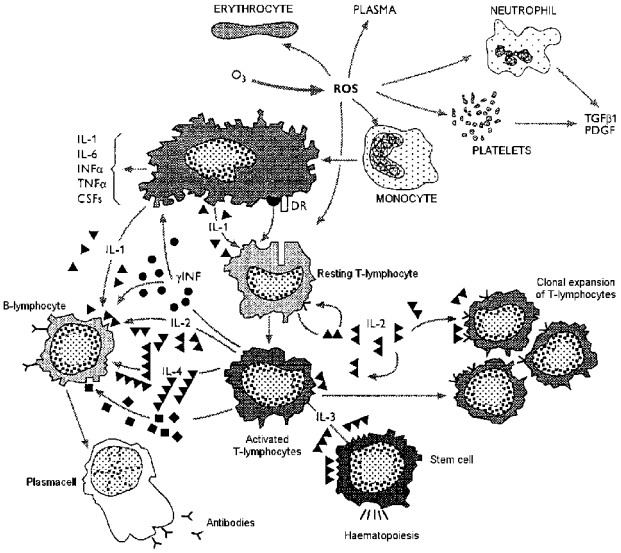

Figure 3: An overall view of interactions among O_3-ROS and immune cells which, after cytokine induction, home into various lymphoid microenvironments and further release cytokines, thus enhancing humoral and cell-mediated immunity.

repeated O_3-AHT appears of great importance because: firstly, it shows that the effect of blood ozonation ex vivo extends to all organs (Table 3 and Fig. 4) and is likely to improve erythropoiesis, mobilisation of stem cells, hence neoangiogenesis in ischemic areas. Secondly, it shows that a judicious use of ozone, in spite of acting as an oxidant, enhances the antioxidant capacity, which represents the critical factor for overcoming chronic viral infections, ischemia and cell degeneration. The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is not farfetched (Bocci,1996) and there is good evidence that this phenomenon is common in the animal and vegetal kingdom (Fig. 5). It is very unfortunate that free-radical experts do not want to distinguish between, the endogenous chronic oxidative stress (COS), occurring every day during a lifetime, and the calculated, extremely brief and exogenous oxidative stress that we induce on blood by using a precise ozone dose.

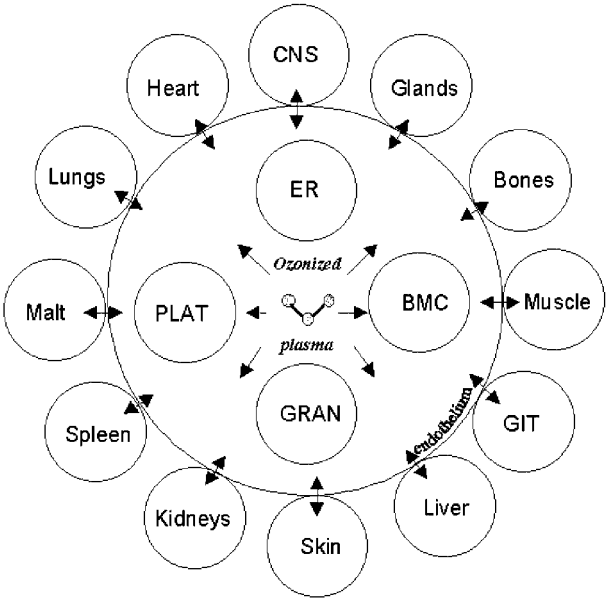

Figure 4: The multivaried biological response of the organism to ozonized blood can be envisaged by considering that ozonized blood cells and compounds interact with a number of organs. Some of these represent real targets (liver in chronic hepatitis, vascular system for vasculopathies), while other organs are probably involved in restoring normal homeostasis. ER: erythrocytes; BMC: blood mononuclear cells; GRAN: granulocytes; PLAT: platelets; GIT: gastrointestinal tract; Malt: mucosal-associated lymphoid tissue; CNS. central nervous system.

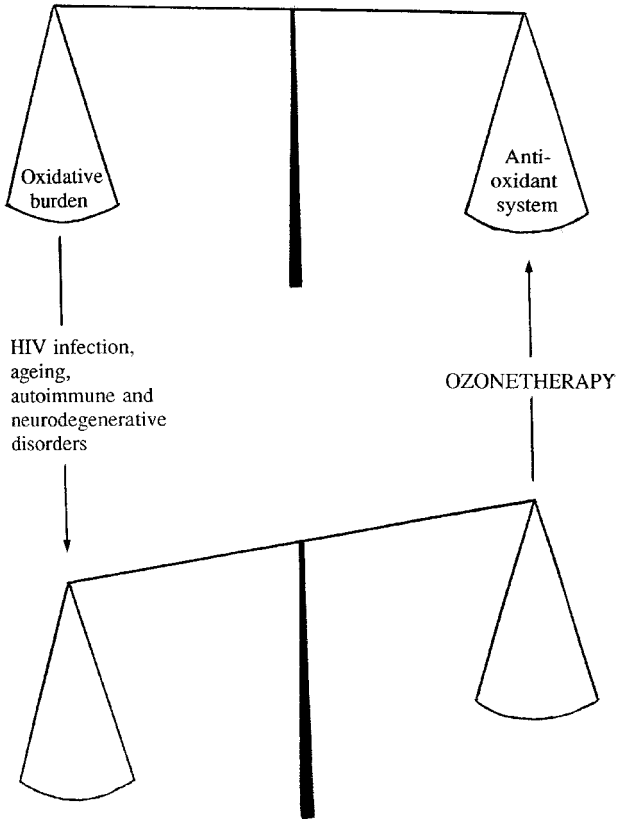

Figure 5: The normal and pathological redox balance. The scheme suggests that, by upregulating the expression of antioxidant enzymes, ozonetherapy may favour normalization of the impaired redox balance.

Which are the routes of ozone administration?

Table 4 shows that ozone can be administered with great flexibility but it should not be injected intravenously as a gas because of the risk of provoking oxygen embolization and uncertain ozone activity. An interesting, recently developed new route, discussed in this book, is an ozone delivery system to be used in dentistry.

On the basis of experimental data and on the average antioxidant capacity of human blood, we have determined the so called 'therapeutic window', that is the range of ozone concentrations (expressed as ug/ ml of gas per ml of blood) within which ozone can exert therapeutic effects without toxicity (Fig. 6). The range is surprisingly wide: 10–15 ug/ml as minimal and 80 ug/ml as the maximum. It is clear that the ozone oxidative activity is efficiently counteracted by the wealth of plasmatic and intracellular antioxidants so that an ozone concentration of 5–10 ug/ml is practically neutralised: only a trace of ROS and LOP become detectable and at the best O_3-AHT may have a placebo effect. As we are particularly conscious of ozone toxicity, we always apply the strategy "start low go slow" and, depending on the stage of the disease and of the patient' s condition, we usually scale up the concentrations from 10–15, then 20, 30 and 40 ug/ml for the 1st, 2nd, 3rd and 4th weeks, respectively. Normally we perform the treatment bi-weekly. Table 5 gives an idea of the ozone concentrations suggested in different pathologies but this remains a problem to be defined.

Table 4: Routes of ozone administration

Parenteral	Topical or Locoregional
Intravenous (IV)	Nasalep†
Intra-arterial (IA)*	Tubalep†
Intramuscular (IM)	Auricular
Subcutaneous (SC)	Oralep†
Intraperitoneal (Ipe)	Vaginal
Intrapleural (IPL)	Urethral and intrabladder
Intra-articular (Iat)	Rectal
a) Periarticular	
b) Myofascial	
Intradisc (ID)	Cutaneous
Intraforaminal (IF)	Dental
Intralesional (ILes)**	

* It is no longer used for limb ischaemia. Hepatic metastasis could be embolized via the hepatic artery.
** Intratumoural or via an intra-abscess fistula
† To be performed during 30–60 sec apnoea

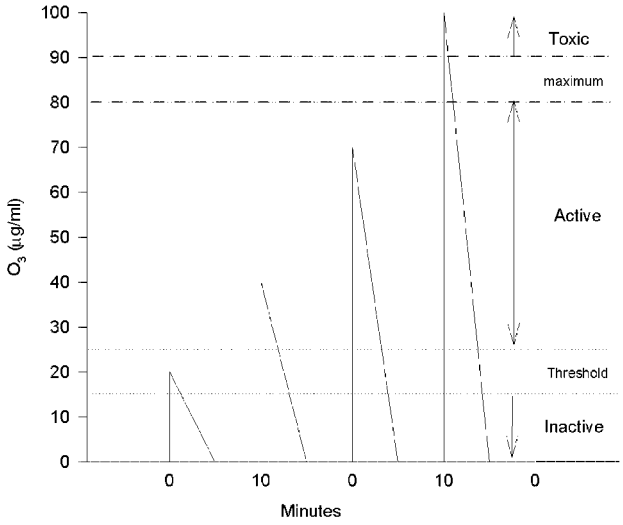

Figure 6: A schematic representation of the "therapeutic window" of ozone in blood.
Concentrations below 15 µg/ml are practically neutralized by the antioxidant system and may act as a placebo. The threshold level varies between 15 and 25 µg/ml depending on the individual TAS. Active doses range between 25 and 80–90 µg/ml. Above 90 µg/ml, an incipient haemolysis warns about toxicity.

When ozonetherapy should be used?

The answer cannot be more adamant: whenever orthodox medicine fails to solve the medical problem!

The physician has the duty to fully inform the patient of all possible and valid options available before beginning ozonetherapy. So far our experience is ample only for vascular diseases and age-related macular degeneration (ARMD) but there are several other indications:

1) Acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi
2) Osteomyelitis, pleural empyema, peritonitis, abscesses with fistulae, bed sores, chronic ulcers, diabetic foot, burns, insect and jellyfish stings, infected wounds
3) Hepatitis, herpetic infections and herpes zoster, papillomavirus infections, fungal (onychomycosis

and candidiasis) and parasitic (giardiasis, cryptosporidiosis) infections
4) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Crohn's disease).
5) Ischaemic diseases (hind-limb ischaemia, cerebral and heart ischaemia, venous stasis)
6) Degenerative disorders: age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, senile dementias, sudden hearing loss, tinnitus
7) Pulmonary diseases: emphysema, asthma chronic obstructive pulmonary disease and acute respiratory distress syndrome
8) Skin diseases (psoriasis, atopic dermatitis)
9) Chemoresistant metastatic cancer; therapy of cancer-related fatigue
10) Orthopaedic diseases the problem of back-ache!). Osteoarthrosis
11) Chronic fatigue syndrome and fibromyalgia
12) Trauma, burn injuries
13) Emergency surgery
14) Before transplantation and before elective surgery.

Conclusions and perspectives

Owing to the obstinate obstruction of official medicine towards ozonetherapy I often ask myself if ozonetherapy is obsolete or worthwhile being pursued. Our many treated patients answer for me and they loudly say that it is very beneficial. Not only have we never observed side effects but very often patients report a sense of wellbeing. The compliance is excellent and the patients, as soon as the therapeutic effect declines, ask for a new cycle. Needless to say that ozonetherapy does not "cure" ARMD or other chronic pathologies but it does improve the condition and the quality of life. We are certainly not blinded by ozonetherapy but the great strides of molecular biology during the last decade have not yet been paralleled by comparable advances in therapeutic innovations. There have been a few successes but also two deaths to warn us that things are not as easy and simple as expected. The gap between the discovery at the laboratory bench and its application at the bedside seems to be widening: at first it was thought that gene therapy would find a prompt application but many unforeseen difficulties still have to be overcome. Let us hope that the endeavour with staminal cells will be quickly successful.

Of course I do not want to diminish very important scientific achievements but simply to point out that we are often unable to predict the pitfalls and serious adverse effects when new treatments are applied from mice to patients. This is probably one reason for the worldwide boom of complementary medicine, not only in underdeveloped countries but also in USA, where homeopathy, phytotherapy and acupuncture are in great demand. Patients are often disappointed with orthodox therapy because of its frequent side effects or because the high-tech therapist shows little concern towards the patient as a human being.

Ozonetherapy is slowly capturing increasing attention in Europe and Asia, since recent developments have clarified biochemical mechanisms of action and the real possibility of taming ozone toxicity. Indeed one important characteristic of ozonetherapy is that, in comparison to other complementary approaches, it can be experimentally verified both at the biochemical and clinical levels. So far the most advanced and reliable approach has been the O $_3$-AHT because a predetermined volume of blood can be exposed to O_2-O_3 in a stoichiometric fashion and the ozone concentration is precisely determined. After 5 min. of gentle mixing the ozonated blood is reinfused into the donor in 10–15 min. Owing to the activation of blood cells and the formation of LOP messengers, this simple procedure has already yielded therapeutic results in vascular diseases and ARMD superior to those achieved by conventional medicine. Moreover the therapeutic modalities, until recently restricted to major or minor AHT and the empirical and imprecise rectal insufflation, have been extended and now include the extracorporeal blood circulation (Bocci and Di Paolo, 2003) and the quasi-total body-exposure to O_2-O_3 (Bocci et al, 1999). The latter procedure is not invasive at all and is associated with a mild hyperthermia that enhances absorption via the skin of oxygen and LOP products. Thus at the newly open ozonetherapy centre at the University Polyclinic, we will be able to select the optimal method for different pathologies, their stage and the patient's condition. The most exciting aspect of ozonetherapy relies on inducing an adaptation and possibly a re-equilibration of a chronic oxidative stress (Fig. 5), moreover different mechanisms of action elicited by different cell types are obviously important in different settings but it is also

quite possible that they act concurrently. This line of thought can explain why a simple gaseous molecule like ozone (that can even be produced naturally *in vivo*!) can have superior therapeutic effects than ordinary drugs. As far as chronic diseases are concerned, the problem is that official medicine tends to treat symptoms rather than the cause(s) of the disease. Besides the fact that the aetiology remains obscure, the treatment is often too limited and remains unsatisfactory. On the other hand, ozonated autohemotherapy, by acting on so many targets, at least in part can recover functional activities that went astray. We believe that the therapeutic power of ozonetherapy consists in simultaneously improving the circulation and oxygen delivery, in enhancing the release of autacoids, growth factors and cytokines and in reducing the endogenous, chronic oxidative stress. Although our actual understanding of ozonetherapy is still incomplete, if we can continue our search, we may find interesting and useful surprises.

Acknowledgements

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CHAPTER 1.2

History of the Clinical Applications of Ozone

Martin Grootveld, Aylin Baysan, Navaede Siddiqui, Julia Sim, Christopher Silwood & Edward Lynch

Introduction

Schonbein first discovered ozone (O_3) in 1840, and its employment in industrial environments has an impressive history. The American Indian, for whom fishing represented a central food source, noted a relationship between a successful catch and a strong odour generated by the action of lightning subsequent to an electric storm, an observation also noted by the ancient Greeks who named this odour "ozein". These cultural groups preferentially fished following electric storms, a custom which prevails even today. This phenomenon is explicable by an elevated generation of O_3 in this biosphere since the upper layer of lake water is enriched with O_2.

The generation of O_3 is achieved by the photodissociation of molecular O_2 into activated oxygen atoms which, in turn, react with a further dioxygen molecule (equations 1 and 2). The two oxygen-oxygen bond lengths in the O_3 molecule are equivalent and intermediate in strength between those of O-O and O=O bonds (Halliwell and Gutteridge, 1989). The decomposition of O_3 to O_2 is exothermic ($\Delta H = -142$ kJ mol.$^{-1}$), although the reaction is very slow in the absence of ultraviolet (uv) light and catalysts, even at temperatures as high as 250°C. UV radiation in the 240–300 nm range promotes its decomposition.

1) $O_2 \rightarrow 2O$
2) $O_2 + O \rightarrow O_3$

Reaction of O_3 with single electron donors generates the $O_3^{\cdot -}$ radical anion (Jacobs, 1986), a transient species which becomes protonated in aqueous solution forming $HO_3^{\cdot}$ which, in turn, decomposes to hydroxyl radical ($^{\cdot}OH$) as shown in equations 3 and 4.

3) $X: + O_3 \rightarrow X^+ + O_3^{\cdot -}$
4) $O_3^{\cdot -} + H^+ \rightarrow HO_3^{\cdot -} \rightarrow {}^{\cdot}OH + O_2$

Hence, the reactions depicted in equations 4 and 5 transform O_3 to an even more powerful oxidant, both *in vivo* and *in vitro*. Indeed, it is well known that O_3 acts as a precursor to a range of radical reactions both *in vitro* and *in vivo* (Goldstein and Balchum, 1967; Goldstein et al, 1969; Roehm et al, 1971; Menzel, 1970, 1971; Menzel et al, 1975; Mudd and Freeman, 1977; Dillard et al, 1978; Halliwell and Gutteridge, 1989). $^{\cdot}OH$ radical is an extremely reactive species that can give rise to cell and tissue damage if its actions are uncontrolled by the presence of sufficient levels of water-soluble antioxidants (Hoppe et al, 1995).

The role of O_3 as a disinfectant for drinking water was first suggested as early as the 19th century in view of its powerful ability to kill or inactivate micro-organisms (Ohmuller, 1892). Indeed, many researchers have found that the employment of this reactive oxygen species (ROS) over limited periods of time can disinfect water supplies effectively, it being an alternative biocidal agent to chlorine (Fether and Ingols, 1956; O'Donovan, 1965; Broadwater et al, 1973). It acts rapidly and in lower concentrations when compared to chlorine, and has no adverse side effects such as taste and malodour which are characteristic of other disinfectants (Berg, 1970). However, high levels of O_3 were found to be unsuitable for disinfection purposes in view of the limited solubility of this agent in water, and the adverse toxicological problems associated with the use of such

high concentrations (Guinvarch, 1959; Toricelli, 1959; Rilling and Viebahn, 1987).

O_3's disinfecting actions arise from its ability to destroy, neutralise or suppress the growth of pathogenic micro-organisms. A 0.35 mg/l concentration gave rise to the reduction of at least 5 log values in populations of approximately 1×10^6 cells of Esherichia coli, Vibrio Cholerae, Salmonella typhi, Yersinia enterocolitica, Pseudomonas aeruginosa, Aeromonas hydrophilae, Listeria monocytogenes and Staphylococcus aureous when compared to 0.50 mg/ml chlorine (with the exception of Vibrio cholerae). Of course, both disinfectants are expected to have been completely consumed during the treatment period (Toricelli, 1959). Both Broadwater et al (1973) and Morris (1971) have suggested that O_3 is an effective cidal agent towards vegetative cells, and also the B. megaterium and B. cereus spores of E. Coli ATCC 9677.

Notwithstanding, considering the level of organic compounds in water, these researchers suggested that this ROS can be applied at higher dosages (0.50–1.00 mg/l) and for longer periods of time (2–10 min.) for practical applications since its consumption by these components prevents full exploitation of its biocidal activities (Rilling and Viebahn, 1987).

The potential toxicological actions of O_3 should not preclude its employment for medical purposes, as emphasised by Bocci (1994). Indeed, as with any therapeutic agent, the dose employed, its site and route of delivery, its perceived role and target site, together with specified means of recognising and dealing with any adverse or toxicological effects encountered, are critically important factors for careful consideration. Hence, selected dose levels of this agent are of potential therapeutic value to the management of viral and circulatory diseases, together with cancer. Indeed, O_3 is expected to have a narrow therapeutic range, i.e., a low therapeutic index (TI), defined as the ratio of the minimum toxic to the minimum effective doses, and hence careful clinical control and monitoring of the dose applied is of critical importance.

Clinical applications of ozone

O_3 has the unique feature of decomposing to a harmless, non-toxic and environmentally safe material (dioxygen). Interestingly, O_3 has been utilised to treat patients with inflammatory bowel disorders, (specifically ulcerative colitis, Crohn's disease and chronic bacterial diarrhoea). The first O_3 generator was developed by Werner von Siemens in Germany as early as 1857, and the first report of it being used therapeutically was for the purpose of purifying blood by C. Lender in 1870. In 1885, Dr. Charles Kenworthy published "Ozone", a document outlining its first potential medical applications. To date, O_3 therapy has been a recognised treatment in sixteen nations worldwide.

Historically, O_3 was first administered by application to external body surfaces to determine its effects on a variety of lesions. Currently, there are several different types of O_3 generators utilised for the purpose of clinical applications: (1) the production of O_3 from O_2 in a narrow frequency bandwidth of uv light; (2) corona discharge involving a tube with a hot cathode surrounded by a screen anode; and (3) a method described by the term 'cold plasma' which involves a device constructed from two glass rods filled with an inert, noble gas excited by high voltage. In this latter method, the voltage jumps between the rods, forming an electrostatic plasma field, which converts O_2 to O_3. The original cold plasma generator, originally developed by Nikola Tesla in the 1920s, is still in use today.

The four primary routes for the administration of medical ozone are: (1) autohaemotherapy, of which there are two classes. Major therapy involves the removal of approximately 200 ml of blood from a patient, adding O_3 and O_2 to it and infusing the mixture back into the individual (heparin is required as an anticoagulant to prevent blood clotting), whereas minor autohaemotherapy involves withdrawing only 5–10 ml of blood from patients; (2) rectal insufflation, in which O_3 and O_2 are administered as a rectal enema. The O_2/O_3 mixture is then absorbed through the larger intestine; (3) O_3 "bagging" which involves the placing of an airtight bag around the area to be treated followed by the pumping of an O_2/O_3 admixture into the bag and absorption of O_3 through the skin; (4) in the form of externally applied ozonatated olive or sunflower oils (in this respect, treatment with O_3 appears to be therapeutically beneficial and cost-effective, although its safety remains questionable).

Autohaemotherapy

Autohaemotherapy has been used since 1950 in central Europe (Garber et al, 1991). Many unrelated diseases such as acute and chronic viral diseases (Knock and Klug, 1990a; Konrad, 1991; Werkmeister, 1991), neoplasia (Knock and Klug, 1990b), vascular disorders such as obstructive arteriopathies, venous insufficiency and vascular degenerative diseases (Konrad, 1991), ulcers and cutaneous infections (Knock and Klug, 1990a) have been treated with O_3. There are several mechanisms of action for autohaemotherapy by which ozonated blood can improve the circulation and oxygenation of hypoxic tissues. However, it is not yet clear whether the increased amount of 2,3-diphosphoglycerate observed remains at higher levels than normal for the remaining cell life-span either after autohaemotherapy (Garber et al, 1991), or following insufflation of O_3 into the colon (Halliwell and Gutteridge, 1987). CD4 cell count, and interleukin-2, gamma-interferon, beta-2-microglobulin, neopterin and p24 antigen activities were found to be unaffected by the application of O_3 during the treatment of 10 patients with HIV infection. In addition, O_3 had no significant effect on haematologic, biochemical or clinical toxicity. It should, however, be noted that erythrocytes are probably O_3's major target cells on consideration of their area of 70 mm^2 in 100 ml of blood. O_3 exerts its oxidising actions in a matter of seconds, and can generate reactive species which are firstly quenched by antioxidant compounds in plasma (Forrester, 1990; Cross et al, 1992). Moreover, cell membrane phospholipids, glycolipids and glycoproteins (Halliwell and Gutteridge, 1987), and also intracellular components such as enzymes and DNA (after exhausting the reserve of intracellular reduced glutathione) can be inactivated. Another aspect is the improved rheology of erythrocytes at the capillary level related to their flexibility and charge modification. A micro-release of adenosine triphosphate (ATP) in the ischaemic environment may cause a local vasodilatation, and hence blood flow can be improved (Washutti et al, 1989).

Major autohaemotherapy was employed in the treatment of acute chronic viral diseases (herpes, hepatitis) and neoplasia by activating induction mechanism for the phagocytic and bactericidal functions of leukocytes, with a synchronous enhancement of immunoglobulin production. In this context, autohaemotherapy is a promising treatment based on the use of inducers to elicit the endogenous production of cytokines. The advantages are lack of toxicity and a resulting equilibrated stimulation of cytokines, the latter being a phenomenon accompanied by improved oxygenation and metabolism (Washutti et al, 1989). Paulesu et al (1991) studied the action of O_3 as a potential inducer of tumour necrosis factor (TNF-α) in human blood and Ficoll-purified blood mononuclear cells (PBMC). All samples were exposed to O_3 concentrations ranging from 2.2 to 108 mg/ml and tested for TNF activity, whilst some PBMC cultures were analysed for their capacity to synthesise DNA. The applied O_3 concentration was found to be critical in terms of TNF production and cell mitogenesis. Furthermore, a high added O_3 concentration in blood was found to be more effective than it was in PBMC. Subsequently, in one of their series of experiments regarding the biological effects of O_3 in human blood, these researchers have also found that there was a significant release of transforming growth factor beta (TGF-β1) in volunteers' blood following exposure to O_3 concentrations ranging from 22 to 126 mg/ml (obtained via the utilisation of autohaemotherapy). In comparison to TGF-β1, TGF-β2 production was not influenced by O_3 exposure. Hence, it appears that blood, in the presence of heparin and 5.00 mM Ca^{2+}, allowed a consistent production of TNF-α and the induction of a low and non-hazardous level of haemolysis. These data supported the notion that autohaemotherapy may promise a valuable therapeutic approach to achieve immunoregulatory effects (Bocci et al, 1993a, 1993b). Indeed, these researchers (Bocci et al, 1994) found that autohaemotherapy had a therapeutic value in viral diseases and neoplasms since O_3 acted as a mild inducer of cytokines. They studied the influence of increasing levels of O_3 exposure on human blood and attempted to correlate the production of cytokines with the depletion of reduced erythrocyte glutathione (GSH) and haemolysis; endogenous thiols such as GSH are readily susceptible to oxidative attack by O_3, (equations 5 and 6, where GSSG represents glutathione disulphide). Erythrocytes were found to be a useful marker of O_3's oxidative activity since they constitute the bulk of blood cells and represent the main target of oxidative damage exertable by this agent. Transient exposure (30 sec.) of human blood to 78 mg/ml O_3 showed no depression in the production of cytokines, even though there was a slight

increase in haemolysis and a small decrease in intracellular GSH. It should also be noted that either a constant (up to 30 sec.) O_3 exposure, or a high O_3 concentration (108 mg/ml) substantially reduced GSH levels and depressed cytokine production (Bocci and Paulesu, 1990).

5) $GSH + O_3 \rightarrow GS^{\cdot} + O_3^{-}$
6) $2GS^{\cdot} \rightarrow GSSG$

The effects of autohaemotherapy on the human hair cycle in 42 subjects suffering from adrogenetic alopecia were studied by Riva Sanseverino et al (1995). The microscopic observation of hairs (trichogram) was carried out before and after autohaemotherapy according to a European Scientific Protocol (O_3 dosage 2,500–3,000 micrograms for each treatment, one cycle consisting of 16 treatments), and the investigators concluded that there was a marked improvement in the hair cycle.

Recently, Cooke et al (1997) revealed that a therapy involving the combination of heating, ozonation and exposure to uv light (H-O-U) may exert a therapeutic effect in the treatment of Raynaud's syndrome. Four patients with severe Raynaud's syndrome for the duration of more than five years and presenting with more than five daily attacks were selected for this study. Patients were treated daily, or on alternate days, for a 2–3 week period by the re-injection of citrated, autologous blood pre-treated with heat, O_3 and uv light. A decrease or abolition of Raynaud's attacks for at least three months was observed following this treatment.

Rectal insufflation

Ozmen et al (1993) investigated the peritoneal cavities of 240 rats following faecal-capsule implantation. Ozonated saline solution proved to be an effective irrigant for diminishing abscess formation in survivors when compared to normal saline, and saline-cephalothin irrigation in the treatment of faecal peritonitis. Subsequently, Romero Valdes et al (1993) reported that the least uncomfortable, harmless and economically-feasible manner of O_3 administration was rectal insufflation in the treatment of 72 non-diabetic patients with obliterant atherosclerosis (rather than the endovenous and intramuscular methods of employing O_3 and conventional medical treatment, the latter serving as a control group). Daily doses of O_3 ranging from 2.7 to 30 mg were employed in the treatment of intractable diarrhoea associated with AIDS, and Carpendale et al (1993) found that rectal insufflation was simple, safe and effective. In view of the fact that O_3 administration by rectal insufflation can infiltrate the parenchyma of the liver to inactivate the Hepatitis B virus (Carpendale et al, 1993), the ability of this oxidant to inactivate HIV or any enveloped virus in the wall of the colon may be possible.

Rodrigeuz et al (1997) recently performed rectal insufflation on a daily basis to patients suffering from senile dementia (vascular, degenerative or mixed aetiology). An improvement was observed according to psychometric and Hamilton tests, and parameters such as the Hachinsry scale and Katz index. Since no side effects were observed, this class of therapy was recommended for the treatment of senile dementia.

Ionozone therapy

Ionozone therapy was first utilised in Germany. It has been found to have a bacteriocidal effect, particularly on staphylococcal, streptococcal and protean infections, and has been employed in the treatment of open wounds, together with ulcers such as varicose, diabetic and pressure sores. An ionozone apparatus was designed to generate steam which was then ionised via passage over a mercury vapour arc (producing a mixture of ionised water, ozone and oxygen) (Dolphin and Walker, 1979; Church, 1980; Kief, 1988; Rodriguez et al, 1997). The physiological effects observed could be considered as a sedatory action on sensory nerve endings, and/or a stimulation of superficial blood flow. However, a bacteriocidal effect was observed in conjunction with the use of antibiotics. Following three years of treatment, Dolphin and Walker (1979) indicated that over 75% of lesions were completely healed and the condition of the skin was found to have no transparent appearance that can often be seen within the short healing period. A preliminary investigation was designed to determine the effect of ionozone therapy for treating skin lesions in elderly people in a geriatric unit (Turk, 1985). Pressure sores were defined as an area of tissue necrosis with underlying ulceration of the skin, a consequence of ischaemia related to the sustained pressure and ulceration of the lower leg regarding venous pressure. Seventeen out of 23 patients showed a rapid improvement and were discharged mobile, although 6

were referred to other units. Of the 11 surviving patients with ulcers, 10 healed rapidly and were discharged (one was referred to another unit). It should also be noted that no member of staff or patient complained of any adverse symptoms when the ionozone apparatus was employed.

A bioozone U type apparatus (produced by B. Prochazka GmbH, Reutlingen, Germany) was employed to examine the effects of an O_3/O_2 mixture in rats (Kuryszko et al, 1995). Rats poisoned with cadmium acetate during 12 weeks, at a dose of 50 mg · dm^{-3} (administered in drinking water), were treated with an O_3/O_2 mixture as an i.p. injection during the last 10 days of the experiment at a daily dose of 1.00 ml of a vehicle containing an O_3 concentration of 40 mg/ml. Two control groups included animals treated with this mixture except cadmium ion, and rats poisoned with this metal ion without the subsequent O_3 treatment. Liver and cardiac muscle were examined using transmission electron microscopy. Morphological traits of a protective effect of the administered O_3/O_2 mixture against cadmium poisoning (expressed as less destructive changes within the endoplasmic reticulum, basal cytoplasm and lysosome of the hepatocytes) were observed in both organs.

Moreover, O_3 therapy has been applied to the forehead of 16 male test persons suffering from acne vulgaris on 7 consectutive days using Vapozone 9 (a commercially-available O_3-generating instrument). Surface lipids on the unchanged skin (casual level), and 2.0 hr. after defatting the skin (replacement sum), were directly extracted and analysed by means of thin layer chromatography (tlc). As expected, a decrease in the content of free fatty acids (presumably unsaturates and polyunsaturates) was induced by this therapy (Gloor and Lipphardt, 1976).

Medical ozone is a mixture of O_3 and O_2 which can be employed for several therapeutic applications. Recently, 50 patients were treated by the employment of intradiscal O_3 inflitration as an alternative to surgery in the treatment of lumbar sciatic pain supported by an intradiscal hernia (D'erme et al, 1998). Following local anaesthesia, 12 ml of a mixture of O_3 and O_2, the former at a concentration of 20–30 rng/ml, was injected into the disk with 18–20 G needles under computer tomography (CT) or fluoroscopic guidance. This treatment was repeated two or three more times at intervals of 3, 15 or 30 days. Following each treatment, CT examinations were conducted and these revealed 82% positive results (36% excellent, 46% good), whilst there were no major changes between pre- and post-treatment CT in the remaining (18% of) cases. However, clinical examinations gave 68% positive results (40% excellent, 28% good) and 32% negative results (10% of patients underwent surgery and 22 were taken under medical and physical treatment). In view of its ease of execution and non-invasiveness, O_3 therapy was found to permit the successful out-patient treatment of lumbar sciatic pain. Moreover, the lack of major complications and the promising results obtained compared favourably with those obtained from other methods such as

chemonucleolysis, percutaneous automated discectomy, together with micro- and conventional surgery. Hence, O_3 therapy can be considered a useful treatment for lumbar sciatic pain, and can, in principle, offer a valid alternative to surgery in many cases.

Potential applications of O_3 treatment in dentistry

When mixed into pyrogen-free water, the half-life of O_3 is 9–10 hr. (at pH 7 and 20 °C), and at 0 °C this value is approximately doubled. Ozonated water has been reported to serve as an effective agent in the dental surgery (therapeutic or otherwise) where it is reported to promote haemostasis, enhance local oxygen supply and inhibit bacterial proliferation. Therefore, O_3 can be applied during dental surgery, or following tooth extraction processes (Turk, 1985; Phillipi, 1997).

Recently, a denture cleaner using O_3 bubbles (concentration approximately 10 ppm) was considered as clinically appropriate in view of its strong disinfecting and deodorising power, and relatively high biological

Table 1: Why oxygen ozone therapy has it not yet been accepted by orthodox medicine?

- Excessive empiricism
- Lack of standardization
- No precise ozone generator
- Lack of solid scientific biological and clinical data
- Ozone toxicity
- The problem of charlatans
- Lack of regulation and disinterest of Health Authorities
- Lack of financial support
- Sceptical scientists

Table 2: How ozone acts

Effector molecules	Cell targets	Biological effects	Therapeutic results
INITIAL PHASE ROS (H_2O_2, etc)	Plasma	Transient reduction of TAS	ROS and LOPs act as messengers
	Erythrocyte	Glycolysis (ATP, NADPH) 2,3, DPG ?	Oxygen delivery to hypoxic tissues
	Leukocytes	NFKB activation Cytokine synthesis	Activation of the immune system
	Platelets	Activation with release of growth factors and autacoids	Stimulation of healing
	Endothelial cells	Release of NO	Improved vasodilation Neoangiogenesis?

safety (Murakami et al, 1996). The effectiveness of this cleanser against Candida albicans was investigated and levels of this microbe were found to decrease to about one tenth of their initial value after 30 min., and to $1/10^3$ following a 60 min. period of exposure.

In view of the projected increase in the size of an ageing dentate population in most industrialised countries, the concept of root caries has prompted an increasing interest in the nature and frequency of oral health problems in this age cohort. Elderly people retain more teeth than they did in past generations, a phenomenon ascribable to an increased public awareness of oral hygiene, a better knowledge of the prevention and treatment of oral diseases, and a greater utilisation of dental services.

With time, root surfaces are exposed to the oral environment as a consequence of periodontal diseases, mechanical injury, surgical treatment, or a combination of all these factors. Exposed root surfaces are then more susceptible to caries, which can rapidly develop with only a small deterioration in the level of oral hygiene, slight dietary changes, or the use of medications causing xerostomia. Such modifications have given rise to an increased incidence of root caries amongst elderly patients (Galan and Lynch, 1993).

Root caries has been defined as lesions resulting from carious attack, and appears as a cavitation in the root structure below the cemento-enamel junction (CEJ), with no initial involvement of the adjacent enamel. To distinguish caries originating in enamel from that commencing in root dentine, the term primary root caries lesion (PRCL) has been introduced (Lynch, 1994).

Recent investigations have demonstrated that exposure of carious dentine specimens to O_3 exposure produced by a novel generating device (HealOzone, CurOzone and KaVo) (2100ppm Ozone delivered at a

Table 3: How ozone acts

Effector molecules	Cell targets	Biological effects	Therapeutic results
Hepatocytes	Improved metabolism	Slight increase of fibrinogen and prothrombin Virucidal effect (?)	
LATE PHASE Lipid signals (LOPs): Hydroperoxides, lipoperoxides, malonyldialdehyde, 4-hydroxynonenal	Reticulo-endothelial system	Upregulation of heme-oxygenase I (HSP-32)	Increased release of CO+bilirubin
Erythroblasts	Upregulation of antioxidant enzymes	Improved transport and delivery of O_2	
Other cells	Upregulation of antioxidant enzymes	Adaptation to COS	

Table 4: Routes of ozone administration

Parenteral	Topical or Locoregional
Intravenous (IV)	Nasalep†
Intra-arterial (IA)*	Tubalep†
Intramuscular (IM)	Auricular
Subcutaneous (SC)	Oralep†
Intraperitoneal (Ipe)	Vaginal
Intrapleural (IPL)	Urethral and intrabladder
Intra-articular (Iat)	Rectal
a) Periarticular	
b) Myofascial	
Intradisc (ID)	Cutaneous
Intraforaminal (IF)	Dental
Intralesional (ILes)**	

* It is no longer used for limb ischaemia. Hepatic metastasis could be embolized via the hepatic artery.
** Intratumoural or via an intra-abscess fistula
† To be performed during 30–60 sec apnoea

Table 5: Tentative guidelines regarding ozone concentrations within the therapeutic window (20–80 µg/ml) to be used in different pathologies with the classical O_3-AHT (blood/gas ratio 1:1), twice weekly. Ozone concentrations are slowly upgraded, no more than 5 µg/ml at a time, to achieve the adaptation to COS in 2–3 weeks

	Proposed O_3 concentrations	
	Initial	Final
Infectious diseases	25	70
Vascular diseases	20	40
Degenerative diseases	20	40
Respiratory diseases	20	40
Autoimmune diseases	40	80
Metastatic tumours	25	70

rate of 615 ccs per min. for periods of between 10 and 20 s) substantially reduced the levels of pathogenic micro-organisms in these samples (Baysan et al, 2000). Indeed, the number of colony units were reduced to <1% of their control (untreated) values at both dose levels applied (Table 1), and these data indicate that O_3 successfully penetrates into the lesion and kills the great majority of micro-organisms therein, presumably via a mechanism involving the rupture of their membranes which involves the chemical modification and subsequent fragmentation of unsaturated and polyunsaturated fatty acids by this ROS. Many double blind, masked, controlled clinical trials have proven the reversal of both root caries as well as pit and fissure caries using the HealOzone system, and these studies and applications will be discussed in detail later in this book.

Conclusions

In principle, the potential toxicological actions of O_3 should not prevent its use as a therapeutic agent. Indeed, at the correct dose, O_3 can be a useful therapeutic agent. The modern development of O_3's application to Medicine began in the 1950s in Europe and has gradually spread throughout this continent and then to Australia, Israel, Cuba, Brazil and Columbia. In World War I, O_3 was used medically to treat wounds and other infections. Over 5,000 physicians worldwide routinely use this ROS in their medical practice. Research concerning the anti-microbial efficacy of O_3 has continued over the last twenty years and has conclusively shown the ability of this agent in both gaseous and aqueous solution forms to exterminate a wide range of bacteria, bacterial spores and viruses (Katzeneleson, 1974; Ishizaki, 1986). However, although the oxidative modification of critical biomolecules is primarily responsible for the biocidal actions of ozone, DNA and further intracellular agents may also be damaged when concentrations of this oxidant at levels greater than those permitted by the European Union are employed. It is the authors' contention that O_3 deserves a place in the management of health and disease, and an increased awareness of the molecular mechanisms underlying the therapeutic and toxicological properties of this oxidant should establish suitable applications and dosage limitations regarding its future clinical use. Indeed, various therapeutic regimens have successfully been tested ex vivo and *in vivo*.

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CHAPTER 1.3

Safety Aspects regarding the Therapeutic Applications of Ozone and Ozonated Culinary Oils in Medicine and Dentistry

Martin Grootveld, Christopher Silwood, Julia Sim, Navaede Siddiqui, Andrew Claxson & Edward Lynch

Introduction

Humans experience a continuous exposure to O_3 throughout their daily lives. Indeed, occupational exposure includes its production by office photocopying equipment, electric arc welding, mercury vapour lamps, laser printers, X-ray generators and alternative high voltage electrical equipment, water purification processes, and the employment of this reactive oxygen species (ROS) for bleaching purposes (Meddows-Taylor, 1947; Dickermann et al, 1954).

Side effects arising from the use (or abuse) of a particular drug or treatment are often malign, and some of these are potentially or actually lethal. Such *adverse reactions* have been defined by the World Health Organisation (WHO) as 'a response to a drug (or treatment) which is noxious and unintended which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the modification of physiological function'. However, there is not necessarily a direct causal relationship between the treatment and the adverse effect, a phenomenon which creates problems for the clinician who may wish to modify a patient's treatment in response to a true adverse reaction to the agent applied or administered, but make no change if there is no such causal link. Furthermore, it is well known that the ability of one patient to pharmacologically deal with and respond to a given agent can differ from another, sometimes markedly so. It is probable that a drug or treatment caused an adverse reaction if (1) the episode occurred during or subsequent to commencing treatment; (2) the episode ceased during or after treatment was halted; (3) the episode recurred on re-commencing treatment; (4) it is known that the episode occurs in response to the treatment; (5) there is no evidence available to suggest that the episode occurs as part of the clinical condition for which the treatment was applied or administered.

Figure 1 shows typical plots of percentage of maximum effect (both therapeutic and toxic) versus log. dose, i.e., pharmacological dose-response curves, for different agents: one with a wide and the other with a narrow safety margin. Clearly, the S-shaped curves are widely separated for the safer drug, but involve much overlap for the agent with only a narrow safety margin. An often quoted example of the latter is the anticoagulant drug warfarin, where careful control of its blood plasma concentration is mandatory.

Although O_3 has extremely powerful microbiocidal properties, such actions are not limited to these organisms but can be extended to all living systems, especially on consideration of its potent oxidising capacity towards a range of biomolecules (many of them critical for efficient physiological function in, say, mammals) and the toxicity of 'unnatural' products arising from such chemical reactions. Hence, it is anticipated that O_3 will have only a low therapeutic index, although its site and/or method of application will, of course, be expected to substantially influence the degree of separation between its therapeutic and toxic pharmacological dose profiles as shown in Fig. 1. For example, the difference between these S-shaped curves for the cutaneous administration of ozonated culinary oils are expected to be more widely separated than that corresponding to the direct, microbiocidal application of gaseous O_3 in the oral environment.

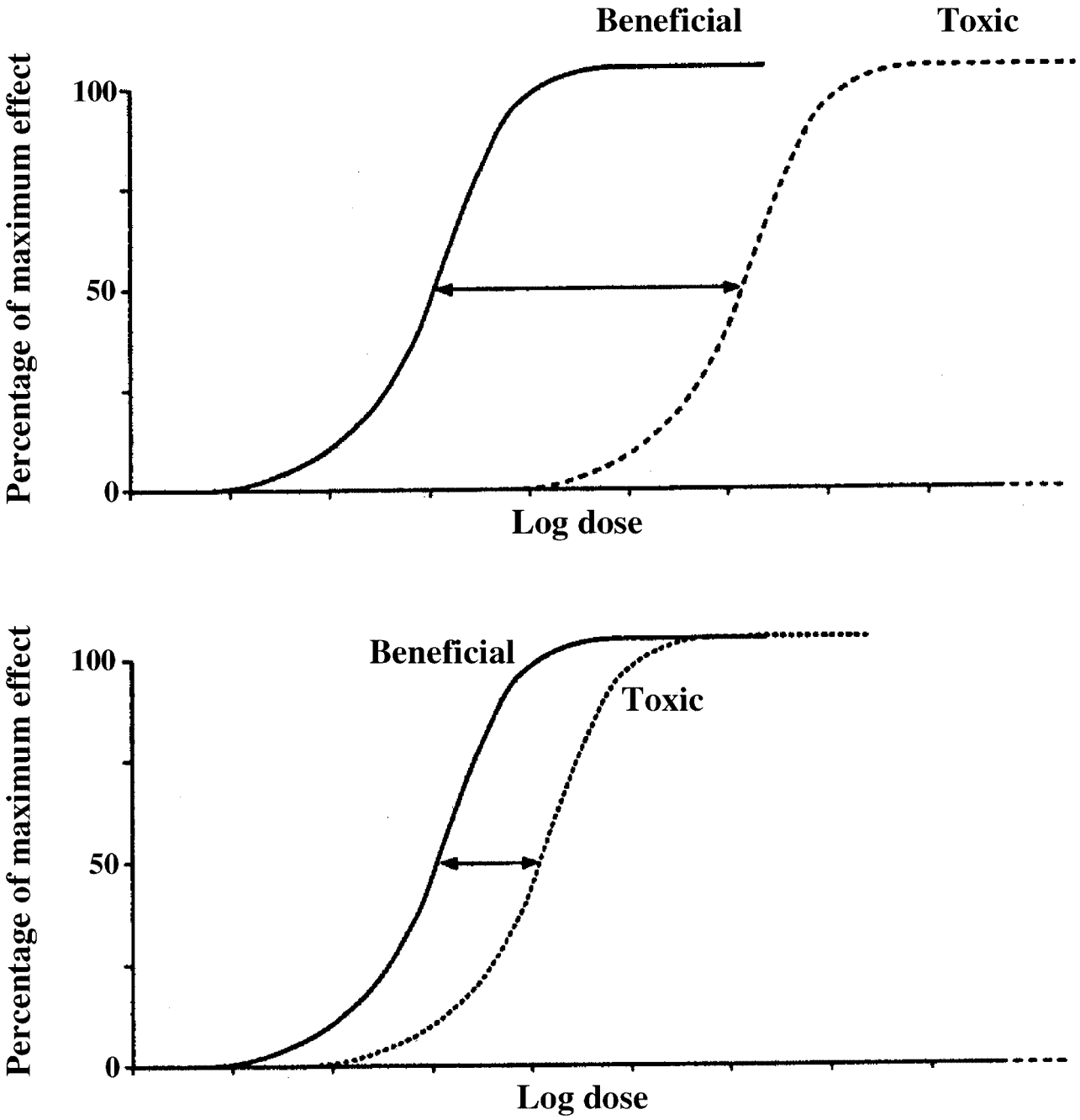

Figure 1: Therapeutic and toxic pharmacological dose-response profiles for agents with wide (top) and narrow (bottom) safety margins.

In this chapter we discuss safety aspects associated with the potential therapeutic applications of O_3 in both medicine and dentistry, with special reference to (1) its chemical reactivity with available biomolecules (focussing especially on its ability to oxidatively degrade unsaturated fatty acids); and (2) physiological effects arising from the exposure of experimental animals and humans to this agent.

Toxicology of O_3 with special reference to its reactivity with biomolecules

In view of its powerful electron accepting (oxidising) properties, O_3 can attack and chemically modify many biomolecules, e.g., both free and protein-incorporated amino acids such as cysteine, methionine and histidine. Protein tyrosine residues can be oxidised by O_3 to form

O,O′-dityrosine species which can cross-link proteins either intra- or intermolecularly via its oxygen-oxygen bond. Perhaps the most well known example of the reaction of O_3 with biomolecules is the ability of this ROS to attack, oxidatively modify and degrade unsaturated fatty acids.

The ozonation of unsaturated fatty acids (UFAs), including polyunsaturated fatty acids (PUFAs), is a complex reaction system which proceeds via the prior generation of ozonides (i.e., insertion of O_3 into one or more >C=C< bonds). Degradation of primary ozonides via scission of the C-C and one of the two O-O bonds results in the generation of a Criegee (biradical) intermediate and an unsaturated fatty acid (UFA)-specific aldehyde or ketone; the intermediate arises from the greater bond strength of the ozonide C-O bond. In the solution state, the Creigee intermediate's excess energy is redistributable and solvent cage effects enhance the probability of it recombining with the synchronously-generated carbonyl compound to produce a secondary ozonide as a major reaction product. Therefore a complex series of products are formed from the ozonation of both mono- (oleic acid) and polyunsaturated fatty acids (e.g., linoleic acid). Figure 2 gives reaction mechanisms for these processes. Cleavage of the primary ozonide generated at the C9 position of oleic acid (18:1(n-9)) gives rise to nonanal and azelaic acid. However, for linoleic acid, the unreacted >C=C< bond in the C3 position of the primary aldehydic intermediate migrates to either the C2 or C4 position in view of the increased stability afforded by conjugation between the >C=C< and >C=O bonds (Morrison and Boyd, 1992). Hexanal is generated from the primary ozonide produced at the C12 position, and dicarboxylic acids such as azelaic acid arise from rearrangement of the Criegee biradical subsequent to stabilisation. Cleavage of the primary ozonide at the second peroxy bond gives rise to further products, i.e., nonanoic acid and 9-oxononanoic acid. Stabilised Criegee biradicals can rearrange to produce a carboxylic acid, or alternatively can react with the solvated aldehyde or acidic hydrogen atoms to generate organic peroxides or hydroperoxides (Bailey, 1978; Tobias and Ziemenn, 2000).

The oxidation of UFAs by O_3 can be examined in simple 'test tube' model experiments. As expected, exposure of methyl linoleate to this oxidant at a level of 0.35 ppm in microimpingers gives rise to the generation of ozonides, and subsequently hydroperoxides (both

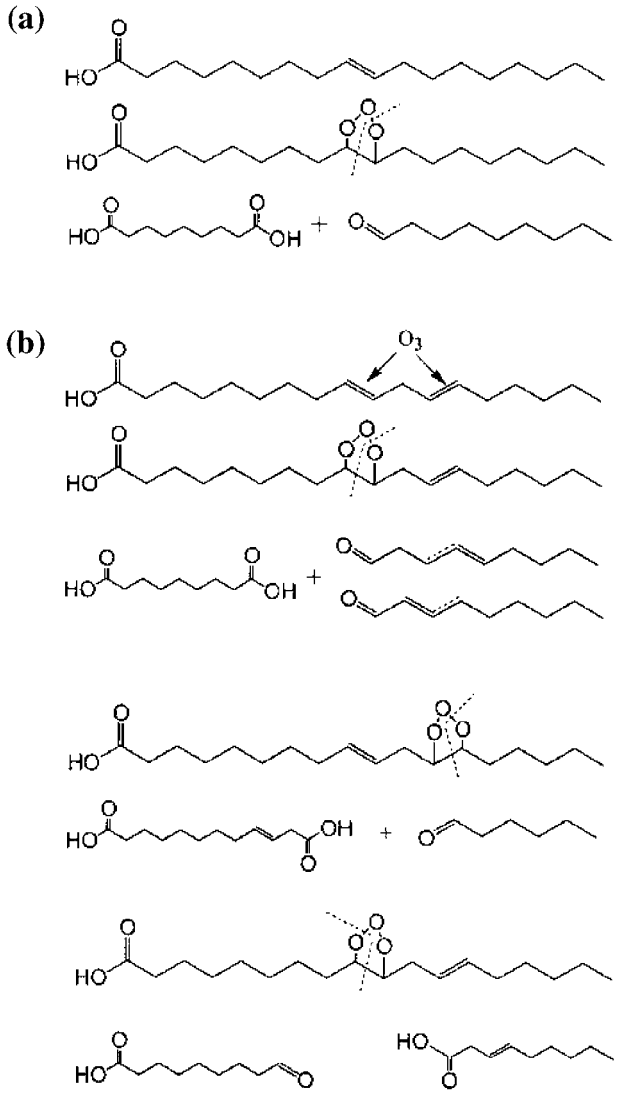

Figure 2: Reaction scheme for the generation of ozonides and their terminal degradation products from (a) oleic and (b) linoleic acid. The position of scission of the primary ozonide O-O bond is indicated by a dashed line. (a) generation of 1-nonanal and azelaic acid from oleic acid ozonide; (b) production of *trans*-2- and *trans*-4-nonenals, 1-hexanal, and nonanoic and 9-oxononanoic acids from the fragmentation of linoleic acid ozonides.

measurable iodometrically), the development of absorption at a wavelength of 223 nm (attributable to isomeric conjugated hydroperoxy- or alternative diene species) and the production of thiobarbituric acid-reactive substances (TBARS), predominantly saturated and unsaturated aldehydes. The rate of hydroperoxide (CHPD) production during the induction period was found to be directly proportional to the square root of the gas-phase O_3 concentration (Mudd and Freeman, 1977).

Predictably, addition of α-tocopherol (vitamin E) at a level similar to that of biomembranes resulted in a prolonged increase in the induction period observed prior to oxidation (Dillard et al, 1978). Selected aldehydes have been employed as biomarkers for ozonation (Pryor, 1981; Cueto et al, 1992), and reaction of O_3 with UFAs and cholesterol in pulmonary lipidic compartments generates specific products isolable from the lungs of rats exposed to this ROS, e.g., heptanal and nonanal (Cueto et al, 1992).

The potentially adverse biological effects exerted by O_3 depend upon the dynamic equilibrium between the concentration of this ROS, the duration of exposure and the nature and concentrations of intra- and extracellular antioxidants. Notwithstanding, the low levels of O_3 experienced during only limited periods of exposure to this oxidant can be prevented from inducing the self-perpetuating, autocatalytic lipid oxidation process (and therefore damage to cell membranes) by the availability of sufficient levels of antioxidants.

Stoichiometric analysis of products serving as indicators of ozonation, together with the 'tracking' of these marker molecules with ^{18}O from ^{18}O-labelled O_3 have revealed that hydrogen peroxide (H_2O_2) and aldehydic products were important mediators of damage to the lung and extrapulmonary tissues when O_3 is inhaled (Koppenol, 1982; Pryor et al, 1991; Santrock, 1992; Teige et al, 1974).

Aldehydic ozonation products have the capacity to exert many further toxicological properties. Indeed, aldehydes derived from the oxidation of PUFAs (especially the α,β-unsaturated ones such as *trans*-2-nonenal) have been implicated in the pathogenesis of atherosclerosis (Witzum and Steinberg, 1991), and its associated pathological sequelae such as ischaemic heart disease and peripheral vascular diseases. Moreover, these agents have also been shown to exert potent genotoxicological (Griffin and Segall, 1986) and pro-inflammatory (Benedetti et al, 1980) actions. Oral administration of the aldehydic lipid oxidation product 4-hydroxy-*trans*-2-nonenal to rats at a dose level of only 2.6×10^{-7} $mol \cdot dm^{-3}$, a concentration not dissimilar to that found in healthy human blood plasma, induced peptic ulcers in these animals (Jayaraj et al, 1986).

The reaction of O_3 with glycophorin inserted into a phospholipid vesicle was investigated by Banerjee and Mudd (1992); the asymmetric orientation of this protein permits an assessment of whether O_3 oxidatively modifies externally exposed amino acids in model membranes and/or those located inside the vesicle. These researchers found that O_3 preferably oxidised the first methionine residue located in the vesicle outside of the model membrane and that residues within the vesicle were protected. Hence, it appears that amino acids are protected against O_3 attack by membrane lipids, i.e., the highly polar O_3 molecule is not able to access those present in the apolar regions of membranes. Furthermore, Uppu et al (1995) exposed human red blood cell (RBC) membranes to low levels of O_3 and oxidative damage to proteins and UFAs present was monitored. These researchers found that O_3-induced oxidative damage to proteins caused significant decreases in thiol group content, the fluorescence of protein tryptophan residues, and the activity of membrane-bound acetylcholinesterase. Oxidative damage to lipids gave rise to changes in some of the UFAs in the lipid fraction of these RBC membranes, and significant amounts of hexanal, heptanal, and nonanal were formed from the ozonation process. There was no decrease in the level of RBC membrane oleate, indicating that the above volatiles predominantly arise from the oxidative consumption of PUFAs. As expected, the extent of product appearance was found to be a more sensitive measure of ozonation than substrate disappearance. These results indicate that both proteins and unsaturated lipids undergo simultaneous and competitive ozonation in human RBC membranes when O_3 was the limited reactant in the membrane.

As noted elsewhere ozonated culinary oils have been applied topically to a range of cutaneous diseases for the purpose of disinfecting lesions and promoting healing processes (Viebahn, 1994). Furthermore, ozonated olive oil has been shown to exert valuable therapeutic actions in patients with intractable fistulae or wounds. Our laboratory has recently demonstrated the rapid, multicomponent ^1H and ^{13}C NMRanalysis of products arising from the reactions of UFAs in intact culinary oils with O_3 (Figs. 3–5). Indeed, ^1H NMR analysis revealed that treatment of sunflower seed, grape seed or olive oils with O_3 gave rise to the consumption of glycerol-bound PUFAs therein (i.e., significant reductions in their *mono-* and *bis*-allylic-CH_2 group resonances located at 2.06 and 2.76 ppm respectively, and also that of the vinylic protons at 5.38 ppm), an observation consistent with their ozonation. Moreover, signals present in the 5.10–5.25 ppm regions of spectra acquired on ozonated

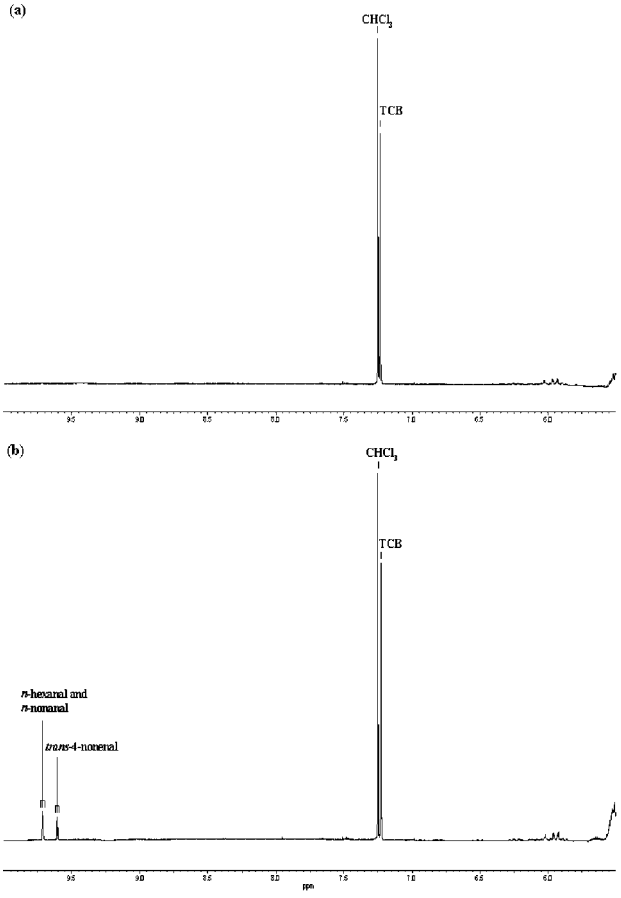

Figure 3: Partial 600 MHz ^{1}H NMR spectra of (a) control (untreated) and (b) ozonated sunflower seed oil. Aliquots (40.0 ml) of a commercially-available brand of sunflower seed oil was divided into two equivalent portions (20.0 ml). The first was treated with O_3 generated by the HealOzone unit (CurOzone, USA) for a period of 10.0 min. (equivalent to a delivery of 8,000 ppm); the second served as an untreated control. 0.25 ml aliquots of each sample was then diluted to a final volume of 0.75 ml with a 5.00×10^{-3} mol.dm^{-3} solution of 1,3,5-trichlorobenzene (internal chemical shift reference and quantitative ^{1}H NMR standard) in deuterated chloroform ($C^{2}HCl_3$), the latter serving as a field frequency lock. ^{1}H NMR spectra of these samples were acquired on a Bruker AMX-600 spectrometer at ambient temperature and an operating frequency of 600.13 MHz. The residual chloroform singlet resonance (δ=7.262 ppm) served as a secondary chemical shift reference. Abbreviations: TCB, 1,3,5-trichlorobenzene.

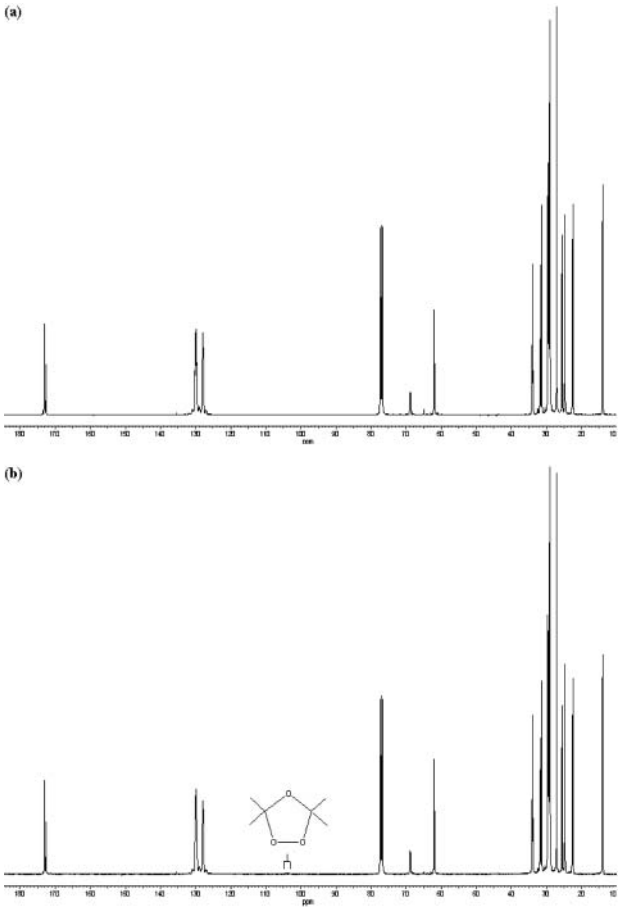

Figure 4: Partial 150 MHz ^{13}CNMR spectra of (a) control (untreated) and (b) ozonated sunflower seed oil. Aliquots (40.0 ml) of a commercially-available brand of sunflower seed oil was divided into two equivalent portions (20.0 ml). The first was treated with O_3 generated by the HealOzone unit (CurOzone, USA) for a period of 10.0 min. (equivalent to a delivery of 8,000 ppm); the second served as an untreated control. 0.25 ml aliquots of each sample was then diluted to a final volume of 0.75 ml with a 5.00×10^{-3} mol.dm^{-3} solution of 1,3,5-trichlorobenzene (internal chemical shift reference and quantitative ^{13}C NMR standard) in deuterated chloroform ($C^{2}HCl_3$), the latter serving as a field frequency lock. ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer at ambient temperature and an operating frequency of 150 MHz. (Abbreviations: as Fig. 3).

sunflower and grape seed oils are assignable to ozonide group protons (i.e., the C9 and C10 position ^{1}H nuclei in oleic or linoleic acid ozonides, and/or the C12 and C13 position nuclei of the latter ozonide). Further O_3-induced modifications to the culinary oils investigated included the production of aldehydes, specifically the *trans*-4-alkenal *trans*-4-nonenal (triplet resonance at 9.65 ppm) and the *n*-alkanals hexanal and nonanal (triplet resonance at 9.74 ppm). As expected, the oxidising capacities of each ozonised oil, i.e. their abilities to oxidise iodide (I^{-}) to tri-iodide (I_3^{-}) in aqueous solution (representing the combined content of ozonides

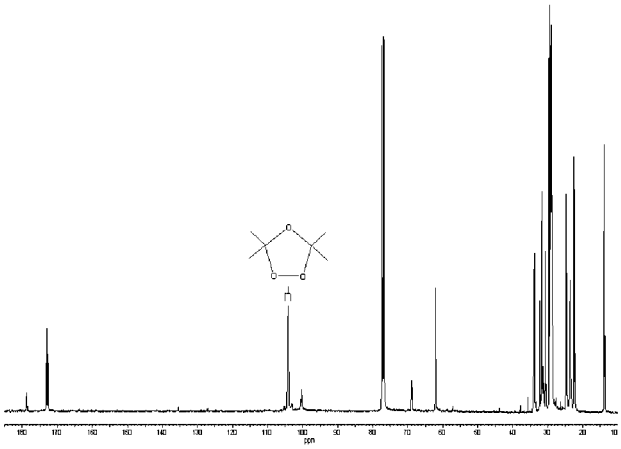

Figure 5: Partial 150 MHz ^{13}C NMR spectrum of a commercially-available gel derived from the ozonation of a culinary oil. Accurately-weighed quantities (*ca.* 120 mg) of the product were dissolved in 0.60 ml of C^2HCl_3 containing 5.00×10^{-3} mol · dm^{-3} 1,3,5-trichlorobenzene (internal chemical shift reference and quantitative ^{13}C NMR standard), the resulting solution thoroughly rotamixed and then transferred to 5-mm diameter NMR tubes. ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer at ambient temperature and an operating frequency of 150 MHz.

and hydroperoxides present) was strongly correlated with the intensities of the ozonide signals present in their ^1H NMR spectra.

The ozonide and aldehydic reaction products detectable are agents which are undoubtedly responsible for the powerful microbiocidal activities of such formulations.

Adverse physiological effects of ozone exposure and their potential molecular mechanisms

Bauer and Brook (1995) reported data regarding personal exposure to O_3 (monitoring conducted with a nitrite-coated filter passive O_3 sampler in two groups of 25 subjects); although O_3 concentrations were low during summer sampling periods, these values showed a strong dependence on collocated *uv* absorption measurements of this oxidant. These researchers estimated that personal values differing by more than 35% represented true differences in exposure which were associated with the time spent outdoors. Hoppe et al (1995) determined lung function parameters, subjective sensation and physiological responses in 40 people exposed to an equivalent distribution of both low and high O_3 levels, and found that on days with moderately elevated concentrations in the environment, this agent exerted a minor influence on pulmonary responses when expressed relative to that of other air constituents in selected locations such as indoor domestic environments or outdoor forestry. These constituents could be those arising from the deposition of O_3 on indoor location surfaces, or its reaction with motor tool exhaust components in the case of forestry workers.

O_3 is so reactive that it cannot penetrate into the air-tissue boundary before it reacts with biomolecules. Therefore, the principle targets for oxidative attack by O_3 probably lie in the fluid layer covering the internal surface of the lung. The lung lining fluid is a patchy and highly dynamic material of variable thickness, consisting of lipids, proteins and antioxidants such as ascorbate, gluathione and uric acid, and the rapid oxidation of organic biomolecules by O_3 is, of course, directly linked to its deleterious toxicological properties. Proteins exposed to low concentrations of this oxidant display the characteristics of denatured macromolecules, and the type of reaction that occurs is indicative of oxidative denaturation involving ionisation of phenolic hydroxyl groups and the uncoupling of polypeptide hydrogen-bonded linkages. Lester et al (1962) studied the reactions of O_3 with plasma proteins in 8 rabbits and concluded that ozone reacted with these macromolecules, generating antigenic species; the antibodies formed reacting with ozonised egg albumin. They suggest that the reaction of O_3 with endogenous proteins may be responsible for the ability of this gas to irritate tissues during repeated exposures. However, low concentrations (1 ppm for a period of 4 hr) have been shown not to exert a demonstrable pulmonary oedema.

In 1992 Cross et al monitored the reactions of O_3 with blood plasma components and found that urate and ascorbate were oxidatively consumed rapidly (the former representing the most important scavenger), with slower losses of protein thiol groups. No oxidation of bilirubin or α-tocopherol was observed, and there was only a small level of PUFA-derived conjugated hydroperoxydienes formed (together with no detectable generation of hexanal, nonanal and 4-hydroxynonenal). In view of these observations, these researchers concluded that lipid oxidation by O_3 should not be assumed to be the key mechanism of its toxicological actions in the respiratory tract.

Histologically, the lungs of mice killed immediately after termination of exposure to O_3 showed moderately enlarged blood vessels and their capillaries contained a conspicuous number of leukocytes with no observable oedema. Lagerwerff (1963) found that there was no change in vital capacity after exposure to levels as high as 0.5 ppm for 3 hr. However, it is possible that breathing the gas through a mouthpiece delivers a higher concentration to the lungs than that obtained via breathing from the atmosphere of a controlled environment. Hence, in view of the lack of control measurements, satisfactory conclusions cannot be drawn from the above findings.

Plopper et al (1973) performed a morphometric evaluation of quantifiable alterations in the lungs of rats on exposure to 3 ppm O_3 for a period of 4.0 hr. Exposure to this ROS was found to alter two components: squamous epithelium and endothelium of the air-blood barrier. The increases in mean thickness of the epithelial and endothelial portions were significantly different, and the lesions consisted primarily of intracellular oedema. However, granular pneumonocytes appeared to be highly resistant to increases in volume. Although the increase in the interstitial portion of the barrier was not a consistent result, this component did not respond during a 12 hr recovery period. The endothelial and epithelial swelling were more peripheral within the acinus than was the loss of epithelium, and the variability of the septal interstitial mean thickness was related to the primary distribution which was adjacent to large airways and vessels.

Exposure of one-month-old macaques to O_3 at a level similar to that of Mexico City, and a pattern mimicking that of Los Angeles (0.5 ppm O_3, 8 hr per day for a 5-day period, followed by 9 days of clean, filtered air; this cycle was repeated for five months and the animals allowed to recover with clean air for six months) gave rise to typical cellular and immune system responses observed with asthma (Plopper, 2001). Indeed, autopsies showed a disrupted and rearranged respiratory system, and smooth muscle (which controls airway constriction and relaxation) increased in mass and was realigned so that it was predominantly perpendicular to the airway. The airways were thinner and of shorter length than those of control (unexposed) animals. This marked reorganisation of airway muscles provides strong evidence that O_3, a primary component of smog, can induce and promote allergic responses that can be responsible for asthma attacks.

The influence of increasing concentrations of O_3 (0, 0.25, 0.50 and 0.75 parts-per-million (ppm)) on sustained auditory and visual attention tasks (vigilance performance) were explored by Gliner et al (1979); EEG signals were categorised between different O_3 levels, at rest, during each task performance and under ambient air conditions, and spectral and discriminant function analyses were performed on these signals. The results acquired from this study showed that O_3 concentrations as high as 0.75 ppm did not alter the performance of visual and auditory tasks. However, a deficit in the performance beyond that of the normal vigilance decline was observed at the highest level of O_3 tested. It is notable that the HealOzone system has been proven to be associated with levels of Ozone below 0.1ppm intraorally and therefore is safe to use on treating early carious lesions in teeth.

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CHAPTER 1.4

High Resolution NMR Investigations of the Mechanisms of Action of Ozone in the Oral Environment: Oxidative Consumption of Salivary, Plaque and Carious Dentin Biomolecules

Martin Grootveld, Christopher Silwood, Julia Sim, Navaede Siddiqui, Andrew Claxson & Edward Lynch

Introduction

In view of its powerful oxidising actions (redox potential of the $O_3/O_3^{\cdot-}$ couple ca. +1.6 V), ozone (O_3) has a very rich chemistry and the oxidation of critical biomolecules undoubtedly accounts for its broad-spectrum biocidal activities. Indeed, O_3 can attack a very wide variety of biomolecules (Cotton and Wilkinson, 1980; Halliwell and Gutteridge, 1989), for example, free or protein-incorporated amino acids such as methionine, cysteine, histidine and tyrosine, carbohydrates, phenolic adducts and, of course, its well-characterised ozonation of carbon-carbon double bonds, e.g., those of polyunsaturated fatty acids (PUFAs). Oxidation of PUFAs by O_3 gives rise to the production of conjugated hydroperoxydiene and subsequently aldehydic species (Pryor et al, 1976), the latter apparently serving as biomarkers of ozonation (Pryor, 1981; Cueto et al, 1992). Interestingly, reaction of water-soluble, single electron-donors with O_3 primarily generates the ozone radical anion ($O_3^{\cdot-}$), a transient adduct which, on protonation, decomposes to hydroxyl radical ($^{\cdot}OH$) and dioxygen (Pryor, 1994) (equations 1 and 2). Hence, some of the reaction products which putatively arise from the interactions of O_3 with molecules present in tissues and biofluids are, at least in principle, identical to those produced from the attack of hydroxyl radical ($^{\cdot}OH$) on such ROS scavengers.

1) $O_3 + e^- \rightarrow O_3^{\cdot-}$
2) $O_3^{\cdot-} + H^+ \rightarrow HO_3^{\cdot} \rightarrow {}^{\cdot}OH + O_2$

Currently, root caries represents a challenging problem to the dental profession in view of a substantial increase in the population of elderly patients during the late 20th century. This condition, as well as pit and fissure caries is primarily ascribable to tooth demineralisation processes induced by organic acids (e.g., lactic and pyruvic acids) generated by bacteria, predominantly *Streptococcus mutans* (Brown et al, 1986) and a recent investigation conducted by Baysan et al (2001) revealed that O_3 exerts a powerful bactericidal action towards this pathogen, together with further micro-organisms associated with primary root carious lesions. Indeed, the application of ozone in dental practices may serve as a viable, cost-effective and convenient means of treating dental caries, and it is conceivable that it could eventually replace conventional 'drilling-and-filling' procedures currently employed by dental surgeons especially for all early carious lesions.

Previous investigations have demonstrated that O_3 exerts powerful bactericidal actions towards *S. mutans*, together with further micro-organisms associated with primary root carious lesions. Therefore, the application of O_3 in dental practices may serve as a viable and convenient means for the treatment of dental caries, and in this investigation we have employed high resolution proton (^1H) nuclear magnetic resonance (NMR) spectroscopy to simultaneously evaluate the oxidising actions of this reactive oxygen species (ROS) towards a wide range of biomolecules in human saliva. We have also conducted a multicomponent ^1H NMR study of the reactivity of O_3 with biomolecules detectable in aqueous extracts of plaque and carious dentin in order to furnish further valuable molecular information regarding the mechanism of action of this oxidant in the oral environment. The therapeutic, aesthetic and bio-

chemical significance of the results acquired are discussed in detail.

Materials and methods

Sample collection and preparation

Unstimulated human saliva samples were obtained from a total of 10 healthy volunteers (5 male, 5 female). Subjects were seated comfortably and then asked to collect all saliva into a cup for a period of 10 min. Immediately after collection, all samples were centrifuged at 16,000 ×g for 30 min. (4 °C) to remove debris.

Aliquots (5.00 ml) of the above salivary supernatant samples were removed and each of them was divided into two equivalent portions (2.50 ml). The first of these was treated with O_3 generated by the HealOzone Unit (CurOzone, USA) for a period of 10 s (equivalent to a delivery of 4.484 mmol. of this oxidant) and then equilibrated at a temperature of 37 °C for a 30 min. period prior to NMR analysis; the second group of portions served as untreated controls.

Single plaque specimens were collected from each of 12 patients, weighed on a microbalance and then divided into two equivalent portions. The first was treated with 4.484 mmol. of O_3 as described above, and the second group of portions served as controls.

A total of 10 soft primary root caries lesions (PRCLs) requiring restoration from teeth were employed for this study since these represent the severest class of lesions found in humans. Plaque was primarily removed using a hand-held standard fine nylon fibre sterile toothbrush with sterile water. Subsequent to drying, a PRCL sample was collected using a sterile excavator from half of the most active part of the lesion. A further specimen was then taken from the other half of the lesion's most active portion. Each group of samples was weighed on a microbalance, the first half treated with O_3 in the manner described above, the second serving as an untreated control for each patient.

Plaque and PRCL samples (both control and O_3-treated) were extracted with 0.90 M perchloric acid ($HClO_4$) as previously described (Silwood et al,1999) and the post-neutralised clear supernatants derived therefrom subjected to ^1H NMR analysis.

Proton NMR measurements

Proton (^1H) NMR measurements on the above samples were conducted on a Bruker AMX-600 spectrometer (ULIRS, Queen Mary, University of London facility, UK) operating at a frequency of 600.13 MHz and a probe temperature of 298 K. Typically, 0.60 ml of sample was placed in a 5 mm diameter NMR tube, and 0.07 ml of $^2H_20$ was added to provide a field frequency lock.

The intense water signal (δ=4.80 ppm) was suppressed by presaturation via gated decoupling during the delay between pulses. Pulsing conditions for one-dimensional (1-D) spectra acquired on salivary supernatant samples were: 64 or 128 free induction decays (FIDS); 16,384 data points; 3–7 μs pulses; 1.0 s pulse repetition rate. Line-broadening functions of 0.30 Hz were utilised for the processing of experimental NMR data. For selected salivary supernatant specimens, the minor-but-broad protein resonances present in control and dentifrice supernatant-treated salivary supernatant samples were suppressed by the Hahn spin-echo sequence (D(90 °x-t-180 °y-t-collect)) (Hahn, 1950) which was repeated 128 times (t=68 ms). Chemical shifts were referenced to external sodium 3-trimethylsilyl (2,2,3,3-$^2H_4$) propionate (TSP; δ=0.00 ppm). Where present, the methyl group resonances of acetate (s, δ=1.920 ppm) and lactate (d, δ=1.330 ppm) served as secondary internal references for the specimens examined.

The identities of biomolecule resonances present in the ^1H NMR spectra acquired on saliva, and post-neutralised $HClO_4$ extracts of plaque and PRCLs, were routinely assigned by a consideration of chemical shift values, coupling patterns and coupling constants.

The relative intensities of selected signals therein were determined by electronic integration, and the concentrations of components detectable were determined by comparisons of their resonance areas with that of a 42.0 mM standard solution of TSP located within a coaxial NMR tube insert. This procedure was employed to avoid broadening of the TSP resonance which may arise from its binding to salivary proteins or alternative macromolecules. Where required, salivary metabolite concentrations were determined by electronic integration of their ^1H resonances and expressing their intensities relative to that of the 'internal-but-isolated' TSP standard. In view of the protein concentration of human saliva (1.40–6.40 g/l in resting specimens, mean value 2.20 g/l) (Jenkins, 1978), which is much lower than that of human blood serum (65–83 g/l) (Lentner, 1981), the minimal broad macromolecule resonance

envelope in single-pulse (one-dimensional) ^{1}H NMR spectra was found not to interfere with the observation and integration of the sharp low-molecular-mass biomolecule signals, and hence all salivary metabolite levels documented here represent those obtained from such spectra. Of course, the levels given reflect only the non-macromolecule-bound fraction of these biomolecules and therefore are expected to be somewhat lower than the total salivary concentrations. Indeed, it has been established that an 'NMR-invisible' pool of protein-bound metabolites such as lactate can be liberated from macromolecular binding sites by the addition of high levels of ammonium chloride (≥0.5 M) to biofluids (Bell et al, 1987).

The intensities of resonances present in the ^{1}H NMR profiles of the plaque and PRCL extracts were also determined by electronic integration and expression of their intensities to that of a pre-added TSP internal standard (final concentration 0.214 mM).

Two-dimensional shift-correlated ^{1}H-^{1}H NMR (COSY) spectra of human salivary supernatants were acquired on the Bruker AMX-600 facility using the standard sequence of Aue et al (1976), with 2,048 data points in the t_2 dimension, 256 increments of t_1, a 3.00 s relaxation delay, and 24 transients.

^{1}H-^{1}H total correlation spectroscopy (TOCSY) spectra were acquired on these samples using the RD-(90°-t_1-spin lock)-ACQ pulse sequence (Braunscheveiler and Ernst, 1983), with 2,048 data points in the t_2 dimension, 512 increments of t_1, a spin lock of 50 ms, a recycle delay of 1.22 s, and 64 transients (also Bruker AMX-600 facility).

Results

High-field, high resolution proton NMR analysis of human saliva

The 600 MHz single-pulse ^{1}H NMR spectra of a typical control unstimulated human salivary supernatant

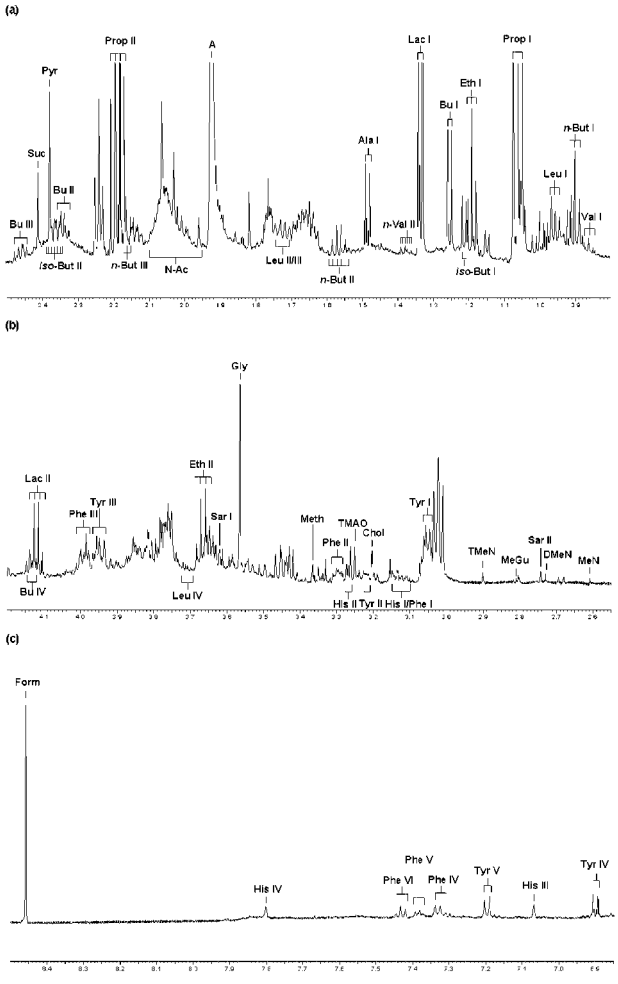

Figure 1: Single-pulse 1D ^{1}H NMR spectrum of a typical human salivary supernatant specimen. (a) complete, and (b), (c) and (d), expanded 0.80–2.50, 2.55–4.20 and 6.85–8.50 ppm regions, respectively, of the 600.13 MHz single-pulse ^{1}H NMR spectrum of a human salivary supernatant specimen (pH value 6.78). A typical spectrum is shown. Abbreviations: A, acetate-C<u>H</u>$_3$; Ala, alanine-C<u>H</u>$_3$; Bu I, β-hydroxybutyrate γ proton; Bu II, III and IV, β-hydroxybutyrate β, β′ and α protons respectively (ABX coupling system); *iso*-But I and II, *iso*-butyrate-C<u>H</u>$_3$ and -C<u>H</u> group protons respectively; *n*-But I, II and III, *n*-butyrate γ, β and α protons respectively; Chol, choline-N$^+$(C<u>H</u>$_3$)$_3$; DMeN, dimethylamine-C<u>H</u>$_3$; Eth I and II, ethanol-C<u>H</u>$_3$ and -C<u>H</u>$_2$ group protons respectively; Form, formate-<u>H</u>; Gly, glycine-C<u>H</u>; His I and II, histidine <u>ABX</u> β protons; His III and IV, histidine imidazole ring protons; Lac I and II, lactate-C<u>H</u>$_3$ and -C<u>H</u> protons respectively; Leu I, II, III and IV, leucine δ, γ, β and α protons respectively; MeGu, methylguanidine-C<u>H</u>$_3$; MeN, methylamine-C<u>H</u>$_3$; Meth, methanol-C<u>H</u>$_3$; N-Ac, spectral region for acetamido methyl groups of N-acetyl sugars; Phe I and II, phenylalanine <u>ABX</u> β protons; Phe III, phenylalanine AB<u>X</u> α proton; Phe IV, V and VI, phenylalanine aromatic ring protons; Prop I and II, propionate-C<u>H</u>$_3$ and -C<u>H</u>$_2$ group protons respectively; Pyr, pyruvate-C<u>H</u>$_3$; Sar, sarcosine-C<u>H</u>$_3$ and -C<u>H</u>$_2$ group protons respectively; Suc, succinate-C<u>H</u>$_2$; TMAO, trimethylamine oxide ON(C<u>H</u>$_3$)$_3$; TMeN, trimethylamine-C<u>H</u>$_3$, Tyr I and II, tyrosine <u>ABX</u> β protons; Tyr III, tyrosine AB<u>X</u> α proton; Tyr IV and V, tyrosine aromatic ring protons; *n*-Val I and II, *n*-valerate δ and γ protons respectively. Assignments of ^{1}H NMR resonances to *iso*-butyrate, methylguanidine, sarcosine, *n*-valerate and N-acetylsugars are tentative in this instance.

sample is shown in Fig. 1. The high- and low-field regions of the spectrum of the control (untreated) specimen contains many prominent, sharp resonances attributable to a wide range of low-molecular-mass components. Indeed, signals assignable to short-chain organic acid anions, amino acids, and carbohydrates are readily observable. In addition to considerations of chemical shift values, coupling patterns and coupling constants, together with comparisons with established literature values (making allowances, where appropriate, for the analytical samples' pH values and the pH-dependence of selected resonances in such spectra), the identities of many of these resonances were confirmed or supported by the employment of further procedures.

Firstly, for agents with singlet resonances, or those with multiplets not readily detectable in the above 2-D spectra in view of their low concentrations in each class of the biological specimens examined, samples were 'spiked' with standard additions of a series of authentic, pure components (addition of microlitre aliquots of *ca.* 5 mM aqueous solutions). In this manner, the identities of acetate, choline, dimethylamine, formate, glycine, methanol, methylamine, pyruvate, succinate, trimethylamine and trimethylamine oxide (species with a single class of uncoupled hydrogen nuclei under the conditions employed in our studies, i.e., one singlet present in the spectrum of each component), and 3-D-hydroxybutyrate, iso-butyrate, histidine, methionine, sarcosine and n-valerate (compounds with >1 class of coupled and/or uncoupled nuclei) resonances was confirmed.

Secondly, two-dimensional (2D) proton-proton COSY and TOCSY NMR spectra of several salivary supernatant, and plaque and PRCL extract samples were acquired and data obtained provided further evidence supporting the identities of many biomolecules with coupled hydrogen nuclei. Indeed, connectivities in these 2D spectra were confirmed the presence of the organic acid anions, n-butyrate, lactate and propionate, the amino acids alanine, leucine, lysine, ornithine, phenylalanine, proline and tyrosine, carbohydrates such as glucose, galactose and N-acetylsugars, and ethanol (data not shown).

The organic acid anions are predominantly derived from microbial metabolism and hence these agents (either individually, or two or more in concert) conceivably serve as chemotaxonomic markers of microbial activity in the oral environment. For example, n-butyrate is generated by the pathogenic micro-organism P. gingivalis (Guerrant et al, 1982).

Also notable in the salivary supernatant spectra acquired is a broad resonance at 2.04 ppm which, in view of previous investigations conducted on a series of further biofluids (human or otherwise) (Bell et al, 1987; Grootveld et al, 1993) is assignable to the acetamido methyl group protons of N-acetylsugars in the highly mobile side-chains of 'acute-phase' glycoproteins (this signal underlies several sharp acetamido-methyl group resonances ascribable to either free N-acetylsugars such as N-acetylglucosamine and N-acetylneuraminate, or those present in low-molecular-mass saccharide fragments, which may arise from the actions of bacterial hyaluronidase and/or neuraminidase, respectively).

Moreover, both methanol and ethanol were detectable in many of the saliva, plaque and PRCL samples subjected to NMR analysis; the former arises from the passive or direct inhalation of cigarette smoke (which results from the combustion of tobacco lignin which contains many methoxy aromatic substituents), and, in the absence of alcoholic beverage consumption, the latter represents a microbial-derived fermentation product.

Multicomponent proton NMR investigations of the oxidation of salivary, plaque and PRCL biomolecules by O_3

600 MHz single-pulse proton NMR spectra of a typical human saliva specimen acquired prior and subsequent to in vitro treatment with O_3 in the manner described in the Materials and Methods section are also displayed in Fig. 2. This powerful oxidant was found to give rise to substantial decreases in the intensity of the pyruvate methyl group resonance (singlet at 2.388 ppm), an observation reproducible in all saliva specimens tested in this manner. These data are fully consistent with the oxidative consumption of salivary pyruvate by this ROS (Herz et al,1997) in accordance with equation 3. Indeed, previous investigations have shown that biofluid pyruvate acts as a powerful endogenous electron donor (i.e., a water-soluble antioxidant) towards hydrogen peroxide (H_2O_2) and is oxidatively decarboxylated to acetate and carbon dioxide on reaction with this ROS. The O_3-dependent decreases observed

3) $CH_3COCO_2^- + O_3 \rightarrow CH_3CO_2^- + CO_2 + O_2$

in the intensities of the pyruvate methyl group resonance were, as expected, accompanied by quantitative increases in that of the acetate methyl group signal.

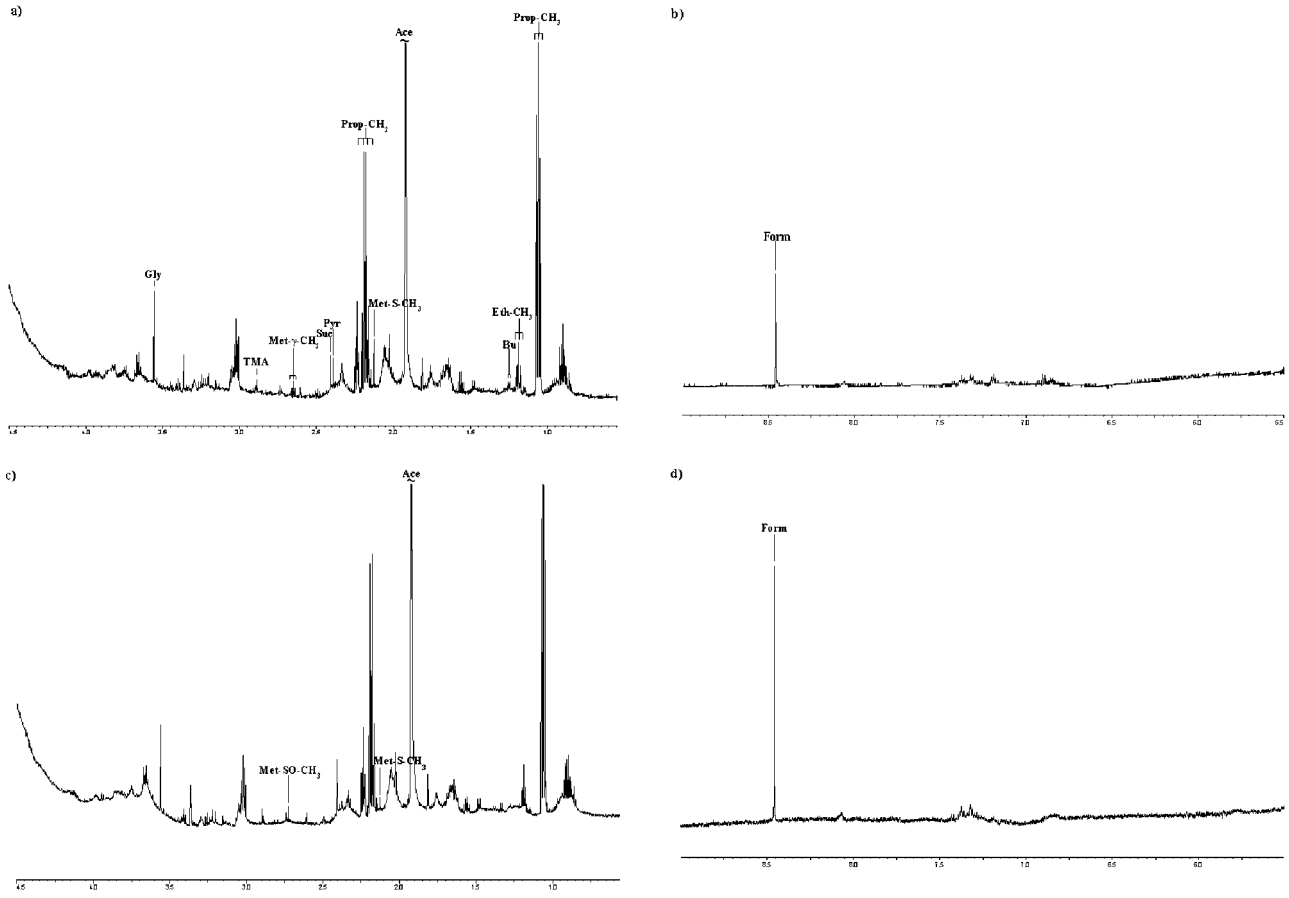

Figure 2: (a) expanded 0.50–4.50 ppm region of the 600 MHz ^{1}H NMR spectrum of a human salivary supernatant sample. A typical spectrum is shown. (b) expanded 5.50–9.00 ppm region of the same sample. (c) and (d) expanded 0.50–4.50 and 5.50–9.00 ppm regions, respectively, of the same sample treated with O_3. Abbreviations: Ace, acetate-CH_3; Bu, 3-D-hydroxybutyrate-CH_3; Eth-CH_3, ethanol-CH_3; Form, formate-H; Gly, glycine-CH_2; Met-S-CH_3 and Met-S-δ-CH_2, methionine-S-CH_3 and -δ-CH_2 respectively; Met-SO-CH_3, methionine sulphoxide-SO-CH_3; Prop-CH_3 and -CH_2, propionate-CH_3 and -CH_2 respectively; Pyr, pyruvate-CH_3; Suc, succinate-CH_2's; TMA, trimethylamine $N(CH_3)_3$.

Substantial reductions in the intensity of the lactate-CH_3 and -CH group signals (located at 1.33 (d) and 4.13 ppm (q) respectively) were also noted following treatment with O_3, indicating that this salivary biomolecule is oxidised to pyruvate which, in turn, is further consumed by this oxidant to yield acetate and carbon dioxide as outlined above. Indeed, we have previously reported that hydroxyl radical ($^{\cdot}OH$) readily oxidises lactate to acetate and carbon dioxide via pyruvate (Herz et al, 1997).

In specimens in which low-molecular-mass methionine was NMR-detectable (n=4), evidence for its O_3-mediated oxidation was obtained, i.e., significant reductions in the intensity of its thioether methyl group singlet resonance at 2.13 ppm, and the generation of signals ascribable to the side-chain methyl groups of its primary oxidation product methionine sulphoxide (singlet located at 2.725 ppm) (Stevens et al, 1992), equation 4, where $R=(^{-}O_2C)CH(NH_3^{+}) \cdot CH_2 \cdot CH_2-$. This observation is of much clinical significance since methionine, present as a residue in many salivary proteins, is liberated as the free amino acid via bacterially-mediated proteolysis and subsequently serves as a precursor to volatile sulphur compounds (VSCs) which are predominantly which are responsible for oral malodour (e.g., methyl mercaptan which accounts for approximately 60% of the VSCs detectable) (Tonzetich, 1977).

4) $R\text{-}S\text{-}CH_3 + O_3 \pi R\text{-}SO\text{-}CH_3 + O_2$

Results acquired also revealed the generation of a resonance located at 5.40 ppm (doublet, j=1.2 Hz) ascribable to allantoin which was present in spectra of all the

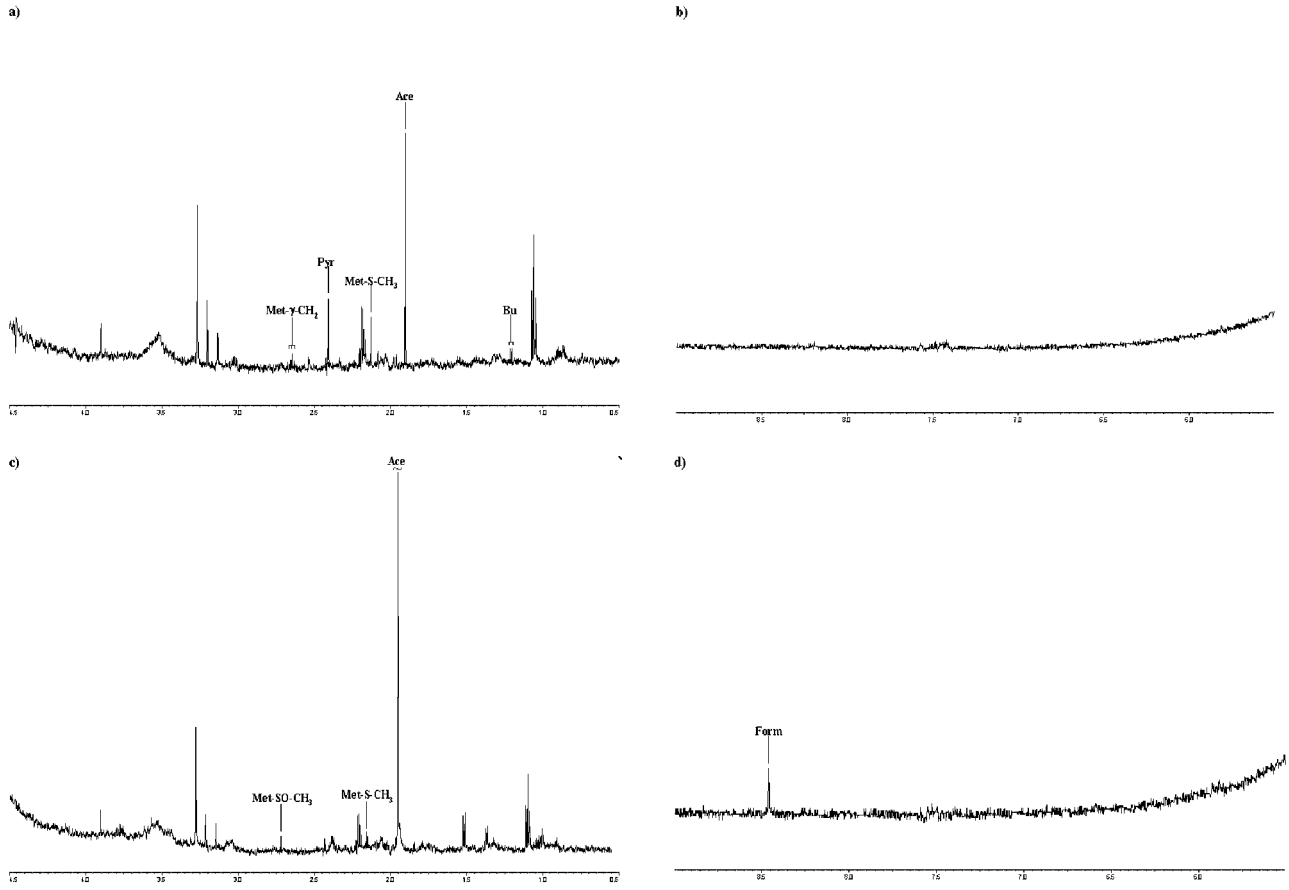

Figure 3: (a) expanded 0.50–4.50 ppm region of the 600 MHz ^{1}H NMR spectrum of a post-neutralised $HClO_4$ extract of a plaque specimen. A typical spectrum is shown. (b) expanded 5.50–9.00 ppm region of the same specimen. (c) and (d), expanded 0.50–4.50 and 5.50–9.00 ppm regions, respectively, of the same specimen treated with O_3. Abbreviations: Ace, acetate-CH_3; Bu, 3-D-hydroxybutyrate-CH_3; Form, formate-H; Gly, Met-S-CH_3 and Met-S-δ-CH_2, methionine-S-CH_3 and -δ-CH_2 respectively; Met-SO-CH_3, methionine sulphoxide-SO-CH_3; Pyr, pyruvate-CH_3.

O_3-treated specimens but absent from their corresponding control specimens. Hence, allantoin appears to represent a very clear 'marker' of the exposure of these biosamples to O_3, and arises from the latter's oxidation of the antioxidant urate which is present at a mean level of *ca.* 70 μM in this multicomponent matrix. This observation is concordant with that of Grootveld et al (1993) who found that radiolytically-generated $^{\cdot}OH$ radical also generates allantoin from urate in inflammatory knee-joint synovial fluid.

Further O_3-induced modifications to spectra which were observed in at least some of the 10 specimens investigated included marked increases in the salivary concentration of formate (s, δ=8.46 ppm) which is known to be generated from oxidative damage to carbohydrates such as glucose, sucrose and fructose (Grootveld et al, 1993) (these increases correlated with reductions in the intensities of the α-anomer H-1 resonances of glucose and other low-molecular-mass carbohydrate species, an observation which reflects oxidation of the latter by O_3); elevations in the intensisties of one or more sharp acetamido group signals in the 2.05–2.10 ppm regions of spectra which are ascribable to the methyl groups of free N-acetylsugars (N-acetylglucosamine or N-acetylneuraminate) and/or those present in low-molecular-mass saccharide oligosaccharides derived from the oxidative fragmentation of salivary or ground substance hyaluronate in accordance with results obtained from previous investigations conducted on the depolymerisation of hyaluronate by $^{\cdot}OH$ radical (Grootveld et al, 1991); reductions in the intensity of the olefinic ^{1}H resonances of PUFAs (either free or as acylglycerol species) were also notable, an observation consistent with their well-known oxidation to ozonides

(Pryor, 1991); oxidation of malodorous trimethylamine, presumably to trimethylamine oxide (i.e., reductions in the intensity of its methyl group singlet resonance located at 2.91 ppm). Furthermore, one of the samples investigated contained glycerol (a component which presumably arises from the subject's use of a toothpaste formulation shortly before sample collection), and its treatment with O_3 gave rise to the consumption of this agent (i.e., a clear removal of its two methylene (3.58 and 3.67 ppm) and single methine group (3.78 ppm) multiplet proton signals), an observation indicating its oxidation to corresponding aldehydic and/or carboxylate anion adducts.

^{1}H NMR analysis revealed that treatment of human plaque specimens with O_3 also gave rise to the oxidative decarboxylation of pyruvate present in these specimens (generating acetate and CO_2, as noted for human saliva). Moreover, the VSC precursor methionine was oxidised to its sulphoxide (Fig. 2).

The ^{1}H NMR profiles of control and O_3-treated PRCL samples (as post-neutralised $HClO_4$ extracts) also provided evidence for the O_3-mediated oxidation of pyruvate to acetate and CO_2, together with its attack of carbohydrates to generate formate (Fig. 3). O_3 was also found to oxidise PRCL lactate, urate, glycosaminoglycans and methionine to acetate and CO_2 (via pyruvate), allantoin, low-molecular-mass-oligosaccharide fragments and methionine sulphoxide respectively.

A further difference noted between the pre- and post-O_3 treated saliva samples were clear O_3-induced

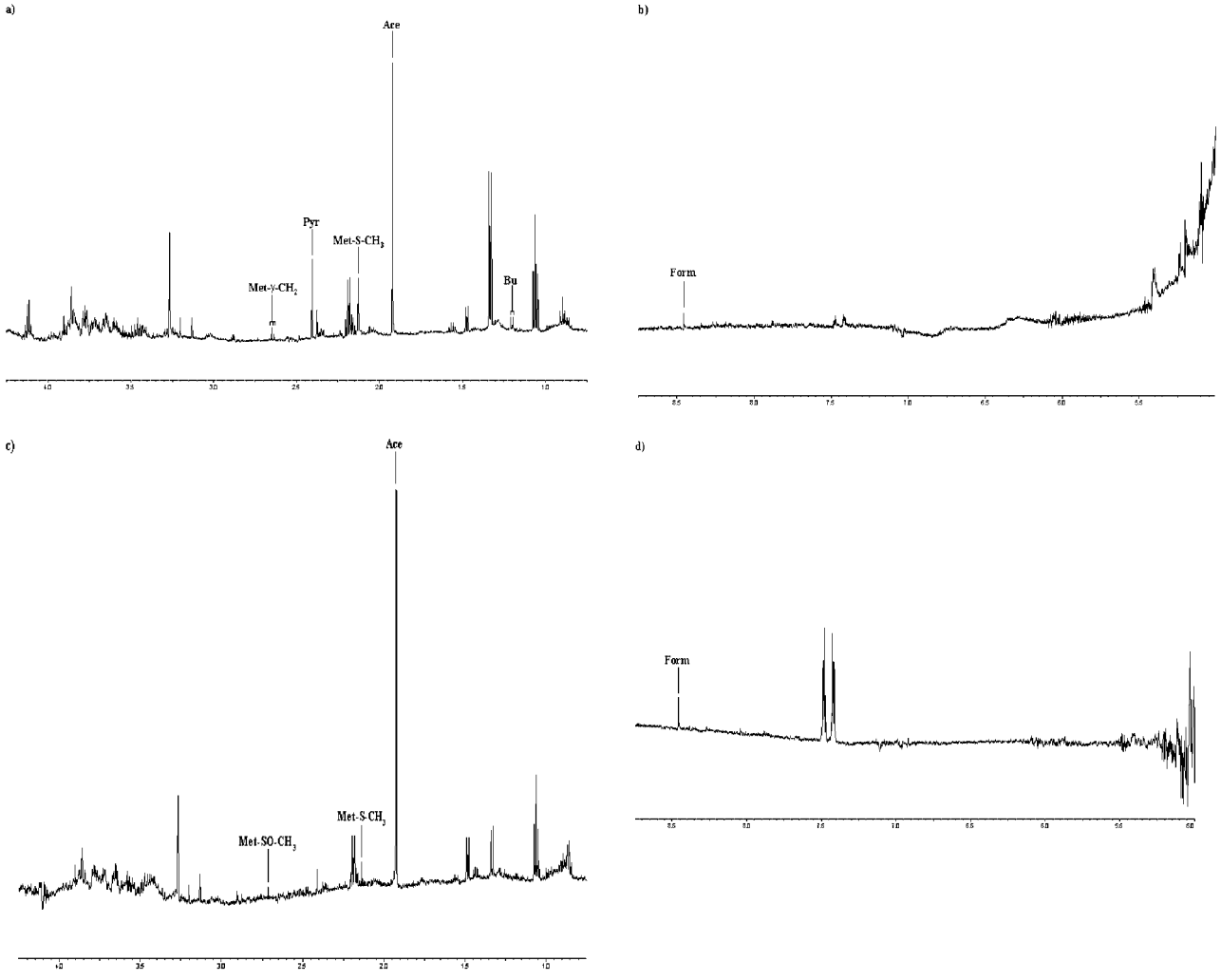

Figure 4: (a) expanded 0.50–4.50 ppm region of the 600 MHz ^{1}H NMR spectrum of a post-neutralised $HClO_4$ extract of a caries specimen. A typical spectrum is shown. (b) expanded 5.50–9.00 ppm region of the same specimen. (c) and (d), expanded 0.50–4.50 and 5.50–9.00 ppm regions, respectively, of the same specimen treated with O_3. (Abbreviations: as Fig. 3).

increases in the normalised intensities of selected low-molecular-mass components which would not normally be considered as oxidation products derived from alternative biomolecules, e.g., glycine, n-butyrate and propionate. These modifications indicate that O_3, $^{\cdot}OH$ radical derived from its single electron reduction product ($O_3^{\cdot -}$), or a combination of these highly reactive oxidants have the ability to release these low-molecular-mass metabolites from protein or alternative macromolecule binding-sites (slow molecular motion of these components when bound to salivary proteins gives rise to short spin-spin relaxation times (T_2) and, consequently, broad undetectable NMR resonances). For example, if a low-molecular-mass biomolecule is bound to a particular amino acid residue of one or more proteins (via electrostatic attraction, H-bonding, Van der Waals forces, etc.) and one or more of the above oxidants has the ability to attack and chemically modify that particular residue, then such a reaction can, in principle, give rise to the mobilisation of the low-molecular-mass biomolecule from its binding-site.

Discussion

High resolution, ^{1}H NMR spectroscopy is a technique which offers many advantages over alternative time-consuming, labour-intensive analytical methods since (1) it permits the rapid, non-invasive and simultaneous examination of a very wide range of components present in biofluids (e.g., human saliva as outlined in this study); and (2) it has little or no requirement for knowledge of sample composition prior to analysis. Furthermore, chemical shift values, coupling patterns and coupling constants of resonances present in ^{1}H NMR spectra of such multicomponent systems provides much valuable information regarding the molecular nature of both endogenous and exogenous chemical species therein.

As demonstrated here, the technique is of much value and utility regarding multicomponent evaluations of the interactions of O_3 with biomolecules present in human saliva, plaque and carious dentin, and the oxidative decarboxylation of salivary pyruvate by O_3 serves as an important example of this which may be of some relevance to its cariostatic properties.

Indeed, pyruvic acid is a very powerful proton donor (K_a=3.20 mM) being much stronger in this capacity than lactic acid (K_a=0.14 mM) (Martell and Motekaitis, 1988) and hence may play an important role in promoting tooth demineralisation processes. Therefore, the oxidation of salivary pyruvate by O_3 may suppress the development and progression of primary root caries lesions since organic acids detectable in human saliva readily diffuse into enamel (in their unionised form). In view of these considerations, O_3 may offer caries-preventative actions and experiments to investigate this further are currently underway in our laboratories.

The production of formate from the attack of O_3 on carbohydrates in general (directly or indirectly, the latter probably involving the attack of $^{\cdot}OH$ radical on these biomolecules), may have a deleterious effect since this organic acid is also stronger than lactate (K_a=0.177 mM) (Martell and Motekaitis, 1988). However the oxidation of pyruvate to acetate (K_a value of the latter= 0.0175 mM) (Martell and Motekaitis, 1988) is clearly of greater significance than the production of formate when expressed in terms of chemical equivalence, although the ratio of the concentrations of pyruvate consumed to that of formate generated multiplied by a constant representing the ratio of the K_a values of their corresponding acid/anion couples (3.20 mM/0.177 mM=18.08) (defined here as the biomolecular O_3 efficacy index (BOE index), equation 5) is a factor which will reflect the overall chemical benefit (i.e., that regarding protection against tooth demineralisation) offered by the exposure of oral biofluids and tissues to O_3. Since O_3-induced formate production is critically dependent on the accessibility of the applied oxidant to carbohydrate species present in human saliva, alternative oral biofluids, plaque and carious dentin, specifically the total carbohydrate concentration/content of these biological samples. Hence, maintenance of low levels of formate-generating carbohydrates in these oral media (by the application of O_3 treatment at a time-point sufficiently separated from that of the patient's last intake of dietary

5) BOE Index=18.08 · (pyruvate consumed)/(formate generated)

carbohydrates, i.e., >2–4 hr.) would appear to offer a further therapeutic benefit to O_3 treatment. It should also be noted that the 'between-subjects' ranges of 'between-days' mean a.m. waking salivary pyruvate concentrations vary from 0.10–10.60 mM (Silwood et al, 2002).

The successful application of the BOE index is also

at least partially dependent on a range of further parameters. Indeed, lactate consumption by O_3, or $^{\cdot}OH$ radical generated therefrom, in the specimens examined, the liberation of organic acids/anions from macromolecule binding-sites in saliva, and the overall buffering capacity of this biofluid (critically dependent on its bicarbonate and phosphate concentrations, amongst other agents which may also exert a buffering effect) are further complicating factors which may confound the future employment of the BOE index. Hence, further investigations regarding the future utility of this efficacy index are required, especially those regarding the overall buffering capacity of human saliva, plaque and carious dentin.

Since both methyl mercaptan (CH_3SH) and hydrogen sulphide (H_2S) are generated from salivary methionine in gram-negative micro-organisms (Tonzetich, 1966; Silwood et al, 2001), oxidative consumption of the latter precursor is of great importance to oral hygiene and periodontology. Therefore, these data suggest that O_3 has the ability to clinically diminish oral malodour via the direct oxidative inactivation of at least one of their amino acid precursors if introduced into the oral environment at tolerable levels. Of course, VSCs themselves are also readily oxidisable by O_3; for example, CH_3SH is primarily converted to its corresponding disulphide (the relatively non-malodorous dimethyl disulphide). Moreover, O_3 can, at least in principle, oxidatively inactivate the VSC-generating enzymes cystine reductase and serine desulphydrase.

Oxidation of malodorous trimethylamine by O_3 is also of some aesthetic and clinical interest since it may represent a toxic, chemotaxonomic marker of bacterial activity, in addition to contributing towards oral malodour.

Data arising from this study are not dissimilar to those obtained from previous NMR investigations conducted to explore the attack of $^{\cdot}OH$ radical and superoxide anion ($O_2^{\cdot -}$) on biomolecules in intact human inflammatory knee-joint synovial fluid and blood serum, observations which support the hypothesis that at least some of the oxidative modifications observed in this work are ascribable to the actions of $^{\cdot}OH$ radical derived from $O_3^{\cdot -}$ (equations 1 and 2). Single electron-transfer agents present in saliva, plaque and PRCL specimens which potentially offer a contribution to this process include cysteine, thiocyanate anion and any dietary Fe(II) (giving rise to corresponding thiyl radical, thiocyanatyl radical and Fe(III) respectively), and further studies to investigate their roles in this process are currently being conducted in our laboratory.

In conclusion, high resolution, high field ^1H NMR spectroscopy offers many analytical advantages with regard to determinations of the nature and extent of salivary, plaque and carious dentin biomolecule oxidation by O_3 administered in the modern dental clinic. Indeed, the multicomponent analytical data provided furnishes researchers with much valuable information regarding its potential therapeutic, aesthetic and, indirectly, microbicidal actions in the oral environment.

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CHAPTER 2.1

Detection Methods of Occlusal Caries for Use in Clinical Practice

Layla Abu-Naba'a, Kim Ekstrand, Hisham Al Shorman, Junji Tagami & Edward Lynch

The advances in dental materials science and techniques for the management of dental caries changed our practice. We no longer drill or extract the tooth once the caries process is detected. Minimal invasive techniques are now available with an armament of non-operative and preventive procedures. The clinical settings utilise various tools to provide the least invasive treatments where a carious lesion is present and preventive procedures to the larger number of unaffected teeth. For a successful caries managing practice, dentists and team provide their patients with the correct diagnosis and risk assessments forming the solid basis on which all the rest is built, including monitoring means for follow-up.

Being a dynamic process under the influence of the ever-present, dental plaque, the carious process was described as an "unpreventable ubiquitous process" (Ekstrand et al, 2001). Although occlusal surfaces account for only 12.5% of those exposed surfaces to cariogenic challenges, 80%–90% of the total carious lesions experienced in children and adolescents occur on them (Anderson, 2002; Ripa et al, 1988). These lesions occur in a wide age range and involve a long process before they are frankly cavitated (Ripa et al, 1988; ten Cate, 2001; Vehkalahti et al, 1991). This means that dentists are most likely to encounter these lesions at different stages in most of their patients. Understanding these facts would allow the clinician to not only avoid supervised negligence but also to treat lesions more conservatively.

Diagnostic tools should be used under optimal clinical conditions. Understandably, an indispensable high level of skill is needed to distinguish the positive carious outcomes from normal variations or other pathological alterations in the tooth that might mimic the carious presentations.

What are the positive signs that should ring the bell?

Diagnosis of caries is one heading on a long list of tasks required from the practitioner performing the dental examination on a new patient. It becomes less attractive when examining the same patient at his 6-monthly or yearly recalls especially if other conditions other than caries are looked for. However, the following signs could be noticed at a glance from an expert clinician's eye, as it should be (Fig. 1). These are:

1. Cavitated occlusal surfaces: This might be the easiest

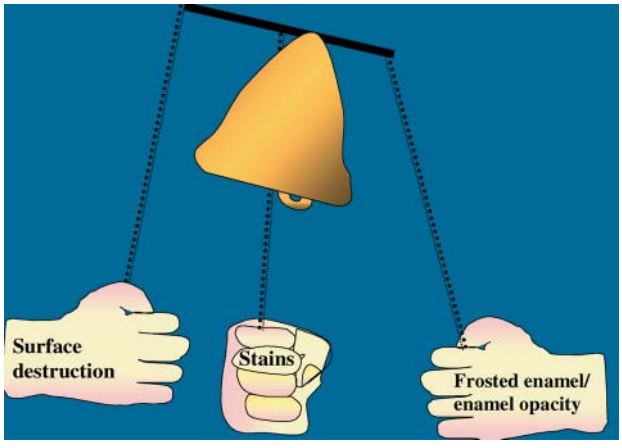

Figure 1: Signs of caries activity.

sign to detect, but at the same time, less prevalent than non-cavitated lesions (Ismail et al, 1992)
2. Opacity of enamel
3. Discoloration of the enamel surface or the dentine undermining the enamel.

Conducting the dental examinations on clean teeth is a key condition that needs to become the norm if the practitioner appreciates that non-cavitated lesions are more prevalent than cavitated ones especially in low caries risk patients. Another comprehension is that the rates at which dentinal lesions develop are faster in the occlusal caries than in the smooth surface caries and dentinal lesions were seen just over one year after eruption (Ekstrand et al, 1997) whilst the smooth surface lesions were noticed after a range of two to four years post eruption (Pitts, 1983).

What should we do once the bell is rung?

Once caries is detected, the clinician should automatically follow the sequence of caries management protocols (Fig. 2). These include:

- Provide further optimal clinical conditions aiding more signs to be detected
- Take out and use the correct tools

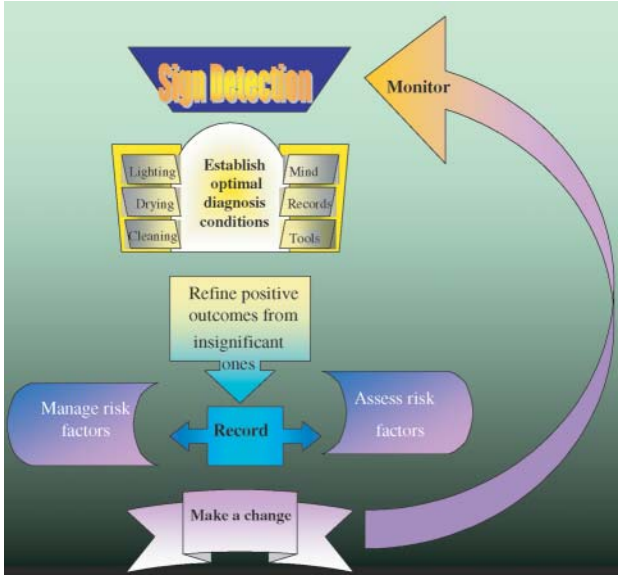

Figure 2: Protocol for caries diagnosis and management.

- Refine the positive tool outcomes due to caries from other variables producing the same clinical presentations
- Reach a diagnosis specific to the lesion in terms of severity (depth, width, length, and mineral density), activity and attributing local risk factors,
- Look for other lesions in surfaces susceptible to the same local risk factors,
- Keep good records
- Point out patient-specific risk for unaffected surfaces
- Convince the patient to alter these factors
- Provide interventions to the active caries process
- Finally, monitor lesions and compare with records.

Optimal clinical conditions for caries diagnosis

Caries-risk oriented thinking

The practitioner should focus on the diagnosis of the lesion taking into account all the local risk factors that are associated with stagnant plaque accumulation. A positive correlation was found between plaque and enamel demineralisations whilst none was found between the clinical accessibility of the fissures for cleaning and demineralisation. So it was suggested that the clinical identification of caries-susceptible areas on occlusal surfaces should be based on the actual plaque accumulation (Carvalho et al, 1992). General plaque cleaning before the initial examination (preferably done by the patient) might help diagnose areas escaping the patients' regular oral hygiene procedures or those missed due to the patient's reduced manual skills. Plaque disclosing would be the clue to areas susceptible to local risk factors, to which the following diagnostic steps are directed. Examples are areas where plaque is protected by a ditched filling or a crowded wisdom. Deep fissures and surface deficiencies might not be obvious unless the plaque is disclosed. Another disclosing method discloses acid producing active plaque, which is stimulated by a sweet rinse or sugar intake. A commercial alginate material (3M) is impregnated with such a disclosing agent where active plaque areas are coloured violet on the alginate impression. Thus the dentists can further refine their choice of the plaque where efforts should be spent and tools directed.

Good dental unit light

This doesn't mean that the light is only on, but means it is correctly directed to surfaces under examination. The practitioner should be familiar with the light's focus point and methods of deflecting light using the dental mirror (Ekstrand et al, 1998b).

Clean tooth

Experts may go to the extreme of recommending any dental examination to be performed on totally clean surfaces after full mouth scaling and polishing. The merits for this are understandable but might not be affordable for each dental examination. For the occlusal surface, cleaning should remove materia alba, plaque and stains. Cleaning using a brush, probe (Ekstrand et al, 2001), (Ismail, 1997b) air-abrasive systems (Banerjee et al, 2000) are recommended. For the latter, care must be taken to control the amount of air pressure and force of abrasive particles to prevent preparation of the tooth while performing the cleaning. Not all stains can be removed from the occlusal surface due to the complexity of the topography and adhesion of the stains. If the lesion is slowly progressing, then the stain might even be incorporated in the structure itself during intermittent cycles of remineralisation. Removal of all stains using air abrasive systems might mean minimal preparation of the tooth. This could only be justified where the dentist had previously diagnosed an active lesion and had decided on a preventive resin restoration or fissure sealant immediately after the examination and approved by the patient. The term air polishing became more popular for devices that use sodium bicarbonate particles instead of the more abrasive alumina particles and have a property of only removing plaque and superficial stains.

Dry tooth

Enamel soaked with saliva has a light refractive index near to the normal dry enamel refractive index. Frosted enamel or opacities appear when pores between enamel crystals increase by the gradual loss of minerals. If these were still small and filled with saliva then the refractive index is near to natural enamel and thus missed in a visual inspection. Frostiness seen after 5 seconds of air-drying was found to be mostly superficial demineralisation (95%). Demineralisation visible without air-drying was mostly deeper but still without dentine demineralisation (Ekstrand et al, 1995).

Good records

It might still be the job of the epidemiologist to diagnose the mere presence of caries in a number of teeth but not for the dental practitioner. The management of the growing number of initial lesions causes the treatment options to shift toward pharmaceutical and preventive regimes. The ultimate efficacy of such decisions is associated with no further progression of lesions.

Because the signs of initial lesions occur on a relatively micro-scale, telltale signs of such activity should be carefully recorded and monitored. It may be even feasible to consider each fissure and pit as a single unit over the occlusal surface, with each having its own degree of infection and would require finding full details of the lesions. Added to this is the fact that *in vivo* studies that had produced significant remineralisation in initial lesions using conventional non-operative methods needed at least 2 years to prove this effect (Curnow et al, 2002). If monitoring takes at least this long to see a difference, then clear and detailed records should be kept for these purposes as the patient shifts between practitioners is likely to happen in this time span. Avoiding supervised negligence is a growing concern for practitioners who are under the close scrutiny of their legally aware patients. This would clearly be avoided by having adequate records. Finally, changes that are associated with remineralisation do not take the tooth back to its normal structure and shape. Stains do not disappear while the frosted appearance most likely does. Roughness of the surface needs time for the occlusal wear to polish it while cavities are never filled – but with plaque. Drawings, intraoral pictures, descriptive records were all used but here we emphasize those records should be detailed and each feature to be graded on its own.

Use of correct tools

The national institutes of health consensus development conference statement dismissed having a magical single correct tool for caries diagnosis. This statement was concerned with the diagnosis and management of dental caries throughout life. In 2001, it stated, "Observations and studies during the past two decades have indicated that diagnostic and treatment paradigms may differ significantly for large, cavitated lesions versus early, small lesions and demineralised areas on tooth surfaces. The essential anatomic-pathophysiologic problem is that the carious lesion occurs within a small,

highly mineralised structure following penetration through the structure's surface in a manner, which may be difficult to detect using current methods. Additionally, carious lesions occur in a variety of anatomic locations, often adjacent to existing restorations, and have unique aspects of configuration and rate of spread. These differences make it unlikely that any one diagnostic modality will have adequate sensitivity and specificity of detection for all sites. The application of multiple diagnostic tests to the individual patient increases the overall efficacy of caries diagnosis."

What are the conventional tools used for caries diagnosis. How could the findings be enhanced by the practitioner using each tool?

Vision

It was thought that initial carious lesions occurring in the fissures are hidden in the walls of deep fissures. This was corrected by the work of Ekstrand and colleagues where they found that the maximum penetration of lesions in the narrow fissure-shaped grooves was in more than two-thirds of the cases at the entrance zone followed by the middle part and few in the bottom. Whilst in the wide groove-shaped grooves, around half of the cases were most severe in the bottom part and some at the entrance with no difference in the depth between the parts. The clinical implication of this observation was important. The extent of the lesion could be detected by careful visual inspection of the surface, as the narrow grooves would have most of the lesion at the entrance whilst the wide grooves were wide enough for the base to be seen. Furthermore, these lesions could be controlled by regular brushing(Ekstrand et al, 1995; 1997; Bjorndal et al, 1995). In more progressed lesions, loss of enamel continuity occurs when there is loss of the dentinal support for the enamel structure, which fractures off because of its brittleness (Robinson et al, 2000). Localised surface destruction corresponded to superficial dentine demineralisation.

Brownish discoloration with or without localised surface destruction accounts for 50% of the cases of dentinal demineralisation (Ekstrand et al, 1995). It starts small and increases with the advancing lesion front. This cavitation is associated with increased microbiological load in the infected dentine and becomes uncleansable (Ricketts et al, 2002a). However, in a study differentiating micro-cavitations where no dentine is exposed and frank cavitations with a dentine base, operative intervention was justified and validated microbiologically (having heavily infected dentine), but only if dentine was exposed in the floor of a cavity or when radiolucency was apparent in the radiographs more than the outer third of dentine as lesions in the outer third seemed to be lightly infected (Ricketts et al, 2002b). Down the line, it was only in advanced cavitated lesions (radiolucency seen on a radiograph in the middle or inner third of dentine) where the lateral spread in the dentino-enamel junction was seen (Ekstrand et al, 1998a) while no lateral spread was seen in non-cavitated lesions (Bjorndal et al, 1999).

Excellent reviews, based on the understanding of this relation between histological events and manifestation of lesions, reported on the high specificity of visual diagnosis (Ie et al, 1994; Ismail, 1997a). When translated to a clinical decision, this meant that teeth were safe from over treatment. However, the low sensitivity of vision to detect signs of early disease often led to many decayed teeth remaining untreated, (Wenzel et al, 1991a), underestimation of caries prevalence (Lussi, 1996), and over-treatment with fissure sealants (Deery et al, 2000).

An early study found no significant correlation between the visual appearance of the site and the level of infection in the dentine. Furthermore, non-cavitated occlusal fissures, diagnosed as carious and required restoration, exhibited a range of visual appearances of which no particular feature was indicative of its condition. Thus, visual examination alone was not helpful for deciding the treatment or preventive option for lesions (Ricketts et al, 1995). This result was further tested and conclusions were contradictory as the external signs of caries were a good indicator of the degree of caries breaks down within the tooth (Ekstrand et al, 1995) and caries activity (Table 1) (Ekstrand et al, 1998b). These promising results would not have been achieved without implementing the optimal clinical conditions under which it could be performed and training the eye to the manifestations of early lesions. Combining other diagnostic tools was an option strongly recommended (Ekstrand et al, 2001).

Table 1: Clinical severity index scores (Ekstrand et al, 2001)

Score	Description	Histopathology
0	No or slight change in enamel translucency after prolonged air drying (>5s)	No enamel demineralisation or a narrow surface opacity
1	Opacity (white) hardly visible on the wet surface, but distinctly visible after air-drying (>5s).	(Active)
		Enamel demineralisation limited to the outer 50% of the enamel layer
1a	Opacity (brown) hardly visible on the wet surface, but distinctly visible after air-drying (>5s) .	(Arrested)
2	Opacity (white) distinctly visible with out air-drying.	(Active)
2a	Opacity (brown) distinctly visible with out air-drying.	Enamel demineralisation (might not be infected) more than the outer 50% of the enamel layer up to the outer third of the dentine layer
		(Arrested)
3	Localised enamel breakdown in opaque or discoloured enamel and or greyish discolouration from the underlying dentine.	Dentine demineralisation (lightly infected) up to the middle third of the dentine layer
4	Cavitation in opaque or discoloured enamel exposing the dentine beneath.	Dentine demineralisation (Heavily infected) up to the inner third of the dentine layer

Can the visual signs detection be improved?

Yes. Dentists used to pick the carious lesion only if cavitated and considered the tooth carious accordingly. Classifications of the site of lesions as class 1–5 added to the clarification of lesion description. The use the validated visual indices detecting frostiness, cavitation and colour change was publicised and finely researched. Training the eye to these features was emphasised as well as using other tools if possible under the optimal clinical conditions described earlier. What else is needed? Much more. If treatments take a non-operative approach then there should be a method of detecting and standardising feature changes. Suppose a tooth was detected by Ekstrand's clinical score index as score 2. You provided strict oral hygiene instructions or used a pharmaceutical approach as applied fluorides or ozone treatments. The patient comes to their 6-month recall or is seen for another treatment in one month. How can the dentist detect reliably if a change had occurred while the tooth seems to be still scored 2?

The next question is how fast do these signs change once active plaque was controlled chemically or mechanically? Where strict oral hygiene was used, a clinical change was only significant in 3 years (Carvalho et al, 1992). For Ozone, a significant change was detected at one month by electrical impedance measurements but not using the Ekstrand's scoring system (Fig. 3). Then there is a need to put these features further into a quantified form to compare on recalls. Methods for this include dividing the tooth into a series of fissures and pits and record for each the dimensions or categories of severity for each feature. The features should be further distinguished if produced by other pathologies or are normal variations of tooth structure. Many studies have described the changes accompanied by arrested lesions as a package of feature changes. Yet, none of them used a comprehensive approach where each feature is recorded and tested separately and further evaluated using other advanced diagnostic tools. Recently, this has been achieved in a longitudinal randomised study where strict criteria were placed for these features and tested using electrical impedance changes after ozone treatment (Table 2) (Abu-Naba'a, 2003).

A summary of clinical features used for occlusal caries diagnosis is illustrated in Fig. 4.

Vision aids

Magnification of the tooth during examination used many optical aids. Some ranged from simple magnifying eye glasses to hand held lenses and head visors. Complicated multiple lenses and mounted or microscopes were also used. But still only few studies have

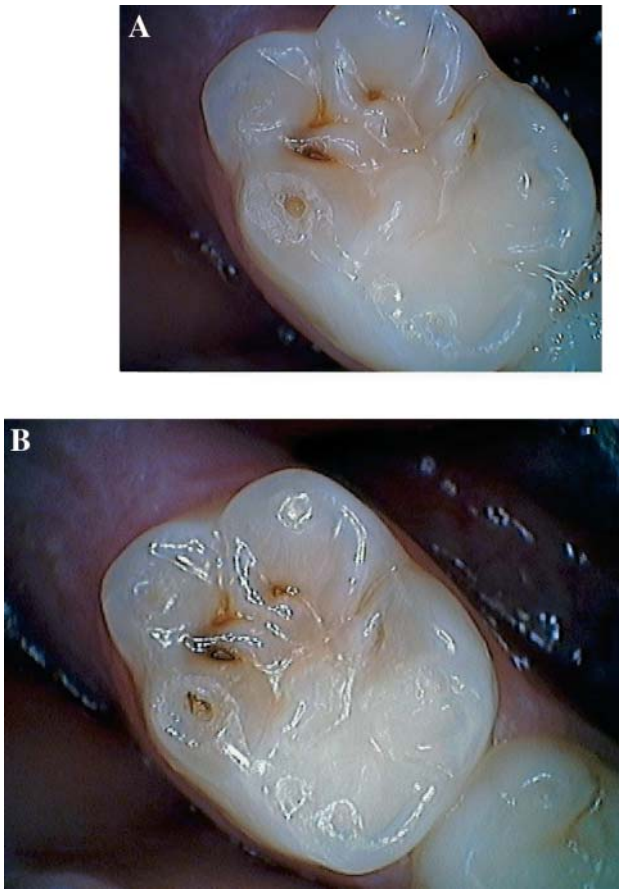

Figure 3: A tooth before (A) and after (B) Ozone treatment. Note tooth became darker, shinier and base of lesions are harder but still have the same Ekstrand's score for each pit or fissure lesion.

compared the effect of such aid on the diagnostic accuracy. The combination of visual inspection with binocular magnification, radiographs and probes were studied. As accuracy of visual inspection was comparable with other studies, all combinations shared the low sensitivity whilst the combination of bitewing radiographs and visual inspection significantly improved the sensitivity (Lussi, 1993). When the restorative decisions were compared before and after using (X3) magnification, 56% more restorative decisions were made (Whitehead et al, 1992). A similar increase was also found (Wenzel et al, 1994). With a lower magnification (X1.25) there were only a non-significant 10.3% more decisions (Lavonius et al, 1997). All studies shared a decreased agreement between visually trained examiners when using the visual aid (Lussi, 1996; Burton et al, 1990). If lesions were further divided as non-cavitated and cavitated, there was an increase in correctly diagnosed lesions

Table 2: Clinical indices used to assess occlusal pit and fissure caries (Abu-Naba'a, 2003)

Surface destruction as seen after further plaque removal by the probe and drying
1. No cavitation
2. Microcavitation
3. Frank cavitation

Hardness perception at the base of the lesion as assessed by dragging a probe from sound fissures into lesion
1. Hard as an adjacent sound fissure or pit
2. Leathery feeling on the end of probe
3. Soft

Clinical judgement
1. Sound
2. Carious
3. Arrested

Colour of the lesion
1. Yellow
2. Light brown
3. Grey
4. Dark brown
5. Black

Frostiness of enamel after 5 seconds drying (length in millimetres or described as
1. None
2. Confined to lesion's margin
3. Covering the inner slopes of adjacent cusps
4. Generalised over the occlusal surface

Enamel Undermining shade (length in millimetres or described as
1. None
2. Confined to lesion's margin
3. Covering the inner slopes of adjacent cusps
4. Generalised over the occlusal surface

Perceived treatment need
1. Requiring no intervention
2. Requiring a pharmaceutical approach but not drilling and filling or preventive resin restoration
3. Possibly requiring drilling and filling or preventive resin restoration
4. Definitely requiring drilling and filling

sensitivity from 0.20 to 0.75, meaning a better performance when diagnosing cavitated lesions (Lussi, 1996). The need for visual aids to detect carious lesions was emphasised with age, as the ability to see close objects is reduced by the reduction of the optic lens to

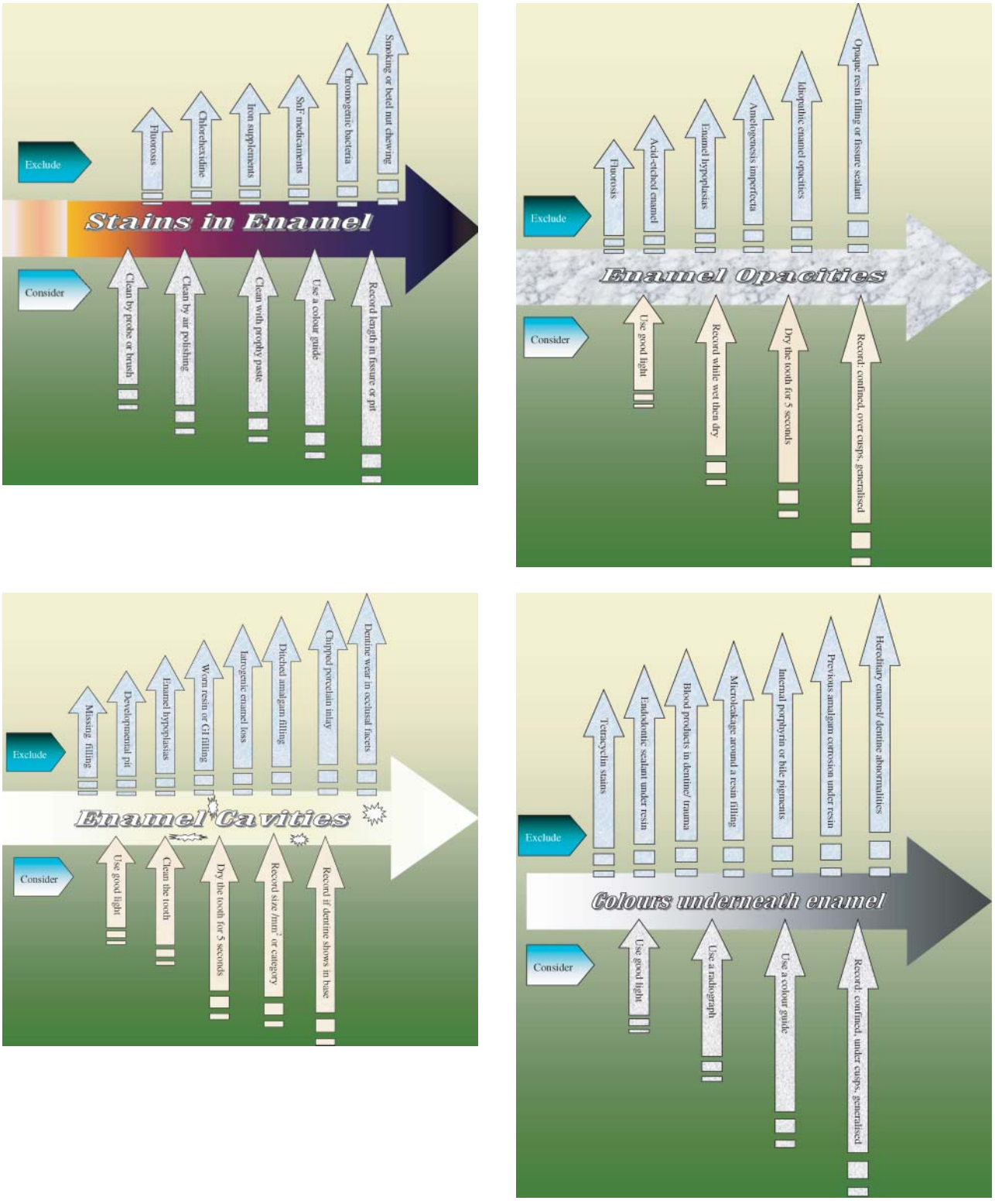

Figure 4: Clinical features used for the occlusal caries diagnosis (a) enamel stains (b) enamel opacities (c) enamel cavities (d) colours beneath enamel. (a) enamel stains; (b) enamel opacities; (c) enamel cavities; (d) colours beneath enamel.

flex and bend to obtain a focus and the best focus point becomes further than the distance of the dentist to the patient (Burton et al, 1990). Still, there seems an agreement by the experts that there should be more studies on the efficacy of such tools and comparing different magnification methods (Kidd, 2001).

Probe

Studies in the last two decades began to question the regular forceful poking motion of probes for occlusal caries diagnosis. The probe didn't add to the sensitivity of vision, and even decreased the sensitivity of visual diagnosis (0.6 versus 0.65). In an *in vitro* validation study using sharp probes to detect fissure caries, less than one quarter of initial and deep lesions was detected (Penning C, 1992). In a review, the probe was put in the bottom when listing available diagnostic systems in the diagnosis of early (non-cavitated) lesions in all surfaces except root caries (Dodds, 1993). Clinical examination was quite variable between practitioners owing to the size and shape of the explorer tip, the force applied, and the judgement of the examiner (Houpt et al, 1985).

The pressure of probing caused other damage. When newly erupted third molars were probed unilaterally, 60% of the fissures showed signs of tissue loss, significantly lower than the 7% in the control group (Ekstrand et al, 1987). In a laboratory study, probing accelerated the rate of subsequent caries progression and possibly damaged sound enamel and increased the chance for isolated lesion development. Initial lesions could be converted into cavities following probing with the size of the defect related to the pressure applied (Yassin, 1995). This might jeopardise the remineralisation ability of the previously intact lesion (Prinz et al, 1999). Probing was also thought to potentially spread infection to sound teeth but contradicting findings were published from the *in vivo* study where the authors concluded that the inoculation by probing was not a likely cause for caries initiation (Hujoel et al, 1995).

All the above does not consign the probe to retirement yet. There is still a lot for it to provide. In cavitated lesions, the information gained, is enormous. The highest increase in diagnostic performances was noticed for the probe amongst other diagnostic tools in these cavitated lesions (Lussi, 1993; Lussi, 1996). Even for non-cavitated, the probe is still accepted by the experts for the removal of plaque from the fissures that might obscure cavities and to improve the access of direct vision. Care must be applied whilst using the probe not to produce damage by disrupting the continuity of the surface (Ekstrand et al, 2001; Lussi, 1996).

The probes role in the last 5 years gained new interest where not only lesions severity is diagnosed but also lesion activity. As lesions are arrested roughness of the porous layers is known to be reduced by the combination of superficial remineralisation and enamel wear by functional occlusion (Carvalho et al, 1992). This principle was used as an essential part of examination for activity of lesions (Nyvad et al, 1999). The way the probe was used was modified from detecting a "tacky" feeling and sticking in the fissures to a light dragging motion. It is the same movement recommended to clean the fissure using a blunt probe. The information conveyed was not only roughness, but also an idea if the base is soft, leathery or hard. Agreements in diagnosis outcome occurred in 94–96% between different examiners or the same examiner at different times. In a recent longitudinal study, where questionably carious lesions were diagnosed using the probe feeling along with other criteria, these had a higher rate of definitive caries diagnosis and treatment after 2 years if the probe feeling was sticky (Hamilton et al, 2002). This altered method using the dragging motion rather than the poking motion was combined to the categorised clinical index (Table 2) to test clinical changes associated with ozone treatment. The main clinical change, which was distinctive for the treatment group, was the probe perception scores. In this pilot study, this perception change occurred early from the first month recall-visit as did the ECM significant change in the main study. Lesions were significantly harder than baseline and from control group since then (Abu-Naba'a, 2003).

A summary of the role of the probe is illustrated in Fig. 5.

Radiographic diagnosis

The use of X-rays to produce diagnostic radiographs has long been used in dentistry. Development of higher speed films and recommendations of guidelines for radiographic imaging aided in the reduction of radiation exposure risks. Only where there is benefits risk assessment should any radiograph be taken and visual inspection was augmented with radiography and not

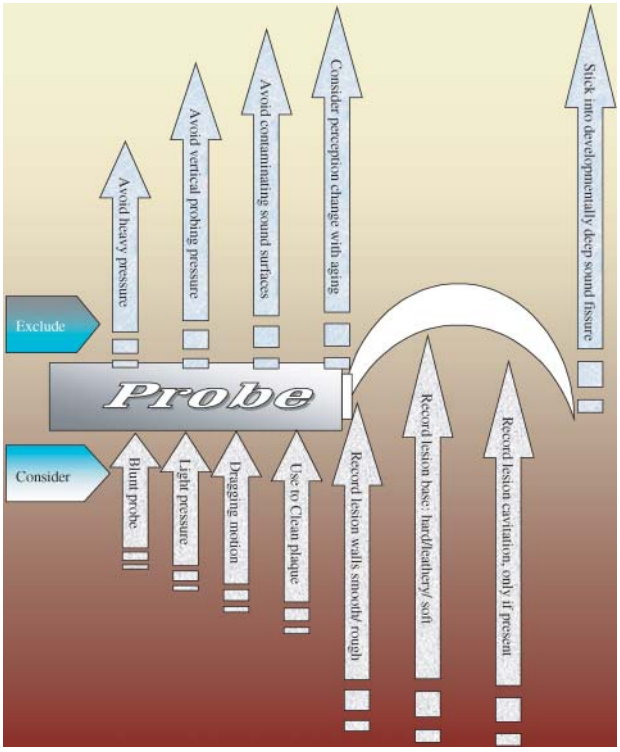

Figure 5: The role of the probe in occlusal caries diagnosis.

replaced by it (Wenzel et al, 1992a). Training had increase accuracy and agreement between examinations done by dental students and experienced radiographers (Lazarchik et al, 1995).

Because of the superimposition of buccal and lingual enamel, early enamel caries were not generally visible occlusally, and early dentinal involvement was difficult to ascertain with radiographs. Bitewings, taken in the lab conditions, had accurately detected around 60% of present lesions and mistakenly detecting lesions in around 10% of sound surfaces. This performance was better than for visual inspection (Ferreira Zandona et al, 1998). When carious lesions reached the dentinal level, conventional bitewing radiographs were better indicators. Where only one-third of occlusal dentinal lesions were diagnosed visually, two-thirds were discovered on bitewing radiographs (Richardson et al, 1996). Another study reported that bitewing radiographs revealed obvious lesions into the dentine in 15% of apparently sound occlusal surfaces (Weerheijm et al, 1992). However, evidence was that radiographs considerably underestimated lesions' size. *In vitro* experiments have shown that, once an occlusal lesion was clearly visible on radiographs, demineralisation has extended to or beyond the middle third of the dentine. On the other hand, false positives can occur with radiographic diagnosis, and where around 7 to 9 out of 10 lesions were true lesions (Ferreira Zandona et al, 1998). Combination of both vision and radiography increased the accuracy of the total diagnosis. Three quarters of lesions and all sound teeth were correctly identified (Ketley et al, 1993). Thus reducing the risk of unnecessary operative intervention but with a significant risk of missing early dentinal lesions.

The term "hidden caries" described the 15% of dentinal lesions undetected clinically but detected using bitewing radiographs (Weerheijm et al, 1989). Once the term was introduced, an increased number of reports detected such lesions (Weerheijm et al, 1990, 1992, 1997; Wenzel et al, 1991b). This category was further studied using the validated Ekstrand's clinical score index and these lesions were found to be scored as carious, with varying degrees of penetration (Ekstrand et al, 1998b). So variation in the dexterity of visual diagnosis and regarding non-cavitated lesions or ignoring them with the discrepancy in radiographic techniques and variable produced a lower the percentage of hidden lesions, if present at all (Machiulskiene et al, 1999).

Reducing human error by quantification of this gradient of grey shades was needed. The information could be captured for computer analysis indirectly from the exposed film or directly using digital sensors. Further contrast adjustments using specialised software allowed the enhancement of the resultant image improving the radiographic diagnosis of early lesions (Verdonschot et al, 1999; Wenzel et al, 1992a). Although these systems were very useful in many dental clinics due to their speed and performance, limitations included the expense of extra equipment and the large sizes of digital sensors.

Comparing images by subtraction was a way to detect if change had occurred to mineral density of tissue. Deduction of the shades differences in serially well-aligned images gave numerical data. The result would be zero if images taken using the same radiographic quality were well aligned and had no change in the mineral content (Nair, 1998). Mineral changes with advancing, regressing or secondary lesions could be picked as a negative or positive subtraction result. Another combination of images was called tuned-aperture computed tomography (TACT). Using the radiation to create a series of sliced images combined to produce a 3D image similar to the CT scan. As expected, this

would enhance the capture of secondary caries (Mjor et al, 1997; White et al, 1998) as well as comparable performance with the diagnosis of small enamel lesions using conventional radiography (Abreu Jr. et al, 1999; Shi et al, 2000). Developments on these new methods are awaited to be usable in conventional clinical settings.

Fibre-optic transillumination (Schneiderman et al, 1997)

This technique depended on the fact that carious enamel had a lower index of light transmission than sound enamel. For occlusal caries, a study compared FOTI with radiographic examinations and concluded that radiographic examination was a better diagnostic system than FOTI (Longbottom et al, 1990). This was not the conclusion of other studies finding the opposite better performance for FOTI. There was still the need to use FOTI as an adjunct to conventional caries diagnosis rather distinct from it (Verdonschot et al, 1992; Wenzel et al, 1992b).

Further quantification of the shadows seen on the tooth was made by the video processing of the image captured using a charge-coupled device (CCD). The light information is transformed to an electrical current and then to colours on the video monitor. This is further analysed by the computer (Schneiderman et al, 1997; Vaarkamp et al, 1997). Further developments might enable it to enter to the clinic.

Is the dental clinic a wealthy place?

Yes. Research has always focused on applying the knowledge of laboratory to the contentment of the practitioners. Know the dentist is well equipped with the latest gadgets and inventions. The dental team is growing with more tasks for each individual to perform. But the thing which most enhances the dental environment is the wealth evidence-based knowledge, which should enrich the diagnosis process of the most prevalent dental disease using the simplest tools. The crown of all is the wisdom to use the correct knowledge in the correct time and place.

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CHAPTER 2.2

Advanced methods of quantification of occlusal caries

Layla Abu-Naba'a, Kim Ekstrand & Edward Lynch

The previous chapter (Detection Methods of Occlusal Caries for Use in Clinical Practice) was concerned with the clinical parameters used for the diagnosis of occlusal caries. The methods mentioned there are mostly dependent on the judgement of the clinician and are, therefore, affected by operator's skills, experience, attitude and similar factors. When more definite and precise study of the carious lesions is required, means for quantification of the lesions are used.

This chapter deals with methods of measuring the carious process directly numerically using specifically designed devices. The clinical relevance of published research is emphasised as not all devices are available for the practitioner, but might be in the near future.

Why the need to quantify?

Quantification of data from the process of disease has long been recommended for research purposes. The clinician applying quantifying methods perceives the advantages of such preference; minimising human error, considering cut-off points rather than ranges, numerical follow-up comparisons, being reproducible, could be put in records and computers, and so on. The measuring device should be sensitive, specific and reproducible using the least number of factors that are subject to human error. Many devices have been developed in the last decade to quantify the caries process. Extensive research not only helped to prove the validity of outcomes obtained from them, but also equally important, made them user-friendlier. Not all devices are marketed for daily use. Nevertheless, research using these devices would have a clear impact on the daily approach to caries management.

How can caries be put into numbers?

As the caries process advances in the healthy tissue, physical changes take place within the structure, and the organic components of the previously mineralised tissue are altered. The organic to inorganic ratio is changed. Properties related to the organic matter may change in regards to structural changes occurring in extreme acidic environments, chemical composition, light interactions, other sources may contribute to the organic material, and so forth. By measuring the physical property related to the amount of these changes, indirectly the caries process would be judged concerning severity and activity. Experienced visual judgement has long been used accompanied by conventional diagnostic tools as discussed earlier detecting carious alteration. Other technologies are needed to accompany these careful judgments, not replacing them, thereby aiding in the final decision.

Laser and light fluorescence

This method measures the fluorescence of the natural tooth structure when induced by light irradiation to discriminate between carious and sound enamel. It is accepted that the resultant fluorescence is lower in areas of reduced mineral content, and that there is a relation between mineral loss and the radiance of the fluor-

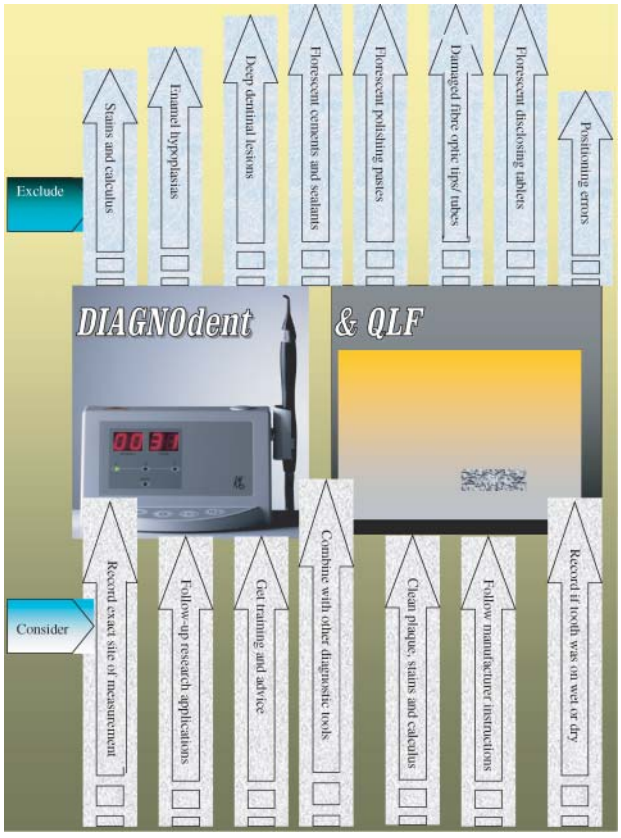

Figure 1: Diagnosis using QLF and DIAGNOdent.

escence. The term quantitative laser fluorescence (Shi et al, 2001a) had been applied to the research method of measuring induced tooth fluorescence after using a laser (488 nm range) to quantify tooth demineralisation and lesion severity. Several studies in which an argon laser light source was used to examine smooth enamel surfaces had shown a strong correlation between a decrease in fluorescence and the degree of enamel demineralisation (al Khateeb et al, 1997). QLF was best suited for longitudinal diagnosis of early lesions of the enamel on accessible smooth surfaces, and many investigations had involved the monitoring of white-spot lesions, such as those observed in orthodontic patients during treatment and after de-bracketing (Emami et al, 1996a).

Laboratory studies of artificial and natural decay of occlusal fissure enamel showed QLF to have better accuracy than visual examination alone or radiographic examination alone but with more false negatives. QLF was affected to some extent by the wet or dry state of the fissure, by stains in the fissure and by fissure morphology. The use of air-polishing to remove plaque improved diagnosis by QLF (Ferreira Zandona et al, 1998).

Some reports suggested that QLF might have been limited to measurement of enamel lesions of at most several hundred micrometres depth and could not differentiate between deep decay, hypoplasia or unusual anatomic features. It also was not designed to discriminate between enamel and dentine lesions. Furthermore, the fluorescence from dentine was not related to dentine demineralisation, so this method was not suitable for measuring dentine demineralisation (Emami et al, 1996b). Recent work on differentiating dentinal and enamel lesions on occlusal surfaces has proved promising and may be as useful for the dental practitioner as well as the researchers. Positioning of the detecting camera and the use of soft ware are also being simplified.

DIAGNOdent system

The DIAGOdent system is a commercial battery-powered quantitative diode laser fluorescence device, which uses a different method than the previous one. It measures not the natural fluorescence of tooth structure but the fluorescence of the products of cariogenic bacteria. A fibre–optic bundle is directed onto the occlusal surface producing light at 655-nm wavelength. A laser probe is designed for the occlusal surface to scan over the fissure area in a sweeping motion. The device then displays two values, the moment value for the probe

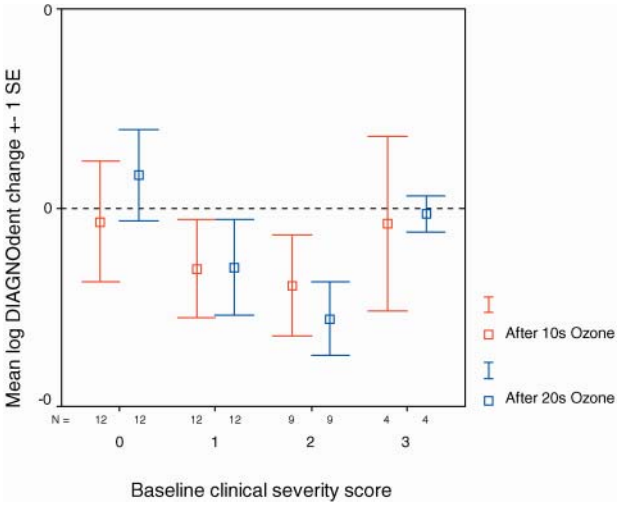

Figure 2: Mean DIAGNOdent readings change categorised by baseline Ekstrand's clinical severity scores after 10 and 20-second Ozone treatment, in-vitro.

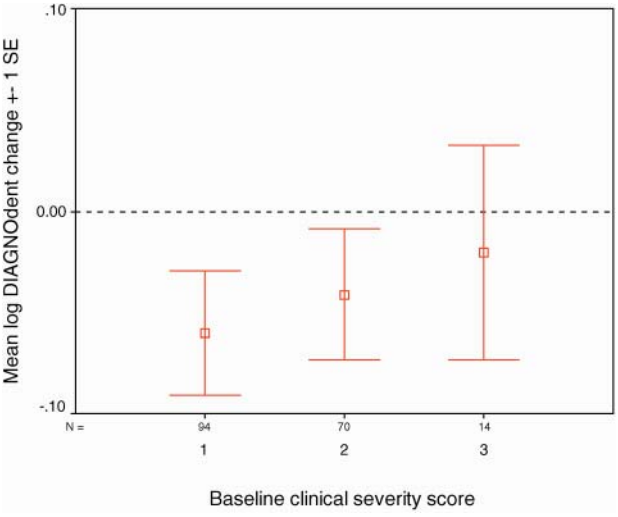

Figure 3: Mean DIAGNOdent readings change categorised by baseline clinical severity scores after 10-second Ozone application time, in-vivo.

position and a peak value recorded from the whole surface.

The diagnostic performance of DIAGNOdent was better in comparison with visual inspection, radiographic examination and electric caries monitoring (Bamzahim et al, 2002b; Anttonen et al, 2003b; Costa et al, 2002). This conclusion had not interpreted itself into fixed cut-off points for dentine and enamel caries due to variation in protocols and validation techniques used in these studies. There was a concern about the accuracy of the device when deep dentinal lesions were measured due to its limited penetration ability, reducing its correlation with histological depth of lesions (Ouellet et al, 2002; Verdonschot et al, 2002). Consequently, it was strongly recommended to use DIAGNOdent in conjunction with other diagnostic tools when diagnosing dentinal lesions (Anttonen et al, 2003a; Attrill et al, 2001; Heinrich-Weltzien et al, 2002; Lussi et al, 2001; 2003; Sheehy et al, 2001).

Limitations of the device to accurately designate carious activity occurs when false positive readings happen around calculus, stains, cements and some composite materials (Cortes et al, 2003b; Krause et al, 2003). Suggestions of inapplicability of the values obtained from previous laboratory studies in the clinical situation were also raised. Some significant changes in DIAGNOdent readings were noticed when formalin was used to store extracted teeth, possibly by altering proteins which induce the fluorescence of carious lesions (Shi et al, 2001b; Tam et al, 2001). Acid etching produced some change which was found to be insignificant (Takamori et al, 2001).

Different cleaning methods changed the DIAGNOdent readings correlation with histology (probe cleaning, air-polishing alone, air-polishing and gentle rinsing, air polishing and the three in one syringe water spray) becoming better using the last two cleaning techniques (Abu-Naba'a, 2003). Ozone altered DIAGNOdent readings with an immediate reduction after ozone treatment for 10 and 20 seconds (Abu-Naba'a et al, 2002b).

In 199 occlusal lesions, cleaning using the air-polishing system increased the correlation of DIAGNOdent readings with the true histological depth as well as with the Ekstrand's clinical score index and the ECM readings (Abu-Naba'a et al, 2002b; Abu-Naba'a, 2003). In a recent clinical study, 34 non-cavitated sound and carious pit and fissures were examined in 24 teeth. The teeth were examined within a one-week interval to test DIAGNOdent repeatability.

Inclusion of sound surfaces in this test was to test of agreement between visits, which had been satisfactory at all severities (Weighed Kappa value, 0.62) (Abu-Naba'a, 2003). 388 non-cavitated pit and fissure carious lesions were examined in one visit using Ekstrand's clinical index, ECM and DIAGNOdent. The scores obtained by the different diagnostic systems used showed significant correlations (Abu-Naba'a et al, 2004).

The immediate effect of ozone for 10 seconds was again tested clinically in 178 lesions from 90 subjects. Another significant drop in DIAGNOdent readings was noticed related to the baseline clinical severity score (Abu-Naba'a et al, 2004).

DIAGNOdent has many advantages. It is commercially available for the practitioners to use. It is helpful where radiographs are not useful in early occlusal decay or when patients refuse radiographs. It also gives instant audible feedback to both clinician and patient and involving them in the treatment decision. There are no known risks from use of the DIAGNOdent and it is also easily portable with no need for computer analysis.

Electric caries monitoring

Carious enamel and dentine are more porous than sound tooth tissue and because they are filled with ion

containing saliva fluids, they are less resistant to a small electric current (White et al, 1978; White et al, 1981). This was the principle behind the invention of an indirect way to measure the progression and activity of carious lesions, the ECM (Bamzahim et al, 2002a).

Many studies were performed to adapt the principle of the electrical conductance property of human dentition to the dental laboratory and clinical situations. These used many prototypes as well as commercially marketed caries detection machines, which not all were available today (Table 3).

Various currents, factors that had to be standardised by the operator, calibration procedures, air supplies and validation outcomes were exclusive to each device (Table 2). Thus, no cross comparison of absolute values between studies that used different devices is possible (Schulte et al, 1999). Nevertheless, main clinical conclusions could be derived form the clinical studies using these devices.

As readings varied according to the varied orientation of the dentinal tubules, the modified ohmmeter assumed that the mechanism underlying electric conductance in dentine was related to the transport of ions in the dentinal fluid (Gente et al, 1991a). This principle was used inversely. For a reading taken within a prepared tooth, it was possible to estimate the situation of the pulp horn tips (Gente et al, 1991c) and calculate the depth of the remaining dentine in crown preparations or in standardised cavities (Gente et al, 1991b). The wetting substance used over teeth affected the readings as the higher NaCl concentrations produced lower impedance values (Schulte et al, 1998a).

A clinical cross-sectional study used the device to compare caries free premolars in children and adults. Lower impedance values were found in children's teeth compared to adults (Schulte, 1997), (Schulte et al, 1998b). Erupting premolars free of soft tissue had electrical resistance values obtained every 3–6 months from the deepest point of the fissure after isolation. Resistance values increased up to 15 months as these premolars matured (Schulte, 1999). A later study on molars found maturation to be over a period of 36 months (ten Bosch et al, 2000a). Using the same device, differences were found between bovine and the higher resistant human enamel (Schulte, 1999).

The Caries Meter-L used a colour display in the form of four lights reflecting the status of the tooth; green for no caries, yellow for enamel caries, orange for dentine caries, and red for pulpal involvement(Ricketts et al, 1995b; Sawada et al, 1986). The device was accurate in discovering around 75% of both sound and decayed surfaces in the enamel caries level or around 93 and 63% at the dentine caries level, which were both much better than achieved by visual inspection.

The Vanguard electronic caries detector overcame the inconsistency of drying by placing the probe tip centrally and coaxially within a steady stream of air (Flaitz et al, 1986).

Other than testing cut-off points, the detector was used to predict the need for a sealant or sealant restoration within 18 months after baseline. ECM was a better predictor of caries than visual inspection and Fibre–optic Transillumination (Cortes et al, 2003a; Fennis-Ie et al, 1998). The device was valuable in measuring early lesions which can be monitored longitudinally, thus, it becomes possible to detect the change towards remineralisation, demineralisation or stabilisation (Ricketts et al, 1995a)

The ECM by LODE diagnostics took a long path where many versions were tested. At the time of this report, the 4th version was available. Areas on the tooth were possible to be measured using a gel conducting media. This total surface reading was limited by micro-cracks and some anatomical deep fissures besides tooth maturation variations(Huysmans et al, 1998). Using this technique, an interesting finding was the variation of conductance readings with the season of observation: In the fall, the resistance was

Table 1: Examples of devices, which used the principle of electrical conductance property of human dentition as an indirect way to measure caries activity

Devices using the electrical conductance property of human dentition
1 The modified AC Ohmmeter
2 Caries Meter L (G-C International Corp., Leuven, Belgium)
3 Vanguard Electronic Caries Detector (Massachusetts Manufacturing Corp., Cambridge, Mass., USA)
4 ECM I, II, III and IV (LODE Diagnostic, Groningen, The Netherlands)
5 Modified Electrochemical Impedance Spectroscopy (EIS)
6 Electrical Impedance Tomography (EIT)

Table 2: Various factors, which might affect the validity outcome of the ECM as a diagnostic tool

Factors	Features
Device specifications	
Current used	• High or low frequency • Alternate or continuous current • Frequency of alternate current
Electrode	• Touching contra-lateral gingival tissue • Touching contra-lateral the cheek • Held by hand
Calibration procedure	• In lab using standard resistance units • Internally calibrated
Air flow control	
Air supplies source	• 3in1 syringe before the measurement • Connected to the tip and active during measurement
Direction	• Operator dependant • Parallel to measuring tip
Air flow meter	• Supplied or not • Could be altered or not
Air flow rate	• Stable flow or not, throughout the drying period • Amount of flow rate per minute
Air flow time	• Time of application was variable
Air operation	• Automated or operator dependant
Measurement specifications	
Measurement display	• Numbers • Symbols and Colours
Ranges displayed	• Small • Large
Type of measurement	• Ordinal • Continuous
Units	• Ω or none
Measurement procedure	
Number of measurements from each site	• One measurement • Multiple and the least single one was chosen • Multiple and the mean was chosen
Protocol for selection of the measuring site	• Most severe as detected by vision, • Most severe as detected by other devices, • Most severe as detected by the device
Area of contact between measuring tip and tooth	
Area of contact with the tooth	• Tip area only (site-specific) • Area of contact fluid (surface-specific)
Type of conducting fluid.	• Saliva of the subject • Various tooth pastes • Saline • NaCl solutions
Area covered by the conducting fluid	• A single lesion area • All fissures and pits. • Including cusp tips and marginal ridges
Consistency of conducting fluid	• Fluid • Gel
Visualisation of area covered by conducting fluid	• Possible by dyed fluid • Not possible (transparent fluid)

Table 3: Mean Dz values measure the accuracy of the methods tested. A higher value means better performance from various diagnostic systems in occlusal caries diagnosis (Ie et al, 1994)

Diagnostic system	n	Mean Dz	±SD
Visual inspection	8	0.71	0.39
Fibre-optic transillumination	2	1.08	0.33
Conventional radiography	10	0.89	0.31
Digital radiography	8	0.97	0.27
Xero-radiography	1	0.73	0
Radiovisiography	3	0.91	0.15
Electrical Resistance Measurements	2	1.30	0

lower than in the spring for the same molars studied (ten Bosch et al, 2000b).

The principle was further extended to measure marginal leakage around fissure sealants in the lab but the clinical situation was still to be considered(Verdonschot et al, 1995). Prediction of caries activity using ECM readings was done. The ECM predicted stability of sound and carious lesions in 75% and 78% of the cases correctly up to 18 months. The remaining percentages were thought to be changed by incidence of new lesions or remineralisation in old ones (Ashley et al, 2000).

Mineral loss by acids was studied using ECM. The acid etching reduced the resistance readings and brown lesions were more resistant to itching than sound and white-spot decayed samples (Huysmans et al, 2000). Acid-produced artificial lesions were monitored by the resistance values which inversely related to lesion depth and mineral loss in both enamel and root samples. It was also inversely related to the time of demineralisation, which extended for 4 weeks. It was concluded that the ECM was valuable in monitoring early demineralisations in artificial caries studies (Yeganeh et al, 1998). Caries removal using Carisolv was as effective in removing carious tissue as conventional drilling as tested by ECM (Moran et al, 1999). Remineralisation using toothpaste was monitored *in vitro* (Petersson et al, 1998). The mean value of the ECM readings increased as the time of soaking in the toothpaste increased. Enamel samples had a mean change that was larger than the mean change for dentine samples. Further applications were suggested to compare different toothpastes. Validation was recommended for these procedures.

Root caries was tested using the ECM in various ways. The ECM readings were compared to clinical classification criteria for root caries (Lynch et al, 1999). Soft dentinal lesion and dark brown lesions had a lower mean integrated value than leathery lesions and light brown lesions respectively. The ECM gained a good reproducibility by different operators. It correlated negatively with histological lesion depth and positively with remaining thickness of the dentine bridge (Wicht et al, 2002). A longitudinal study found earlier that the ECM could detect the deterioration of root lesions *in vivo* (Yeganeh et al, 1997). Probing was found to break the superficial layer over root lesiosn and predisposed to further demineralisation and cavitation. ECM was able to demonstrate this as the mean resistance value for lesions which have been probed and further demineralised was significantly lower (Prinz et al, 1999).

ECM VI used the vanguard method of drying, as ell as a standardised method where the 'Standard ECM Scale' procedure was followed. A total drying and measuring time was fixed at 5 seconds and airflow was fixed at 5 L/min. an Integrated Resistance value across the total drying was displayed with an alternating mean resistance value of the last second of drying (the End value). Three to five readings were recommended for each lesion (centre, north, south, east, and west) and the average was calculated to represent the tooth. The tooth was suggested to be re-wetted by saliva, *in vivo*, at least 5 seconds between successive readings. The end value is the reading with the maximum number of constant factors mentioned in Table 2.

Non-cavitated occlusal carious lesions in 388 teeth from 90 subjects were further examined in one visit following the previous protocol. The effect of 5 seconds re-wetting between the readings was found to be a sufficient wetting agent that was specific for each individual (Abu-Naba'a, 2003). When half of the lesions in the previous test further received 10-second Ozone treatment, possible immediate changes were tested. No significant ECM change was found immediately after Ozone treatment (Abu-Naba'a et al, 2002a).

The ECM was found to be a technique sensitive device. As the ECM is a valuable device in monitoring lesions, the repeatability can be improved by observing different factors affecting the resistance readings. It was highly repeatable when excluding readings affected by the technique errors (Abu-Naba'a, 2003). These repeatability tests although satisfactory concluded the importance of the training of the investigator as the repeatabil-

ity might significantly increase by conforming to the following:

- Knowledge of the principle and methodology of application
- Familiarity with the display of different measurements and the relationship of the integrated value to the end value when using the standard scale measurements
- Understanding of the readings variation form sound to diseased or within the diseased tissue
- Understanding the effect of the soft tissue or saliva contact to both tooth and the tip apparatus.

Studies to develop and evaluate a new method of spectroscopic Electrical Impedance Tomography (EIT) have been taking place. It aims to reconstruct cross-sectional maps of site-specific electrical impedance spectra (EIS). The tomographic representation will allow diagnostic interpretation of changes in tissue impedance among different locations, instead of being dependent on a single quantitative reading. It would then be used for the detection of small carious lesions (Huysmans et al, 1996; Longbottom et al, 1996).

Summery

Many dental practitioners might have a chance to use some of these advanced detection tools. Understanding the following facts would help maximise the benefit gained for the diagnosis of caries:

1. Diagnosis of presence and absence of disease is only a part of the dental caries management process. The responsibility lies on the practitioner to choose which action should follow.
2. Combination of more than one diagnostic tool is strongly recommended for diagnosis process.
3. Each diagnostic tool, conventional or advanced, has some limitations to correctly diagnose lesions at different levels of severity. Optimising the conditions used in the clinical situation would help minimise these limitations.
4. Diagnosis of caries activity proves to be more important than caries severity. Monitoring on the diagnosis and treatment outcomes is indispensable.

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CHAPTER 2.3

Use of the DIAGNOdent in Detecting and Monitoring Caries Lesions and Residual Caries for Ozone Treatment

A. Lussi & P. Francescut

Introduction

The onset of caries is characterised by demineralisation of dental hard tissues. Optimal fluoridation with respective oral hygiene habits and diet may stop the progression of a lesion and even allow its remineralisation. The aim of modern dentistry must be a preventive approach rather than invasive repair of the disease. This is only possible with respective preventive measures. Besides oral hygiene and diet fluoride applications are regularly recommended. Further, antibacterial acting varnishes or gels are used. Due to its very good antibacterial properties the application of ozone has the potential to replace them. In order to monitor remineralisation of such lesions after or during ozone treatment, several methods could be used. One of them is the laser fluorescence based DIAGNOdent.

Background of the DIAGNOdent

Enamel consists mainly of a carbonate-substituted calcium-deficient hydroxyapatite and a small amount of water and organic matrix. Sound enamel shows a small baseline fluorescence that differs from that of a decayed tooth. Spectral investigations on teeth with carious lesions revealed that a good contrast between sound and carious regions can be achieved when fluorescence is excited in the red and detected in the near infrared region. In this case fluorescence is much more intense for carious compared to sound enamel (Hibst and Paulus, 1999; Hibst et al, 2001).

White spot lesions formed *in vitro* without bacterial involvement as well as very early white spot lesions formed *in vivo* do not result in a significant increase in fluorescence compared to sound surfaces (Lussi et al, 2001). On the other hand, distinct fluorescence of the caries process is observed in more advanced stages (e.g. D_2, D_3) (where microorganisms are involved). This fact lead to the assumption that besides light scattering, bacteria or their metabolites could contribute to the increased fluorescence of these lesions (Hibst et al, 2001). Candidates for such bacteria metabolites could be porphyrins. Porphyrins occur as intermediate steps in the synthesis of haeme, and are also produced by several types of oral bacteria. In an earlier work porphyrins could be extracted from carious lesions and were demonstrated to be useful in differentiating caries from sound tooth by violet (406 nm) excited fluorescence (König et al, 1993). Although fluorescence yield is maximal for this short wavelength excitation, porphyrins were known to show also some fluorescence when excited by red light. These molecules contribute to the signal obtained from caries (Sailer et al, 2001). Whether these are the dominant or even the only fluorophores, or whether there are also other components resulting in red excited caries fluorescence, has to be evaluated in further research.

On the basis of these findings an instrument for caries detection was developed (DIAGNOdent, KaVo, Biberach, Germany), containing a laser diode (655 nm, modulated, 1 mW peak power) as excitation light source, and a photo diode combined with a long pass filter (transmission >680 nm) as detector. The excitation light is transmitted by an optical fibre to the tooth, and a bundle of 9 fibres arranged concentrically

Figure 1: DIAGNOdent showing real time and maximum (peak) digital display. The device consists of a probe, a fiber-optic lead, and a unit containing the electronics and the laser diode.

around it serves for detection. The long pass filter absorbs the backscattered excitation and other short wavelength light and transmits the longer wavelength fluorescence radiation. In order to get rid of the long wavelength ambient light also passing through the filter, the laser diode is modulated and only light showing the same modulation characteristic is registered. Thus the digital display shows quantitatively the detected fluorescence intensity (in units related to a calibration standard). It shows both a real time and a maximum value (Fig. 1). A tapered fiber-optic tip (tip A) has been designed for the detection of fissure caries and a flat tip for smooth surface caries (tip B) (Fig. 2).

Use of the DIAGNOdent in daily clinical practice

Generally, teeth to be assessed should be cleaned and dried in order to reach optimal conditions for regular visual inspection, which should be the first diagnostic step. Thorough cleaning is a prerequisite for accurate caries detection. Drying makes decalcifications visible. It lowers the refractive index of the intercristalline spaces from 1.33 for wet demineralised tooth surfaces to 1.0 for dried demineralised tooth surfaces, thus making the opaque appearance of the decay clearly visible (Basting and Serra, 1999). If there is doubt about the status of health at any particular site then more sensitive equipment should be used as a second opinion. This approach allows the dentist to combine the advantages of higher specificity and speed of clinical inspection with the higher sensitivity of the new devices. The assessment of a tooth with the laser fluorescence system is as follows: After calibration with a ceramic standard (Fig. 3) the fluorescence of a sound spot on the smooth surface of the tooth is measured to provide a baseline value (Fig. 4). This value is then subtracted electronically from the fluorescence of the site to be measured. In order to get the maximum extension of caries, the instrument has to be tilted around the measuring site (Fig. 4). This ensures that the tip picks up fluorescence from the slopes of the fissure walls where the carious process most often begins. A rising tone starting with a value of 10 helps the examiner to find the maximum fluorescence value of the site under study.

Figure 2: The two tips for detection of occlusal (left) and smooth surface (right) caries.

Figure 3: The ceramic standard is used for the periodical calibration.

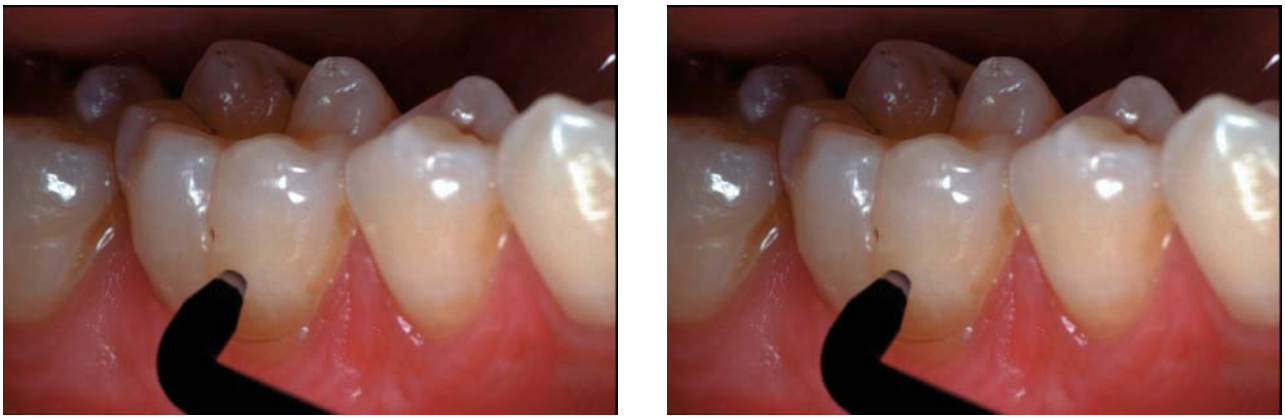

Figure 4: Measuring procedure on an occlusal surface. a) Fluorescence of a sound spot (baseline value). b) Assessment on the measuring site.

Detection of caries

Currently, DIAGNOdent has been tested on occlusal and smooth surfaces (Lussi et al, 1999, 2001; Shi et al, 2000, 2001; Attrill and Ashley, 2001; El-Housseiny and Jamjoum, 2001; Pereira et al, 2001; Bamzahim et al, 2002; Anttonen et al, 2003; Côrtes et al, 2003; Lussi and Francescut, 2003) and compared to visual inspection, histology, radiography and quantitative light-induced fluorescence. Table 1 gives an overview of the specificity- and sensitivity values assessed in different studies. Good intra-examiner reproducibility on occlusal and accessible smooth surfaces was reported *in vivo* under daily practice conditions (Lussi et al, 2001; Sheehy et al, 2001; Heinrich et al, 2002; Pinelli et al, 2002) and *in vitro* (Lussi et al, 1999; Attrill and Ashley, 2001; Shi et al, 2001; Lussi and Francescut, 2003) (Table 3). The only investigation which assessed inter-examiner reproducibility showed on smooth surfaces a substantial Kappa value of 0.77 (Pinelli et al, 2002).

In one of the above mentioned studies general dental practitioners assessed a total of 322 occlusal surfaces (Lussi et al, 2001). Caries extension was determined after operative intervention and served as the gold standard. Although the dentists were trained to distinguish dentinal caries from enamel caries a more accurate gold standard would have been possible after extraction of the teeth. The recommendations derived from this study for the clinical use of the DIAGNOdent are given in Table 2 (Lussi et al, 2001). The border-line reading for operative intervention, set at a (peak) value of about 30, reduces the sensitivity of the device but increases its specificity. A higher setting of this 'trigger' for operative intervention also represents a safety factor for cases with stained fissures or fissures with calculus. These recommendations were later confirmed by others (Heinrich et al, 2001; Anttonnen et al, 2003). Anttonnen and co-workers (2003) reinforced that strict instructions about cut-off values cannot be given, and hence the values given here are to be taken as guidelines. The published border-line values obtained *in vitro* should not be transferred to the *in vivo* situation. Firstly, the fluorophores do change their characteristics as a consequence of the storage of the extracted teeth (unpublished observation) and, secondly, histological examination of test teeth *in vitro* is capable of identification of even minute changes in dentine.

It has to be kept in mind that involvement of dentine should not indicate immediate operative intervention in all circumstances. The decision triggering restorative treatment is dependent upon a range of other variables, such as the patient's case history, fluoride and dietary status, as well as perceived caries activity and the status of the surface. Triggers for restorative intervention in daily practice are therefore at a higher DIAGNOdent value than the ones quoted above (Lussi et al, 2001). In no case, early detection of caries should be an excuse for early operative intervention. This study also showed that a second method has an additional diagnostic yield. Out of the 322 occlusal surfaces, 100 had dentinal caries. These teeth were also assessed by visual inspection, bitewing radiography and DIAGNOdent. Twenty-nine of these 100 lesions were detected by visual inspection. This number increased to a total of 71 lesions correctly detected when bitewing radiography was the second opinion. Using laser fluor-

Table 1: Sensitivity and specificity of the DIAGNOdent assessed in different studies

Diagnostic threshold	Condition	Sensitivity	Specificity	n	Gold standard	Author
Primary teeth						
Occlusal surface (D_3): (*in vitro*)	dry	0.79	0.84	58	Histology	Attrill & Ashley, 2001
Occlusal surface (D_2): (*in vitro*)	dry	0.75	0.68	95	Histology	Lussi & Francescut,2003
Occlusal surface (D_3): (*in vitro*)	dry	0.82	0.85	95	Histology	Lussi & Francescut,2003
Permanent teeth						
Occlusal surface (D_2): (*in vitro*)	dry	0.83	0.72	105	Histology	Lussi et al, 1999
Occlusal surface (D_2): (*in vitro*)	moist	0.87	0.78	105	Histology	Lussi et al, 1999
Occlusal surface (D_3): (*in vitro*)	dry	0.84	0.79	105	Histology	Lussi et al, 1999
Occlusal surface (D_3): (*in vitro*)	moist	0.76	0.87	105	Histology	Lussi et al, 1999
Occlusal surface (D_1): (*in vitro*)	dry	0.95	0.50	46	Histology	El-Housseiny & Jamjoum, 2001
Occlusal surface (D_3): (*in vitro*)	?	0.19	0.98	230	Histology	Pereira et al, 2001
Occlusal surface (D_3): (*in vitro*)	dry	0.80	1.00	87	Histology	Bamzahim et al, 2002
Occlusal surface (D_2): (*in vitro*)	dry	0.46	0.95	76	Micro Rx	Shi et al, 2000
Occlusal surface (D_2): (*in vitro*)	moist	0.42	0.95	76	Micro Rx	Shi et al, 2000
Occlusal surface (D_3): (*in vitro*)	dry	0.82	1.00	76	Micro Rx	Shi et al, 2000
Occlusal surface (D_3): (*in vitro*)	moist	0.80	1.00	76	Micro Rx	Shi et al, 2000
Occlusal surface (D_3): (*in vivo*)	dry	0.92	0.86	332	Biopsy procedure	Lussi et al, 2001
Occlusal surface (D_3): (*in vivo*)	dry	0.93	0.63	281	Biopsy procedure	Heinrich et al, 2002
Smooth surface (D_3): (*in vitro*)	dry	0.75	0.96	40	Micro Rx	Shi et al, 2001

D_1: No caries is classified as 'sound'; enamel caries (D_1, D_2) and dentinal caries (D_3, D_4) is classified as 'decayed'.
D_2: D_0, D_1: 'sound', D_2, D_3, D_4; 'decayed'.
D_3: D_0, D_1, D_2: 'sound'; D_3, D_4: 'decayed'.

escence as the second opinion a total of 92 dentinal lesions were correctly detected (Lussi et al, 2001).

It is important to note that calculus, plaque, hypomineralisation, composite filling materials, remnants of some polishing pastes, and stains may produce fluorescence (Lussi et al, 1999, 2001; Shi et al, 2000; Sheehy et al, 2001) which could cause false-positive readings.

Table 2: Guidelines for the clinical use of the DIAGNOdent (Lussi et al, 2001)

Value	Action
0–13:	Usual preventive measures
14–~20:	Intensified preventive care is advised
21–~29:	Intensified preventive or operative care is advised depending on the patient's caries risk, the recall interval, etc.
≥=~30:	Operative care (and intensified preventive measures) are advised

Note that the cut-off value is a range (~).

Monitoring the carious process

Due to the very good intra-examiner and good inter-examiner reproducibility (Table 3), the device should be useful for longitudinal monitoring of the carious process and thus for assessing the outcome of preventive interventions. The correct assessment of a site is dependent on the correct calibration and tilting procedure. In contrast to the calibration, tilting has to be undertaken on each site under study, which needs some seconds. Therefore, this method makes it difficult for large numbers of assessments where time is often a limiting factor. A second prerequisite is the exact (re)positioning of the device during monitoring. The previously mentioned very good reproducibility could, in epidemiological or large-scale clinical studies, be hindered by the difficulty of reproducibly placing the probe on the tooth surface. This is most difficult on the occlusal surface and probably only possible when a positioning system is coupled with the device. This could

Table 3: Intra-examiner reproducibility of the DIAGNOdent assessed in different studies

Primary teeth			
Occlusal surface (D_3): (*in vitro*)	Kappa value	0.72	Attrill & Ashley, 2001
Occlusal surface (D_2): (*in vitro*)	Kappa value	0.81	Lussi & Francescut, 2003
Occlusal surface (D_3): (*in vitro*)	Kappa value	0.81	Lussi & Francescut, 2003
Permanent teeth			
Smooth surface: (*in vitro*)	Intra-class Corr. Coeficient	0.94	Shi et al, 2001
Smooth surface: (*in vivo*)	Kappa value	0.75	Pinelli et al, 2002
Occlusal surface (D_2): (*in vitro*)	Kappa value	0.88	Lussi et al, 1999
Occlusal surface (D_3): (*in vitro*)	Kappa value	0.90	Lussi et al, 1999
Occlusal surface (D_3): (*in vivo*)	Kappa value	0.93	Lussi et al, 2001
Occlusal surface (D_3): (*in vivo*)	Kappa value	0.88	Heinrich et al, 2002
Occlusal surface (?): (*in vivo*)	Spearman corr.	0.89	Sheehy et al, 2001

Kappa (χ)=1: perfect agreement χ>0.75: excellent agreement 0.4< <χ=0.75: good agreement (Fleiss, 1981).

be a drawing, a photograph of the site to be inspected and reinspected during a longitudinal clinical trial. Placing on oral or facial smooth surface lesions should be easier. Placement of the tip on approximal surfaces is hard to achieve because the tips available today are too big to reach the contact point and its surrounding area where the caries process most likely begins. A very thin lance-like tip should be developed in order to reach the approximal space.

It is important to bear in mind that during the ozone treatment, the cavity will remineralise and often extrinsic stain will discolour it. This colour change could lead to false positive DIAGNOdent readings. In these cases, hardness of the cavity floor should be evaluated and considered as a parameter of dentine remineralisation rather than DIAGNOdent readings.

Detection of residual caries

The previous guidelines mostly refer to lesions that cannot be easily detected to the naked eye (the so-called hidden caries) or non-cavitated lesions. But can the same procedure be applied for caries detection while drilling? How can dentists be sure if the cavity floor is free of bacteria or not? Practitioners usually assess the status of the prepared cavity with the aid of an explorer and optical judgement (consistency and colour of the cavity floor) or by detector dyes. Even when the tactile and optical criteria were shown to be adequate to ensure the removal of most of the infected dentine (Kidd et al, 1993), these are still clinical and subjective parameters, dependent on the operator's ability and experience. Structural factors typical of the dentinal tissue could also lead to differences in hardness (Jones and Boyde, 1984; Mjör and Nordahl 1996; Marshall et al, 1997; Banerjee et al, 1999). Moreover, acute and chronic caries would exhibit different discoloration and softening patterns that complicate the differentiation of the bacterial invasion front in the cavity (Fusayama et al, 1966).

Even dyes (0.5% basic fuchsin, 1% acid red, Lissamine, Coomasie or Carbolan green) are considered nowadays as not absolutely reliable for they mark the demineralised but still remineralisable organic matrix of the lesion rather than the presence or absence of bacteria. Thus, their use results frequently in unnecessary removal of sound tissue (Kidd et al, 1993; Yip et al, 1994; Ansari et al, 1999; Mc Comb, 2000).

A third approach is needed to selectively identify and differentiate sound from infected dentine. High autofluorescence signals emitted from softened carious infected dentine were detected by confocal laser scanning microscopy (Banerjee et al, 1999). It seems reasonable to test the response of the DIAGNOdent laser based device in the caries detection of remnant dentine during excavation. In fact, some authors have carried out in-vivo and in-vitro studies using DIAGNOdent as the only tool for the detection of residual caries during cavity preparation (Reich et al, 1999; Braun et al, 2000; Lussi et al, 2000). Besides DIAGNOdent, further three methods were compared by Lennon et al. (2002) in an in-vitro experiment: Visible fluorescence, visual tactile examination and caries detector dye.

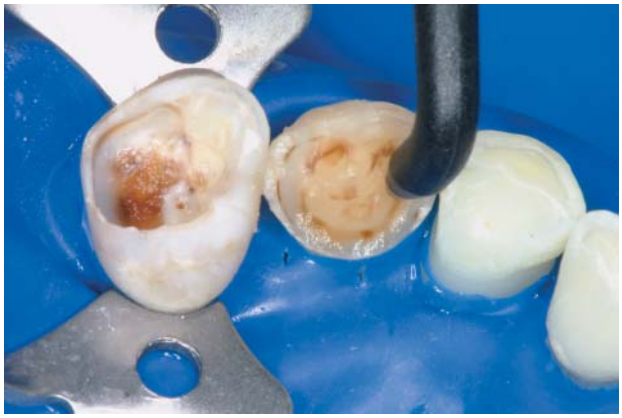

Figure 5: Measuring procedure in a cavity.

In all these studies each cavity was measured with DIAGNOdent before and/or after excavation. The device proved to be efficient in the detection of caries in dentin. When lesions approached the pulp, however, higher unexpected DIAGNOdent values were obtained in some cases (Reich et al, 1999; Braun et al, 2000; Lussi et al, 2000). That lead to the assumption that autofluorescence of the pulp could additionally inflate the final fluorescence response (Reich et al, 1999; Lussi et al, 2000). However, none of these studies pointed out the presence of discoloration that could also increase fluorescence values and result in false positive answers. It can also be speculated that in the *in vivo* studies, the operators drilled conservatively closer to the pulp, probably leaving some amount of infected dentin on the cavity floor.

Neither intra- nor inter-examiner reproducibility tests for DIAGNOdent were performed in these studies. However, the good to excellent intra-and inter-examiner reproducibility demonstrated on primary caries would render the device a useful tool to follow up changes in the caries status (in cases where the cavity is not filled). Combined with ozone treatment, DIAGNOdent would register changes in mineralisation of the treated lesions along time.

Practitioners could also take advantage of this property applying it also during dentin excavation. The measurement procedures are basically the same as for primary caries detection on not drilled lesions as explained above. Every measurement should be always preceded by a calibration of a sound spot on the smooth surface of the tooth (baseline value). This value should be subtracted from the fluorescence value obtained at the chosen site (or sites) in the cavity. The DIAGNOdent tip (tip A is desirable as with its smaller size the depth of the cavity is easier to achieve) should pivot around the site. If more sites in the same tooth are to be measured, the device should be reset. A new calibration on the sound surface of the tooth is not needed (Fig. 5).

When used in epidemiological or long-term studies, it is essential to precisely locate the site or sites to be scanned (by drawings or photographs). Moreover, a thorough register of the measurements obtained before each ozone application is indispensable. Only in that way, an accurate follow up of the mineralisation along time is possible.

In order to better understand the influence of ozone on the caries process, a preliminary experiment was carried out in our clinic. A total of 4 sites (in-vivo) and 14 sites (in-vitro) on different occlusal surfaces with different caries status were cleaned and measured with DIAGNOdent. Immediately afterwards, all the teeth underwent a 40 seconds ozone therapy. Finally, each site was measured again with DIAGNOdent at regular periods of 2, 5, 15, 30 and 60 minutes. The results are shown in figure 6.

Dentine decayed teeth showed a sudden decrease in fluorescence in the first minutes after ozone application (2 to 15 minutes). After that time, fluorescence increased gradually but did not reach the value obtained before ozone application. On the other hand, the fluorescence response from sound teeth and teeth with enamel caries did not change much along time.

Therefore we recommend taking the measurement with DIAGNOdent prior to the ozone application. The second measurement (follow-up) should be carried out on the following visit and not immediately after ozone treatment.

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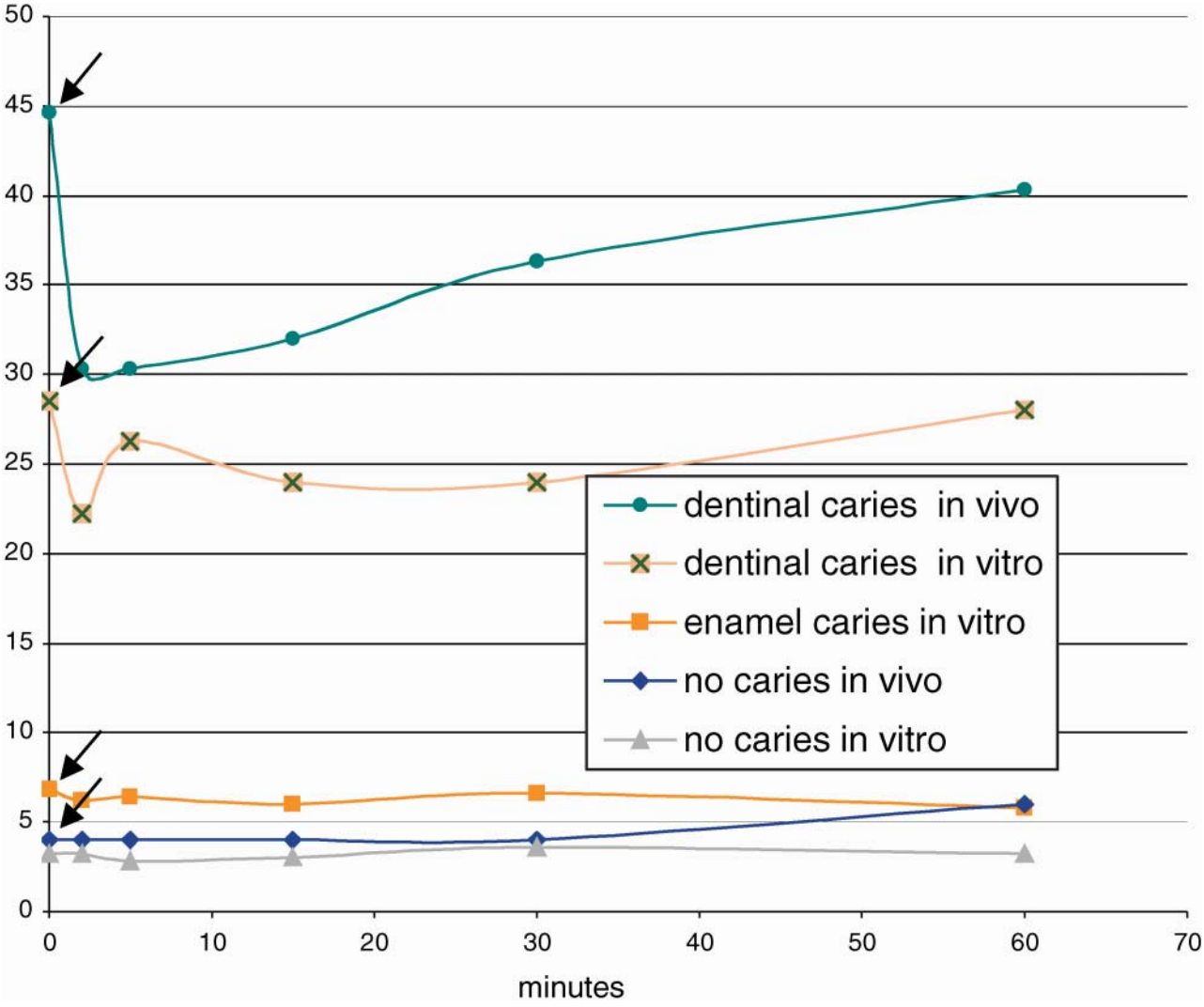

Figure 6: DIAGNOdent values along time after ozone application (*in vivo* and *in vitro* measurements). The arrows show the values obtained before ozone application.

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CHAPTER 2.4

Equipment Available to Deliver Ozone in Dentistry

Julian Holmes & Edward Lynch

Ozone could be described as a schizophrenic gas!

On the one hand, ozone has a long history of beneficial use in the medical and alternative therapy fields. For over 150 years, millions of people have benefited from the effects of ozone to eliminate disease and encourage natural healing. There is a vast amount of research and published data showing the effects of ozone on micro-organisms, fungi and viruses, and these have been discussed at length in other chapters of this book.

On the other hand, ozone is toxic and will attack human lung tissue. The UK Health and Safety at Work Act of 1974 requires every employer to ensure, so far as is reasonably practicable, the health of all his/her employees and others who may be affected by the work he/she undertakes. The Act also places duties in respect of health and safety matters on the self-employed. The UK Factories Act 1961 requires factory occupiers to take all practicable measures to protect employed persons against inhalation of fumes. The general policy adopted by the Health and Safety Executive is that exposure to hazardous substances should be kept as low as is reasonably practicable and in any case exposure should be kept within published standards by the application of engineering controls or other suitable control techniques. The Health and Safety Executive publishes, in guidance notes in the EH series, information on exposure limits applied in the UK. The recommended exposure limit for ozone in the work place, in the United Kingdom is 0.1 ppm (0.2 mg/m3) calculated as an 8-hour time-weighted average concentration. There is also a short-term exposure limit for ozone of 0.3 ppm (0.6 mg/m3) calculated as a 15-minute time-weighted average concentration.

Used properly, ozone has to one of the safest treatments that dentists and doctors around the world have and yet it still raises a great deal of controversy in the medical world, often being viewed as a 'quack' treatment modality. This book and this chapter will help to put ozone into the context of treatment modalities that are available for potential patients.

Because of the inherent dangers of ozone gas, the design of any equipment for use in the oral cavity must therefore be free from creating a hazard to the operator, the support team, and most important, the patient being treated. It is dangerous to release ozone into the oral cavity. As a member of one of the most reactive gasses, ozone will form concentration equilibrium within seconds throughout the oral-nasal complex and the lungs, as well as the area being occupied by the patient and the treatment team.

It is interesting to note that in 1978, a USA FDA report highlighted that 1.5 million people were hospitalized in the USA due to side effects from medication. On the other hand, medical ozone has been legally used in clinics worldwide on a daily basis since the forties. In Germany, Ozone side effects are typically minor irritations that are caused by incorrect application and quickly disappear. This side effect rate is incredibly far, far, lower than US drug therapy side effect rates wherein each year approximately 140,000 people die from prescription drug usage.

In May of 1980 The German Medical Society stated 384,775 patients were treated with ozone with a minimum of 5,579,238 applications and the side effect rate

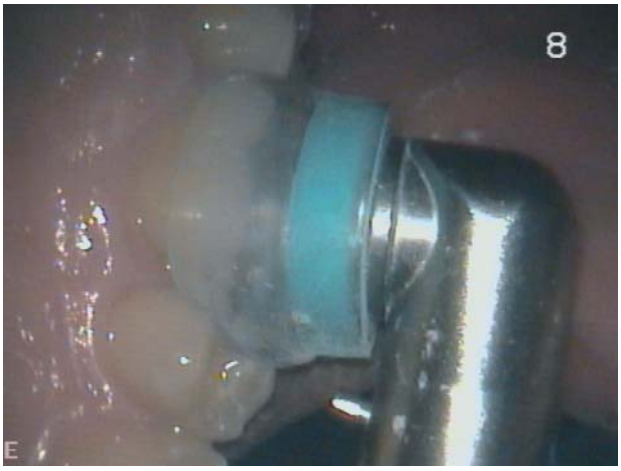
observed was only .000005 per application. Used properly, ozone has to one of the safest treatments that dentists and doctors around the world have.

There is currently only one device that has CE and MDA approval for the treatment of caries in the mouth, and this is the HealOzone. It is manufactured by Curozone Inc, Canada, and marketed by KaVo GmbH, Germany. Initially it is only available in selected countries around the world. Due to FDA regulations, it will not be available in the USA until 2005 onwards until the USA clinical studies have been reported.

The HealOzone is essentially a self-contained device for the manufacture of ozone gas. There are a plethora of devices that can produce ozone, but all depend on positive pressure to push air or oxygen through a production unit. This uncontrolled production of ozone is unsuitable for use in the mouth for reasons already discussed here and elsewhere in this book. The HealOzone has a handpiece connected to the main unit by a hose. All the connections are quick-release couplings, and all parts, except the cups, can be autoclaved. Check the handpiece seals and hose seals regularly, as worn or damaged seals will cause a vacuum air leak and prevent ozone generation. Lubrication of the fittings will help ease connections and prevent seal damage.

The handpiece has a single-use silicone rubber cup that is easy to remove and replace. The delivery cups come in a variety of 5 cup sizes; 3 mm, 4 mm, 5 mm, 6 mm and 8 mm. Each is colour coded. The cups are made of two welded components; the first is a stiff ring that locks into the retention groove on the ozone hand piece. The second component is a soft silicone ring that forms the delivery cup; this adapts and seals around the lesion or tooth to be treated.

The cup is placed over the area of caries to be treated, and when operated, the HealOzone creates a suction inside the cup. The HealOzone can be user-programmed to deliver 10, 20, 30 or 40 seconds of ozone at a fixed concentration of 2,100 ppm of ozone



±5% at a flow rate of 615 cc/minute. If the integrity of the seal around the lesion is intact, then air is drawn through the hose, which in turn switches on the ozone production. If the seal breaks down for any reason, then the production of ozone will cease.

If the seal is broken, the cup's position can be slightly re-positioned, and once the seal has been re-established, the delivery of ozone will recommence. The unit 'bleeps' every second, as well as a visual display on which the time count-down is shown. At the end of the timed ozone delivery, the unit continues to suck for 10 seconds before the complete sequence ends and the unit returns to stand-by for further ozone applications. Unused ozone is drawn from the cup through the hose, through a biological filter, and then finally through a cytalytic converter. This breaks down any unreacted ozone into oxygen. At this point, air and oxygen are returned to the surrounding air in the treatment area.

There are two further items that need user intervention. On the right hand side of the HealOzone, set behind the side door, are an air drier and the biological filter.

It is essential that dry air is used to create ozone. A silica-gel dessicant column is linked into the air intake. Air is drawn through this into the plasma unit. In the Mark II version of the HealOzone, a new air drier is bright blue, and as moisture is drawn out of the air as it passes through, it turns to light blue then to pink. In the Mark III and later models, the dessicant is a bulk-dessicant type, and the manufacturer states that this should last 2–3 months, depending on the ambient humidity of the operating area.

The time each dessicant drier unit lasts is determined by the humidity of the air around the HealOzone. If it HealOzone is located in a surgery that is air conditioned, then the air drier will tend to last longer. However, if the surgery area also has an autoclave, or a water distiller, then the room's humidity will be higher, and the driers will need to be replaced on a more frequent basis. The best results are gained by changing the filter regularly, as the older or wetter the desiccant is, the lower the ozone concentration and the less destructive the effect on the bacteria will be.

The biological filters are designed to stop any oral fluids being drawn up through the cup, down the connecting hose and into the HealOzone unit. New users of the system will find initially that treatment in the lower arch tends to draw in oral fluids into the hoses and filter. As the system works on a suction principle, the location of the biological filters is essential to prevent poisoning of the catalytic converter. The hydrophobic waste filter should be changed when nearly full. It has two functions:

- To collect any waste liquid which may be drawn through the vacuum system from the mouth
- The hydrophobic membrane protects the destructor filter and vacuum pump from liquid by shutting down when it comes into contact with liquid.

When changing the hydrophobic waste filter, be careful not to kink the pipes on fitting, as this will prevent flow.

Regular service maintenance of the HealOzone unit by trained technicians is essential for safe working conditions. The HealOzone electronics count the number of treatments given. As this number reaches a threshold, the unit will first request that a service is performed by

calling a KaVo engineer, and if this is not carried out within an additional threshold number, the unit will shut down until the system is serviced and reset. This service cannot be carried out by dental personnel. Specialist equipment is used to examine and adjust as necessary the concentration of ozone and the flow rate. The catalyst is renewed, and the unit examined to ensure that is safe to continue to use.

The unit requires a full service every six months, to:

- Replace the destructor filter.
- Check that the ozone concentration level and flow rate is correct.
- The flow rate and concentration will reduce slightly with use and will need readjusting by an engineer. This service cannot be carried out by dental personnel. Specialist equipment is used to examine and adjust as necessary the concentration of ozone and the flow rate.

The HealOzone works at 2100 parts per million of ozone with a flow rate of 615 ccm. Any remaining high level of ozone concentration must be destroyed after use. The destructor filter, manganese dioxide, breaks down the ozone into oxygen and returns it to the treatment area atmosphere.

- The destructor has a life of approximately 5,000 cycles or 6 months. Its efficiency reduces over time, or the longer it is used. It will eventually pass high levels of ozone into the working area, which could be hazardous to health.
- The Mark II and III will display a prompt to the user at 4,500 cycles if not serviced before. At 5,000 cycles the unit will stop working, ensuring no threat of ozone dispersal into the environment.
- The first service at 6 months is free-of-charge and KaVo will contact the user on the due date.
- At the end of the guarantee period you will be offered a Service Contract. If you would like information earlier please contact KaVo direct. A 3-tiered Maintenance Contract is available.

Setting up the HealOzone unit for treatment

Setting up the HealOzone has to be one of the easiest tasks out of all the dental equipment that is used in a modern dental surgery.

- Remove all packaging.
- Attach the hose to the console and the handpiece to the hose as shown in the instruction manual.
- Plug the power cord into the back of the console.
- Fit the Hydrophobic Waste Filter – remove the waste filter from the package and connect the twist lock onto the connector and push the tubing end onto the barbed fitting. As shown on the diagram in the unit. Take care not to twist or kink the tubing, and make sure all the tubes are firmly pushed home onto the spigot ends.
- Install a Desiccant Cartridge – remove and discard the plug from the desiccant cartridge. Push the tubing end onto the barbed fitting as per the diagram.
- Connect the foot pedal to the rear of the console, if required.

Your system is now ready for use.

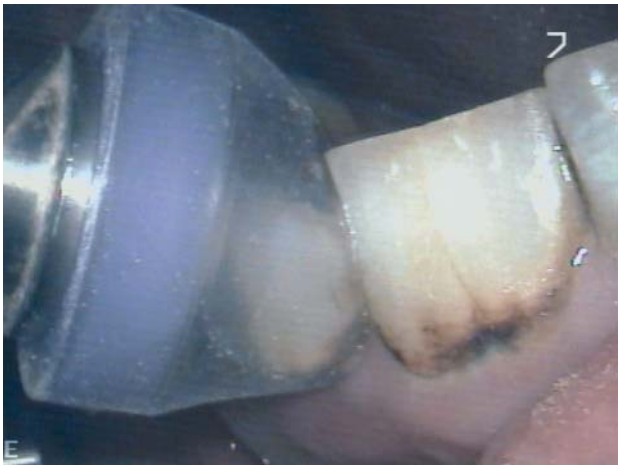
Using the HealOzone unit

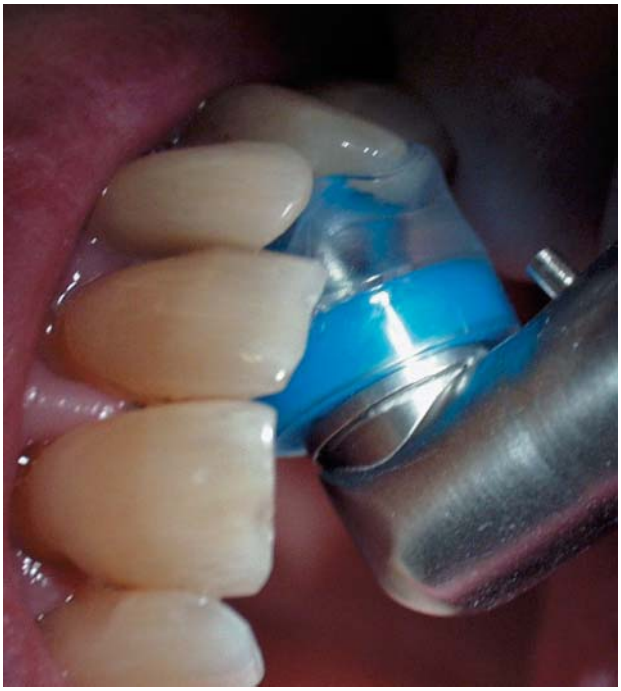
- Turn the unit on using the power switch located on the left side of the rear panel.
- Select the appropriate delivery cup dependent upon size of treatment area.

Note: Delivery cups are designed for single use and may distort if used more than once. There are 5 cup sizes; 3 mm, 4 mm, 5 mm, 6 mm and 8 mm. In very general

terms, the 5mm cup tends to fit deciduous molars and permanent pre-molars. The 6 mm cup tends to fit permanent molars. The 3 and 4 mm cups are useful for buccal areas and the 8 mm cup can be used to treat interdental caries between two anterior deciduous teeth, provided a seal can be obtained, or large permanent molars.

- Affix the selected delivery cup to handpiece head.
- Press the Reset button to bring up cycle time screen.
- Select a dosage time by pressing the TIME/PRIME button. The cycle times are preset at 10,20, 30 and 40 seconds on the Mark II version, and 20, 30, 40 and 60 seconds on the Mark III.



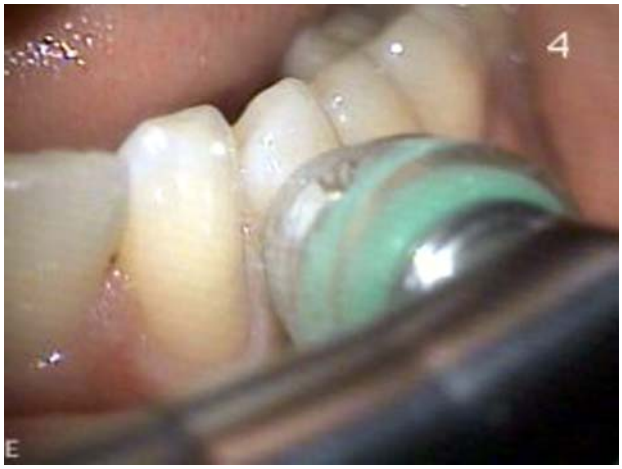


- Once cycle time is selected create and maintain a seal with the cup. Some users have found it easier to start the unit, and then place the cup on the lesion to be treated. In this way, the audible second bleep can be used as a guide to the attainment of a seal.
- Press the START button or foot control to start ozonation. The vacuum pump inside the unit will start.
- The ozone generator will only begin if a seal is attained. Then the count down on the display will start and the unit will sound a bleep every second.
- A longer beep will sound when the ozone dosage time is complete, the unit will then begin a 10 second ozone evacuation cycle.
- The ozone cycle can be stopped at any time by pressing the STOP button or foot pedal.
- Further doses can be applied by pressing the Start again.

Guidance notes for the HealOzone user

Detailed application notes are available from the www resource, **www.the-o-zone.cc**A 5-part series of pdf files by Dr. Julian Holmes shows a number of clinical cases, with commentary on how to create the delivery seal around various types of carious lesions. Over time, these pdf files will be updated and added to.

1. Assess patient motivation to adhere to one's professional guidance and advice. **HealOzone** creates an environmental condition within and around the tooth, which enables Nature to allow natural remineralisation to occur. If the patient doesn't ap-



preciate that their compliance is also part of the Treatment Plan, the process may be flawed.

2. Perform a thorough dietary analysis of all potential patients, prior to commencement of treatment.
3. Encourage patients to attend a pre-**HealOzone** treatment entry session, with the practice Hygienist.
4. On presenting and prior to all recall appointments; ensure thorough and intensive prophylaxis of all the dentition with **PROPHYflex**, prior to taking **DIAGNOdent** readings, especially lower lingual and upper buccal posterior surfaces. Stains or presence of debris will produce elevated **DIAGNOdent** readings and lead to false positive caries diagnosis.
5. Select the lingual surface of a clean, healthy lower anterior tooth for the "Set 0" or "Tooth Constant" function of **DIAGNOdent** – allows greater accuracy in diagnosis. Utilise the caries "Clinical Severity Index", as developed and modified from the original 7-level 'Ekstrand Index', by Prof. Edward Lynch and Dr. Julian Holmes. This is also available as a file from www.the-o-zone.cc in pdf format.
6. If solely relying upon DIAGNOdent readings, the following guidelines will prove helpful:

   - Assessment="D1" or "D2" (**DIAGNOdent** *reading 14 to 24*) 20-second application of **HealOzone**
   - Assessment="D3" (**DIAGNOdent** *reading 25 to 30*) 30-second application of **HealOzone**
   - Assessment="D4" (**DIAGNOdent** *reading above 30*)

   Conservative, minimally invasive preparation, followed by a 40 second **HealOzone** application.
7. Generally speaking, expose the lesion to ozone for at least 20, preferably 40 seconds. Even longer doses should be applied if it is considered that Ozone gas might have difficulty in reaching a subsurface non-cavitated carious lesion, or if the caries forms part of a particularly deep or extensive lesion. Studies suggest that Ozone may penetrate up to 5 mm into carious tissue. If it is believed caries extends beyond 5 mm, it is desirable to remove only sufficient diseased material to enable the Ozone effect. The more original tooth structure that remains will give rise to a less invasive procedure and a stronger tooth upon which to place a restoration. **HealOzone** has no adverse effect on healthy tissues found within the oral cavity, so it is impossible to overdose.
8. With this treatment regime, the primary outcome variable must be "Clinical Reversal" of caries, by whatever means one currently employs in the diagnosis of this disease. If using radiographs, Ozonated and remineralised tooth tissue displays the same density as the healthy surrounding tissue. The lesion therefore disappears radiographically.
9. If obtaining cup seal proves difficult, block-off one side of embrasures with a light-curable liquid dam (or similar). These materials are excellent for obtaining the seal around orthodontic arch wires of fixed appliances. Simply peel away dam, post Ozonation. Preferably leave all exposed lesions to re-mineralise for four to eight weeks, prior to considering functional and/or aesthetic restoration. Should a finishing restoration be placed immediately into an Ozone treated lesion, the bond will likely fail prematurely, owing to the soft nature of the demineralised enamel/dentine structure. Should it not be possible to leave a lesion open, due to food impaction or similar reason, consider using GC's **Fuji VII** encapsulated *(pink)* Glass Ionomer. This is a high-fluoride containing material, which allows passage of essential minerals through its structure, thus assisting natural re-mineralisation. On recall, it may be necessary to use air abrasion to remove the pink **Fuji VII** material to make a new caries assessment. When caries is deemed reversed and hard, the cavity may be filled with an aesthetic material of choice. If small, an acid-etched and bonded flowable composite would be used. For large lesions that require restorative care, then a filled composite can be used. Consult the publication 'Reality' for a complete rating of available materials to use.
10. Emphasise the necessity of patient compliance with dietary advice, oral hygiene and at home Patient Kit instructions. Whilst good results may be obtained without use of the **HealOzone** Patient Kit, to obtain excellent results in the shortest possible time, it is important to tip the balance in favour of remineralisation with the best possible minerals, such as Calcium, Fluoride, Sodium, Phosphate and Zinc. Unfortunately, these stronger minerals are not always available from some patient's diets. In addition, use

of these strong tasting dentifrices remind and encourage the patient to maintain their new, improved levels of oral hygiene. Recently published studies from Belfast University and others would seem to emphasise that the addition of the remineralising pastes and fluids leads to the best results.

11. Reduction in consumption of fermentable carbohydrates is very important. Recommend that acid containing or carbonated drinks be avoided, as well as continuous sucking of sweets/mints/medical lozenges.
12. Temper patient expectation with realism. **HealOzone** is not the panacea of life! In some cases, ozone is unsuitable without traditional dental care. In the larger cases, or where the tooth structure is badly broken down, it may not be possible to obtain or create a seal. In others, ozone may be considered as an adjunct to dental care, after conventional tissue removal has taken place.
13. Teeth will become healthier and stronger, but Ozonation may require to be combined with conventional dental treatment if lesions are extensive and deep into the dentine. Additionally, aesthetic treatment to mask staining taken-up during the remineralisation process, may be required. Also make sure patients understand that for some of the larger lesions, repeated treatments may be required. In a recent study (Holmes J, 2003) buccal lesions were treated at start, 3, 6, 12 18 and 24 months.
14. Even if **HealOzone** treatment fails, one may still resort to conventional treatment. Patients have nothing to lose and potentially everything to gain. However, once a small filling is placed, the treated tooth is condemned to a lifetime of ever increasing-sized fillings, crowns, bridges, dentures or implants with corresponding trauma, plus the financial burden on both the individual patient and Dental Health Service.

HealOzone – modus operandi

- Lower organisms such as bacteria and viruses do not possess enzyme coats. This allows Ozone to easily penetrate their cell membranes and render them sterile. Higher organisms actively produce enzyme coats, which prevent reaction with Ozone. This protects these cells from attack. Interestingly, Ozone does target and attack *diseased* human cells, which possess deficient cell wall enzymes and/or disrupted DNA or RNA strands. This explains why clinical Ozone treatment is toxic to infecting organisms but not to the patient. *(Use in "dry sockets" and general "soft tissue" indications, etc.)*
- Bacterial caries causes an acidic pH in the mouth that impedes or prevents re-mineralisation. Ozone kills micro-organisms, which allows buccal pH levels to rise and return to normal, thus allowing natural re-mineralisation by calcium, fluoride, sodium, zinc, phosphates etc., to occur.
- Metabolites *(waste products)* of micro-organisms include Pyruvic Acid, which again provides a niche environment for even more sinister "acidogenic" and "acidoduric" organisms. Ozone oxidises the Pyruvic Acid, turning it into neutral Acetate and CO_2, thus removing the niche environment for these types of bacteria. Commensal *(non-parasitic)* bacteria may then colonise the lesion, without undue concern – the patient has bought some more time to modify their diet and/or oral hygiene regimen.
- Proteins *(Amino Acids)* strongly adhere to all exposed dental surfaces. Here they also impede re-mineralisation. Ozone "cleaves" or breaks down the chains that bind Amino Acids together, thus dislodging them from their niche environment. Re-mineralisation can thus occur more easily.
- Ozone penetrates into cracks and dentinal tubules, where again it seems to cleanse these channels, allowing minerals to flood in without resistance *(Immediate pain relief associated with cracked crown syndrome, hypersensitive tooth necks, etc.).*
- Ozone adversely affects intracellular anti-oxidant enzymes, which are the proven cause of life-long oxidative stress, responsible for degenerative diseases and aging.
- Ozone facilitates the elaboration *(releasing)* of Nitric Oxide within macrophages *(Human immune system white blood cells etc.).* This enhances the scavenging function of macrophages, making the human immune system more efficient. Prof. Bocci of Italy has also done much work with regard to the beneficial effects of Ozone on diseases within the blood system. Pulpitis and Ozonation close to the pulp chamber may be approached with confidence.
- Whilst Oxygen is a "Free Radical" species, Ozone is *NOT.* Free Radicals are broken down by Ozone into

safer compounds. Those thus affected include:

- Nitrogen Dioxide
- Sulphuric Acid
- Nitrous Oxide
- Carbonic Acid
- Sulphur Dioxide
- Carbon Monoxide

Cleaning and sterilisation

- Remember that ozone is a great sterilisation medium. The ideal way to sterilise the inner surfaces of the hand piece and the tubing is simply to start a 60-second ozone cycle. Place a delivery cup on the hand piece (any size cup will do for this operation), use a hard surface like a glass slab, and once a seal has been obtained, run the HealOzone for 60 seconds.
- Clean the outer surfaces of the handpiece and the hose.
- Disconnect the handpiece from the hose.
- Sterilise the handpiece and hose separately, prior to use and between patients, at 134°C.

Before Using your HealOzone, it is important that you read the manual. KaVo, Dr. Julian Holmes and Prof. Edward Lynch also run courses; there is a basic introduction day course, and a course for the more advanced user. This unit requires training, and this should not be over looked.

Costing options in the dental practice

(All costings are expressed in GB Pounds)

In the UK, dental practitioners are charging a wide range of fees for ozone treatment, from £10.00 per tooth treated, to £95.00 per treatment. An average figure seems to be £35.00. If used correctly, not only is ozone treatment a winner for the patient, in that the tissue destruction is minimal, but it is also a financial winner for the dental practitioner investing in this technology.

Prof. Edward Lynch and Dr. Julian Holmes have collated their research over the last 3 years. If conventional amputation dental care is examined first, the cost breakdown and profit are as follows. Remember, these figures are averages, so there will be a wide variation in the figures quoted below.

– Single surface composite filling	£98.00
• Time	30 minutes average
• Materials	
– needle	£00.02
– r/dam	£00.10
– local anaesthetic	£00.15
– acid etch, resin, brushes	£01.00
– composite	£04.50
– incidentals	£07.00 (equipment w/tear)
– wages	£30.00
– Profit	£54.00 approx

- ***or £108.00/hour profit***

The average fee for a single surface composite filling is approximately £98.00 (UK Pounds). It takes on average 30 minutes to complete. By the time the costs of equipment, materials and wages have been added into the equation, the hourly profit is approximately £108.00.

Lets compare this to a selection of 4 small occlusal, first stage enamel carious lesions. On the DIAGNOdent, it measures 20–22. From the studies of Prof. Adrian Lussi in Berne, Switzerland, we know the demineralisation in these lesions is almost at the EDJ. The research tells us that 20 seconds of ozone is required, but to make sure, the HealOzone is set at 40 seconds. Four small lesions are found, and after ozone treatment, Fuji-VII (GC, Japan) is going to be placed to continue the re-mineralisation process, as well as prevent further ingress and impaction of food and micro-organisms.

– Ozone treatment	(4 teeth)	£140.00
– Fuji7 sealants	(4 teeth)	£120.00
• Time	20 minutes average	
• Materials		
– materials		£40.00/ patient
– incidentals		£07.00 (equipment w/tear)
– wages		£15.00
– Profit		£190.00 approx

- ***or £550.00/hour profit***

The procedure is explained to the patient, the teeth are cleaned, measured with the DAIGNOdent and ozone treated. Remineralising washes are applied, and then FujiVII is applied. The whole procedure takes on average 20 minutes, there has been minimal tooth destruction, and the patients' best interests have been served. The practice has a potential hourly income of £550.00. In some UK dental practices, ozone treatment is being done as part of other treatment. For example, a crown needs to be prepared on a heavily filled molar. As the anaesthetic is taking effect, ozone is applied to lesion(s) before the crown preparation proceeds. When integrated into routine dental care, ozone treatment becomes a very profitable investment for the practitioner.

There are various leasing options. In the UK, Prof. Lynch and Dr. Holmes have taken a wide range of leasing figures, and the tables below should be used as a guide to the costs. For those dentists who are outside the UK, you should consult with your KaVo representatives, or your dental finance companies.

Examples of leasing options are the daily cost of a HealOzone unit.

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HealOzone machine, complete with 1 handpiece	**£11,950.00**
DIAGNOdent caries-detecting LASER, with x3 tips	**£999.00**
PROPHYflex prophylactic polishing system with x2 nozzles	**£599.00**
Value Added Tax @ 17½ %	**£2,370.90**
Grand Total *Prices Correct at January 2004*	**£15,918.90**
÷1,000 =	**£15.92**
× £20.14 Leasing Rate over 5 Years = Monthly Repayment =	**£320.61**
× 12 Months = Yearly Repayment =	**£3,847.28**
÷ 52 Weeks = Weekly Cost =	**£73.99**
÷ 5 Days = Daily Cost =	**£14.80**
– 40% Personal Tax Allowance = Net Daily Cost including VAT =	**£8.88**

HealOzone machine, complete with 1 handpiece	£11,950.00
HealOzone Gold Service Contract	£630.00
DIAGNOdent caries-detecting LASER, with x3 tips	£999.00
PROPHYflex prophylactic polishing system with x2 nozzles	£599.00
Value Added Tax @ 17½ %	£2,481.15
Grand Total *Prices Correct at January 2004*	£16,659.15
÷1,000 =	£16.66
× £20.14 Leasing Rate over 5 Years = Monthly Repayment =	£335.52
× 12 Months = Yearly Repayment =	£4,026.18
÷ 52 Weeks = Weekly Cost =	£77.43
÷ 5 Days = Daily Cost =	£15.49
– 40% Personal Tax Allowance = Net Daily Cost including VAT =	£9.29

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CHAPTER 3.1

Evidence-Based Research into Ozone Treatment in Dentistry: An Overview

Layla AbuNabaa, Hisham Al Shorman, Julian Holmes & Edward Lynch

Recent history of ozone

Production

Ozone is a gas that is produced in the upper atmosphere. It is heavier than air and thus affected by gravity. As ozone descends towards the earth, it binds to any pollutant in the atmosphere that comes in touch with it, thereby cleansing the air. If ozone binds the water molecules present in water vapour in the atmosphere, it forms hydrogen peroxide, a component of rain water. That rain water is cleaner than tap water is demonstrated by plants, which grow better when irrigated with rain water than with tap water.

Ozone is created in nature in three ways: first, by lightening, which provides the wonderful fresh smell after a thunderstorm; second, through waterfalls and crashing surf, which accounts for the energetic feeling and calm experienced near these sites; and third by photons from the sun that split nitrous oxide, a pollutant formed by the combustion of hydrocarbons in the internal combustion engine. This type of ozone accumulates in smog due to temperature inversion and is a lung and eye irritant. This duality of ozone and its effects are the reason why the media focus on the last and the healing property of ozone tends to be ignored.

Medical applications of ozone

Ozone has a long history in research and medicine. In 1785, Van Marum noticed that air near his electrostatic machine acquired a characteristic odour when electric sparks were passed. In 1801, Cruickshank observed the same odour at the anode during electrolysis of water. Finally, in 1840, Shonbein named the substance that gave off this odour, 'ozone', from the Greek word 'ozein', to smell.

In 1857, Werner Von Siemens designed an ozone generator that has since greatly evolved. The cylindrical dielectric type, sometimes called the 'Siemens Type' ozone generator, makes up most of the commercially available ozone generators in use. Since the discovery of the benefits of ozone, many articles were published and the substance came to be used in water purification, technology, and medicine. In 1902, JH Clarke's *A Dictionary of Practical Materia Medica*, described the successful use of ozonated water in treating anaemia, cancer, diabetes, influenza, morphine poisoning, cancer sores, strychnine poisoning, and whooping cough. *A Working Manual of High Frequency Currents*' was published in 1911 by Dr Noble Eberhart, head of the Department of Physiologic Therapeutics at Loyola University. Dr Eberhart used ozone to treat tuberculosis, anaemia, chlorosis, tinnitus, whooping cough, asthma, bronchitis, hay fever, insomnia, pneumonia, diabetes, gout, and syphilis. In 1913, the Eastern Association for Oxygen Therapy was formed by Dr Blass with some German associates.

During World War 1, ozone was used to treat wounds, trench foot, gangrene, and the effects of poison gas. Dr Albert Wolff of Berlin also used ozone for treating colon cancer, cervical cancer, and decubitus ulcers in 1915. In 1920, Dr Charles Neiswanger, the President of the Chicago Hospital College of Medicine, published *Electro Therapeutical Practice*. The title of Chapter 32 was 'Ozone as a Therapeutic Agent'. In 1926, Dr Otto Warburg of the Kaiser Institute in Berlin announced that the cause of cancer is a lack of oxygen at the cellu-

lar level. Dr Otto Warburg received the Nobel Prize for Medicine in 1931 and again in 1944. In 1929, a book called *Ozone and Its Therapeutic Action* was published in the USA. It listed 114 diseases and how to treat them with ozone. The authors were the heads of leading American hospitals.

The Swiss dentist Dr Edwin Fisch used ozone in medical practice before 1932, and introduced it to the German surgeon Erwin Payr who used it from that time on. Aubourg and Lacoste were French physicians who used ozone insufflations from 1934 to 1938.

The use of ozone in the USA can be traced back to the 1940s. In 1948, Dr William Turska of Oregon began using ozone with a machine he had designed himself. In 1951, he wrote an article 'Oxidation'. He was the first to inject ozone into the portal vein, thereby reaching the liver (a technique that is no longer practised).

In 1953, a German doctor, Hans Wolff, used ozone in his practice. He wrote the book *Medical Ozone* and trained many doctors in ozone therapy. In 1957, Dr J Hansler patented an ozone generator that has formed the basis of the German expansion of ozone therapy over the past 35 years. Today, over 7000 German doctors use ozone therapy daily. Hans Wolff introduced the techniques of major and minor autohaemotherapy in 1961. In 1964, spontaneous flocculation in ozone contact chambers led to construction of an ozone plant in France to enhance particulate removal, and in 1965 in Scotland, ozone was used for colour control in surface water for the first time. At the same time, Swiss research led to the use of ozone to oxidize micropollutants such as phenolic compounds and several pesticides.

In 1977, Dr Renate Viebahn provided a technical overview of ozone action in the body. In 1979, Dr George Freibott began treating his first acquired immune deficiency syndrome (AIDS) patient with ozone, and in 1980, Dr Horst Kief reported success in treating AIDS with ozone. In 1987, Dr Rilling and Dr Viebahn published *The Use of Ozone in Medicine*. In 1990, the Cubans reported their success in treating glaucoma, conjunctivitis, and retinitis pigmentosa with ozone.

Safety of medical applications of ozone

In 1978, a US Food and Drug Administration (FDA) report revealed that 1.5 million people were hospitalized in the USA due to side effects from medications. On the other hand, medical ozone has been legally used in clinics worldwide on a daily basis since the 1940s. In Germany, side effects of ozone are occasional minor irritations caused by incorrect application that quickly disappear. This rate of side effects is far lower than the rates of side effects of US drug therapy. Ozone has been found to be an extremely safe medical therapy, free from side effects. In 1980, in a study carried out by the German Medical Society for Ozone Therapy, 644 therapists were asked about their 384,775 patients, comprising a total of 5,579,238 ozone treatments administered. There were only 40 cases of side effects, which were all operator/administrator caused and represents the incredibly low rate of 0.000007%, and four fatalities. It is essential that ozone gas should never be directly injected intravenously due to the potential for air emboli that can be fatal. Ozone administered by routes other than intravenous gas delivery is thus the safest medical therapy ever devised. Professor Velio Bocci from the University of Siena, Italy, has published excellent research objectively quantifying the therapeutic benefits of ozone (Bocci, 1996a,b, 1997, 1999; Bocci and Aldinucci, 2004). In 1992, the Russians revealed their techniques of using ozone bubbled into brine to treat burn victims with astonishing results.

Ozone in water purification

Ozone has played a significant role in the waste treatment process in the past and will continue to do so in the future. The utilization of ozone in industrial situations has a long and impressive history, one that predates current environmental concerns.

One of the commonest uses of ozone is in the treatment of water. Scientists and doctors studied the ozonation system at the Oudshoorn plant in the Netherlands and later constructed a 19,000 M3/day (5 mgd) plant using ozonation for disinfection at Nice, France in the early twentieth century. Nice is often referred to as 'the birthplace of ozonation for drinking-water treatment'.

When ozone is used to treat drinking water, it is effective in eliminating colour, taste, and odour. Its competitor, chlorine, which is used in many facilities as a disinfectant, has recently been found in scientific studies to have a tendency to create carcinogens as it breaks down. For this reason, there is increased pressure to reduce or eliminate chlorine as a primary disinfectant for water. Ozone is 150 times more powerful than chlorine and acts 3500 times faster. It eliminates harmful metals as well, by causing these metals to clump

together which allow them to be large enough to filter out. Because ozone has a short life, it quickly converts to pure oxygen and thus adds much-needed oxygen to the water. Since ozone water purification systems require no chemicals, they are healthier and very cost-effective in long-term applications.

Worldwide applications of ozone

After 125 years of usage, ozone therapy in medicine and various other fields has been widely accepted in many nations around the world, including Germany, France, Italy, Russia, Romania, Czech Republic, Poland, Hungary, Bulgaria, Cuba, Japan, Mexico, and in some states of the USA.

Dental caries

A prevalent disease

Tooth caries (dental caries) is the most frequent and widespread nutrition-related disease in industrialized countries. Despite improvements in oral health (Robinson et al, 2004), the prevalence of dental caries remains high, and it can be a problem from birth. Left untreated, caries can progress through the tooth structure, ultimately resulting in loss of the tooth.

The niche theory

The initiation and progression of caries is a complex process involving the accumulation of microorganisms in a localized environment and leading to loss of minerals from the tooth and progression of caries through the tooth structure. According to the 'niche environment theory', a 'bacterial niche' is established within a carious lesion. Bacteria are far from the 'simple bugs' as they are often referred to. They have survived for millions of years, whereas humans, in comparison, have existed for a minute period of time. The dental profession should not be surprised to learn that bacteria set up complex interactions with other bugs, 'talk' to neighbouring colonies when times are good, and call for help from others when their host attempts to change their environment. Protein coatings, plaque, and debris are known to protect bacterial colonies by reducing the effect of pharmaceutical agents designed to eliminate them.

Progression of the carious lesion occurs when conditions are suitable for acidogenic bacteria to release acid as a metabolic byproduct. The acid produced may lead to a breakdown of mineralized tooth structure. At times, an equilibrium situation may occur when the rate of remineralization equals the rate of demineralization.

Current management and limitations

The management of dental caries is a complicated issue being dependent on the stage at which caries is identified, the type of caries and the assessment of risk and prognosis. The overall aim of management strategies is to maintain a functioning set of teeth. Within this, treatment may be aimed at:

- prevention of caries developing in the first place or
- prevention of progression of existing caries or
- restoration of the tooth once substantial caries has occurred.

The overall management strategy not only involves the application of treatments by the dentist but also relies on the patient to make behavioural changes and apply 'at-home' treatment.

Treatment of non-cavitated occlusal pit and fissure caries

When caries is at an early stage of development various strategies may be employed including behavioural modification by the patient and treatment with sealants, fluoride, chlorhexidine, and antimicrobial agents. Combinations of these treatments may well be used. The assessment of specific caries risk factors may also be important to prevent the progression of caries.

Behavioural modification

Changes in a patient's diet and brushing teeth with a fluoride-containing toothpaste have important roles in caries treatment and these habits may be established by the use of good dental health education (National Institutes of Health (NIH) consensus statement, 2001).

Sealants

Sealants are applied to the pits and fissures of teeth and are an effective barrier method for caries prevention,

preventing impaction of food and growth of bacteria (NIH consensus statement, 2001; Hiiri et al, 2004). The sealants used may be glass ionomers or one of several types of composite resin. The resin-based sealants differ in their means of polymerization and fluoride content.

Sealants still have disadvantages, which limit their applications. They have a limited lifespan and should be reapplied as required (Kilpatrick et al, 1996) as they are only effective if remain intact (NIH consensus Statement, 2001). Even then, caries may continue beneath the sealant, which may be due to micro-leakage around the edges of the sealant that can allow the ingress of food substrates to the carious micro-organisms (Knight, 2003).

Fluoride

Fluoride can be applied by the patient as toothpastes, mouthwashes, or tablets or applied professionally in the form of gels, varnishes, and sealants. The effect of fluoride is predominantly topical, promoting remineralization of early caries and increasing resistance to demineralization (Marinho et al, 2004). Although fluorides are available in a number of formulations, they rely on patient compliance for applications such as toothpastes and mouthwashes and adverse events such as fluorosis may occur, which in its mildest form will cause staining and pitting of the teeth. However, reported data are scarce.

Chlorhexidine

Chlorhexidine is one of a number of antimicrobial agents that have been studied in the treatment of caries. It can be applied as a varnish, mouthrinse, or gel and clinical data are promising (NIH consensus statement, 2001). A disadvantage is its limited effect on selected microorganisms (Mash, 1992) which may further develop resistance (Matthijs et al, 2002). Aesthetic effects, including staining also limit their use.

Another clinical problem with pharmaceutical approaches such as chlorhexidine is the difficulty in suppressing or eliminating microorganisms for a long enough period of time to allow caries reversal and remineralization of the tooth. After treatment, microorganisms may proliferate and re-colonize the lesion.

Treatment of cavitated fissure/occlusal pit caries

If caries has progressed into the dentin, with the formation of a cavity then the current treatment of choice is restorative, often referred to as 'drill'n'fill'. This will involve removal or amputation of carious tissue using a drill, followed by restoration of the cavity with a 'permanent' filling material and will require the injection of a local anaesthetic. This procedure may also be deemed appropriate if caries is present in the dentin, without obvious signs of a cavity.

Restorations generally consist of a mouldable material that is placed into the cavity, where it sets to become hard and is retained by the remaining tooth structure. A number of restorative materials are currently available including amalgam, composite resins and glass ionomers, however, amalgam is the most commonly used in the National Health Service (NHS), the national health provider in UK.

Restorative treatment with amalgam

Amalgam is an alloy of mercury, silver, and other metals that has been available for over 100 years, although its formulation has changed considerably. It is the material of choice if aesthetics are not a consideration, being the most hard wearing and cheapest material available (York University NHS Centre for Reviews and Dissemination, 1999).

These treatments are invasive procedures requiring injection/anaesthetic as they cause pain and discomfort. Removing caries involves amputation of tooth tissue. Some healthy tissue may be removed in the process to ensure that caries is halted, known as 'extension for prevention'. Such procedures weaken the tooth structure. The restoration does not bond with tooth tissue but requires mechanical undercuts for retention, which further weakens the tooth's structural integrity. Pulpal damage may occur due to heat and pressure of the drilling process (Maragakis et al, 2001). Amalgam has limited lifespan, for reasons including occurrence of secondary caries around the restoration and fracture of restoration. Then re-restorations are required. Furthermore the procedure is time consuming with an average chair time of 35 minutes (van Nieuwenhuysen, et al

2003). Re-restorations tend to increase in size since further drilling of tooth material will be required. This increases the risk of tooth fracture and damage to the pulp, which may necessitate the need for a crown, root canal treatment, or tooth extraction in the future (York University NHS Centre for Reviews and Dissemination, 1999).

Randomized controlled trials report amalgam survival rates of 72% at 15 years' follow-up and median survival times of 5–12.8 years (Jokstad and Mjor, 1991; van Nieuwenhuysen et al, 2003), while it is estimated that an amalgam restoration may last up to a maximum of 40 years. Given this limited lifespan, re-restoration may be required.

Many patients suffer from fear and anxiety of teeth being drilled and from receiving local anaesthesia, and this can lead to patients avoiding dental treatment altogether (Milsom et al 1997, 2003).

Treatment of root caries

Preventive treatment

Treatment options include the use of remineralizing fluorides, chlorhexidine and root sealants. However, there has been relatively little research into preventive management for root caries compared with enamel caries (i.e., pit and fissure caries) (Baysan et al, 2001).

It has been suggested that an antimicrobial approach to the treatment of root caries would be appropriate to bring about reversal and remineralization of the caries. This has been demonstrated to some extent by use of chlorhexidine and fluorides on root caries. However, no study has approached a caries reversal rate of 100% with these preventive regimens.

Restorative treatment

Restorative treatment of root caries involves caries removal and subsequent restoration, for example, with a glass ionomer or composite. However, this is challenging due to problems in particular with visibility, proximity to the pulp, and isolation from oral fluids, and the restorations require frequent re-restoration (Lynch and Baysan, 2001; Baysan, 2002).

Ozone in the treatment of dental caries

Many reports and studies have been conducted using ozone for oral and dental applications (Table 1). The chapter presents an analysis the study designs, lists the main findings, and projects further needs of new research complying with standards for clinical studies.

Overview of the included trials

Studies were identified that reported on the safety and effectiveness of treating root and pit and fissure caries with the HealOzone (KaVo GmbH, Germany) device. The studies were published as full papers in refereed journals or were presented as abstracts or posters at conferences. In addition, three studies have been done as part of PhD and MPhil theses, all of which were awarded without criticism and examined by world authorities in cariology. The details of the clinical studies are presented in Table 1.

Trial sample sizes ranged from eight to 377 patients, with occlusal pit and fissure caries in both deciduous and permanent teeth and root caries. The time between initial treatment and reassessment varied from 1 month to 21 months, and some trials reviewed patients more than once. Treatment time with ozone varied between studies from 10 to 40 seconds. All patients received initial treatment with ozone, and in some circumstances, patients were re-treated at each review. Trial comparators were generally no treatment or placebo treatment with air applied through the HealOzone device. Only five studies referred to current treatment options, including sealant and 'drilling'n'filling', of which three had patient-centred outcomes as their primary study result. The major outcomes of the trials include changes in caries severity and reduction in total caries-associated microorganisms.

In some instances, more than one report was found for a single study. These reports appear to present data collected at different follow-up times after the start of the study and if appropriate, the most recent report presenting data at the longest follow-up has been presented. In some cases, studies presented appear to be ongoing such that some patients have yet to be followed up.

The clinical effectiveness data will be discussed separately for root caries, non-cavitated, and cavitated pit and fissure caries. Adverse events and patient-centred outcomes will also be discussed. It should be borne in mind that direct comparisons between studies is difficult because of the variations in study design discussed above. Other applications for ozone will also be presented (Table 2).

Table 1: List of studies applying ozone treatment to different dental disciplines

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
Ozone safety									
1.	Baysan A, Lynch E. Safety of an Ozone delivery system during caries treatment in-vivo. J Dent Res, 80: 1159; 2001.	2001	40	80	10 & 20 Seconds	Root Caries	Both application times produced O_3 aroungd the application cup below the FDA & EU permissible O_3 levels in air	None	
2.	Ebensberger U, Pohl Y, Filippi A. PCNA-expression of cementoblasts & fibroblasts on the root surface after extraoral rinsing for decontamination. Dent Traumatol 18: 262–266; 2002.	2002			O_3 in water	Extracted teeth, cementobalsts & fibroblasts	Marked by Proliferating Cell Nuclear Antigen (PCNA): Irrigation with ozonized water showed higher labelling indices in comparison with saline, but this could not be statistically substantiated (P=0.24). Ozonized water, not being isotonic, had no negative effect on periodontal cells remaining on the tooth surface after irrigation for 2 min.	N/a	None
3.	Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. Journal of Endodontics, 778–781; 2004. Part 1	2004			O_3 in water	L-929 mouse fibroblasts	The metabolic activity of fibroblasts was high when the cells were treated with ozonated water, whereas that of fibroblasts significantly decreased when the cells were treated with 2.5% NaOCl	N/a	None
1H NMR Studies on Caries Biomolecules									
4.	Smith C, Lynch E, Baysan A, Silwood CJ, Mills B, Grootveld M. Oxidative consumption of root caries biomolecules by a novel anti bacterial Ozone delivery system. J Dent Res, 80: 1178; 2001.	2001				Analysis of root caries by high field 1H – NMR spectroscopy to detect oxidation of biomolecules by O3.	O3 caused oxidative decarboxylation of pyruvate & oxidative attack of carbohydrates to generate formate. O3 also oxidised PRCL lactate, urate, glycosaminoglycans & methionine to yield acetate & CO2, allantoin, low-molecular-mass saccharide fragments & methionine sulphoxide, respectively.	None	None
5.	Lynch E, Silwood CJL, Smith C, Grootveld M. Oxidising actions of an Anti-Bacterial Ozone-Generating Device towards Root Caries Biomolecules. J Dent Res, 81: A-138; 2002.	2002	20	20	5 Seconds	Analysis of 12 soft root caries by high field 1H –NMR spectroscopy to detect oxidation of biomolecules by O3.	Results obtained revealed that in addition to giving rise to the oxidative decarboxylation of pyruvate (generating acetate and CO2 as products), and the attack of carbohydrates to produce formate, O3 also oxidised PRCL lactate, urate, glycosaminoglycans and methionine to yield acetate and CO2 (via pyruvate), allantoin, low-molecular-mass saccharide fragments and methionine sulphoxide, respectively.	None	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
6.	Lynch E, Silwood C, Smith C, Grootveld M. Oxidising actions of Ozone towards Root Caries Biomolecules. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no 197; 2002.	2002		18	10 seconds	Analysis of Soft root caries by high field 1H –NMR spectroscopy to detect oxidation of biomolecules by O3.	Bacterial- or yeast-derived organic acid anions (formate, acetate, propionate, n-butyrate, lactate, and pyruvate), amino acids (predominantly alanine and glycine) and carbohydrates such as glucose were present. O_3 gave rise to the oxidative decarboxylation of pyruvate (generating acetate and CO2), carbohydrates to produce formate, also oxidised lactate, urate and glycosaminoglycans to yield acetate and CO2, allantoin and low-molecular-mass saccharide fragments respectively. VSC precursor methionine was oxidised to sulphoxide. O_3-mediated oxidation of 3-D-hydroxybutyrate	None	None
7.	Lynch E, Silwood CJ, Abu-Naba'A L, Al Shorman H, Baysan A, Holmes J, Grootveld M. Oxidative Consumption of Root Caries Biomolecules using Ozone. Journal Caries Res, 38: 364; 2004.	2004	30	30	30 Seconds	Analysis of root caries by high field ^{1}H -NMR spectroscopy to detect oxidation of PRCL biomolecules by O_3.	O_3 caused oxidative decarboxylation of pyruvate & oxidative attack of carbohydrates to generate formate. O_3 also oxidised PRCL lactate, urate, glycosaminoglycans & methionine to yield acetate & CO2, allantoin, low-molecular-mass saccharide fragments & methionine sulphoxide, respectively.	None	None
1H NMR Studies on Saliva Biomolecules									
8.	Lynch E, A Baysan A, Silwood C, Grootveld M. Therapeutic oxidising activity of a novel anti-bacterial Ozone-generating device on saliva. J Dent Res, 77: 1187; 1998.	1998	20		30 seconds	Analysis of saliva by high field 1H -NMR spectroscopy to detect oxidation of biomolecules by O3	O3 produced oxidative de- carboxylation of pyruvate, & oxidation e sulphur compound methionine to its corresponding sulphoxide, oxidative consumption of polyunsaturated fatty acids O3. Moreover, -mediated oxidation of 3-D-hydroxybutyrate was also obtained.	None	None
9.	Lynch E, Smith E, Baysan A, Silwood CJL, Mills B, Grootveld M. Salivary Oxidising Activity of a Novel Anti-bacterial Ozone-generating Device. J Dent Res, 80: 13; 2001.	2001	20		10 seconds	Analysis of saliva by high field 1H –NMR spectroscopy to detect oxidation of biomolecules by O3	Oxidation e sulphur compound methionine to its corresponding sulphoxide, oxidative consumption of polyunsaturated fatty acids O3.	None	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
10.	Claxson AWD, Smith C, Turner MD, Silwood CJL, Lynch E, Grootveld M. Oxidative Modification of Salivary Biomolecules with Therapeutic Levels of Ozone. J Dent Res, 81: A-502; 2002.	2002	20	20	10 seconds	Analysis of saliva by high field 1H-NMR spectroscopy to detect oxidation of biomolecules by O3.	In addition to the complete oxidative consumption of salivary pyruvate (mean±s.e. salivary level prior to treatment 1.75±0.62 mM) and partial oxidation of methionine, results acquired revealed (1) marked elevations in the concentration of formate (a product derived from the oxidation of carbohydrates), (2) substantial reductions in lactate level (up to 92%) via its oxidation to pyruvate, which in turn is oxidised to acetate and CO2, (3) the oxidation of 3-D-hydroxybutyrate to acetoacetate, which liberates acetone on decomposition, (4) the generation of allantoin from salivary urate (up to levels of 40 μM), (5) the production of low-molecular-mass saccharide fragments from hyaluronate, and (6) oxidation of malodorous trimethylamine (initial salivary concentration 78±23 μM) to its corresponding, non-malodorous N-oxide.	None	None
11.	Turner M, Silwood CJL, Grootveld M, Lynch E. Oxidative Consumption of Biomolecules using Ozone. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 594; 2002.	2002			10 seconds	5.00 ml of aqueous solutions: sodium pyruvate, D-glucose, L-cysteine and L-methionine (5.00 mM) were prepared in 40.0 mM phosphate buffer (pH 7.00) rigorously deoxygenated with argon gas)	As expected, treatment of aqueous solutions of L-methionine with ozone confirmed oxidation to its corresponding sulphoxide. H NMR analysis demonstrated that exposure of aqueous solutions of L-cysteine to ozone generated its corresponding disulphide, cystine, as a major product. H NMR analysis also showed that ozonolysis of D-glucose generated formate as a major reaction product, i.e., a concentration of 1.29 mM was produced from the 5.00 mM glucose substrate, an observation consistent with previous studies conducted on the interactions of ROS (e.g., radiolytically-generated .OH radical) with carbohydrates in general. Treatment of pyruvate with this oxidant produced acetate and CO2 via an oxidative decarboxylation process	immediate	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
12.	Turner M, Grootveld M, Silwood C, Lynch E. Oxidative Consumption of Biomolecules by Therapeutic Levels of Ozone J Dent Res, 81: A-272; 2002.	2002			10 seconds	Aqueous solutions containing sodium pyruvate, α-D-glucose, L-cysteine and L-methionine (5.00 mM) were prepared in 40.0 mM phosphate buffer (pH 7.00) which was rigorously deoxygenated with argon gas prior to use.	Attack of O3 on α-D-glucose gave rise to formate as a major product, i.e. 1.21±0.11 mM (mean±s.e.) generated, and treatment of pyruvate with this oxidant produced acetate and CO2 via an oxidative decarboxylation process (93±4% yield under our experimental conditions). Moreover, the amino acid volatile sulphur compound (VSC) precursors cysteine and methionine were converted to their corresponding primary oxidation products cystine (100% yield) and methionine sulphoxide (98±2% yield) respectively.	immediate	None
13.	Silwood C, Smith C, Turner M, Grootveld M, Lynch E. Oxidative Modification of Salivary Biomolecules with Ozone. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 593; 2002.	2002	16	0.60 ml saliva	20 seconds	Analysis of saliva by high field 1H-NMR spectroscopy to detect oxidation of biomolecules by O3.	After complete oxidative consumption of salivary pyruvate and partial oxidation of methionine, results acquired revealed (1) marked elevations in the concentration of formate (a product derived from the oxidation of carbohydrates), (2) substantial reductions in lactate level via its oxidation to pyruvate, which in turn is oxidised to acetate and CO2, (3) the oxidation of 3-D-hydroxybutyrate to acetoacetate, which liberates acetone on decomposition, (4) the generation of allantoin from salivary urate, (5) the production of low-molecular-mass saccharide fragments from hyaluronate, and (6) oxidation of malodorous trimethylamine to its corresponding, non-malodorous N-oxide.	None	None
Ozone effect on dental plaque									
14.	Shargawi JM, Theaker ED, Drucker DB, MacFarlane T, Duxbury AJ. Sensitivity of Candida albicans to negative air ion streams. J Appl Microbiol, 87: 889–897; 1999.	1999			O_3 in Negative air ions (NAIs) at different emitter distances, exposure times, relative humidities & under aerobic & oxygen-free conditions		O_3 levels increased with increasing exposure times ($P<0.01$) but were significantly reduced as emitter distance increased ($P<0.01$). When utilized in a nonventilated room, levels of O_3 produced did not exceed recognized safety limits.		

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
15.	Grootveld M, Lynch E. Mills B, Smith C, Baysan A, Silwood C. Therapeutic Oxidation Of Human Plaque Biomolecules by an Anti-Bacterial Ozone-Generating Device. BSDR Abstract no. 292; 2001.	2001	12		10 seconds	Analysis of plaque by high field 1H –NMR spectroscopy to detect oxidation of biomolecules by O3	O3 treatment gave rise to the oxidative decarboxylation of the electron donor pyruvate (generating acetate and CO2 as products), and the oxidation of the volatile sulphur compound precursor methionine to its corresponding sulphoxide. Moreover, evidence for the O3-mediated oxidation of 3-D-hydroxybutyrate was also obtained	n/a	None
16.	Grootveld M., Baysan A, Silwood C, Lynch E. Oxidation Of Human Plaque Biomolecules by an Anti-Bacterial Ozone-Generating Device. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 595; 2002	2002	12		10 seconds	Analysis of plaque by high field 1H –NMR spectroscopy to detect oxidation of biomolecules by O3	O3 treatment gave rise to the oxidative decarboxylation of the electron donor pyruvate (generating acetate and CO2 as products), and the oxidation of the volatile sulphur compound precursor methionine to its corresponding sulphoxide. Moreover, evidence for the O3-mediated oxidation of 3-D-hydroxybutyrate was also obtained	None	
17.	Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of Ozone on survival & permeability of oral microorganisms. Oral Microbiol Immunol, 19: 240–246; 2004.	2004			ozonated water (4 mg/l) for 10 s	Streptococcus mutans, And dental plaque from human subjects	1. breakage S. mutans seen by electron microscopy. 2. Ozonated water inhibited the accumulation of experimental dental plaque in vitro. 3. Human dental plaque exposed to ozonated water in vitro had no viable bacterial cells detected.	None	
Assessment of Anxiety & Fear with Ozone Treatment v Traditional Dental Therapy									
18.	Al Shorman H, Abu-Naba'a L, Lynch E. Patient's Attitude to Treatment of Pit & Fissure Caries with Ozone. Caries Res, 36: 187; 2002.	2002	49	n/a	n/a	Pit & Fissure Caries	98% happy with treatment, 94% happy with time, 94% would choose O_3 even if higher fee, 94% would recommend it for a friend or relative. 100% would choose O_3 treatment again, & 100% not anxious after O_3 treatment	same visit	None
19.	Domingo H, Smith C, Freeman R, Lynch E. Patients attitudes to managing caries with Ozone. J Dent Res, 81: A-183; 2002	2002	99	n/a	n/a	Patients had drilling & fillings previously & now O_3 treatment by the same dentist	happy or satisfied with 1. O_3 treatment 99% 2. time 97% 3. if cost was more than conventional treatment 95% 4. Recommend to a friend/ Relative 97% 5.Receive treatment once more 100% 6. Patients reported a reduction in anxiety associated with the O_3 treatment ($p<0.05$).		

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
20.	Megighian GD, De Pieri A, Lynch E. Patients attitudes to managing caries with ozone in private practice The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 167; 2002	2002	50	n/a		Ozone vs previous conventional drill and fill	100% were happy or satisfied with the Ozone treatment, amount of time the Ozone treatment required and not anxious after the Ozone treatment and reported less anxiety after, compared with before, the Ozone treatment and would recommend it to a friend or close relative and receive it once more . 96% were satisfied to choose this treatment even if the Ozone treatment cost more than regular treatment, 80% of patients reported a reduction in anxiety ($p<0.05$).	immediate	None
21.	Domingo H, Abu-Naba'a L, Al Shorman H, Smith C, Freeman R, Lynch E. Patients attitudes to managing caries with Ozone. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no 435; 2002 & J Dent Res, 82: C-535; 2003	2003	99	n/a	n/a	Patients had drilling & fillings previously & now O_3 treatment by the same dentist	happy or satisfied with 1. O_3 treatment 99% 2. time 97% 3. if cost was more than conventional treatment 95% 4. Recommend to a friend/ Relative 97% Receive treatment once more 100% 5. Patients reported a reduction in anxiety associated with the O_3 treatment ($p<0.05$).		
Comparison of Caries Detection Techniques									
22.	Abu-Naba'a L, Al Shorman H, Lynch E. The Effect of Ozone Application on Fissure Caries QLF Readings. J Dent Res, 81: A-386; 2002	2002	0	242	10 & 20 Seconds	Primary Pit & Fissure Carious Lesion on freshly extracted teeth	DIAGNOdent readings correlated with histological exam, Airpolishing system improved the performance of visual & DIAGNOdent scores, 10s O_3 reduced DIAGNOdent readings immediately & more reduction was by 20s	n/a	None
23.	Abu-Naba'a L, Al Shorman H, Lynch E. In-vivo treatment of occlusal caries with Ozone: Immediate effect & correlation of diagnostic tools. Caries Res, 36: 189; 2002.	2002	58	236	10 seconds	Primary Occlusal Pit & Fissure Caries	ECM readings are inverslay related to caries severity. Clinical severity scores, ECM & DIAGNOdent readings correlate significantly at baseline. 56% of lesions had immediate DIAGNOdent readings reduction after O_3. ECM readings have not immediately changed after O_3 treatment	n/a	none
24.	Megighian GD, Bertolini L. In vivo Treatment of Occlusal Caries with Ozone: One & Two Months' Effect with Light-induced Fluorescence (QLF) as Diagnostic Methods. J Dent Res, 82: B-354; 2003.	2003	80	300	20,30 & 40 seconds	Primary Occlusal Fissure Carious Lesions	A significant overall reduction of QLF readings was produced after Ozone treatment at one month. The percentage of teeth which produced this reduction was over 80%. After two months QLF readings showing reduction was over 90% ($p<0.05$). Ozone treated lesions significantly clinically reversed ($P<0.05$) whilst control lesions did not change.	2 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
25.	Marashdeh MM, Abu-Salem OT, Lynch E. Ozone Treatment of Occlusal Caries in Primary Teeth: Immediate Effects and Correlation of Diagnostic Methods AADR Abstract no. 683; 2003.	2003	17	50	10 seconds	Occlusal Carious Lesions	Using a paired samples T-test; ECM readings were not altered immediately by the Ozone treatment ($p>0.05$). However, the DIAGNOdent readings were significantly reduced after Ozone treatment ($t=2.408$, $p<0.05$). Moreover, ECM and DIAGNOdent readings were significantly correlated with the clinical classification ($p<0.05$, $p< 0.01$ respectively).	immediate	None
26.	Marashdeh MM, Abu-Salem OT, Lynch E. Ozone Treatment of Occlusal Caries in Primary Teeth: Immediate Effects & Correlation of Diagnostic Methods. IADR Abstract 2003.	2003	17	50	10 Seconds	Occlusal Caries Primary Teeth	Reduced DIAGNOdent Readings after O_3 treatment	n/a	None
27.	Abu-Naba'a L, Al Shorman H, Lynch E. Immediate Effect of Ozone Application In-vivo on DIAGNOdent Readings. IADR Abstract no. 3469; 2004.	2004	90	394	10 Seconds	Primary Occlusal Pit & Fissure Caries	O_3 reduced Diagnodent vales ($p<0.05$), ECM DIAGNOdent, visual score correlated with each other at baseline+K31	n/a	None
28.	Dahnhart JE, Jaeggi T, Scheidegger N, Kellerhoff N, Francescut P, Lussi A. Treating Caries in Anxious Children with Ozone: Parents' Attitudes after the First Session. J Dent Res, 82: B-265; 2003	2003	20	n/a	n/a	O_3+ART	75% afraid of dental care before O_3 treatment, 75% would recommend to relative/friend. 80% willing to pay more vs. drill & fill	n/a	None
29.	Johnson N, Johnson J, Domingo H, Lynch E. Comparison of Conventional Treatment vs. Ozone for Occlusal Caries with Ozone Therapy. J Dent Res, 82; B-2755, 354 2003.	2003	40	n/a	n/a	Ozone and conventional drill and fill	The conventionally treated (i.e. 'drill and fill') patients averaged 35 minutes per patient with a SD of 10 minutes whilst the group treated by therapeutic ozone averaged 8 minutes with a SD of 2 ($P<0.05$). The actual time taken in the application of ozone was less than one minute per tooth.	immediate	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
30.	Johnson N, Johnson J, Johnson K, Abu-Naba'a L, Al Shorman H, Freeman R, Lynch E. Patients' Attitudes to Dental Treatment Using Ozone vs. Conventional Treatment. J. Dent. Res, 82: A-679; 2003	2003	100	n/a	n/a	O_3 treatment vs drill & fill	83% were anxious about teeth being drilled and 80% were nervous about local analgesia. Having received a verbal explanation of the ozone process, only 33% remained slightly nervous prior to the procedure; the reminder having no anxiety. Immediately following ozone treatment, 100% were feeling no anxiety and were very satisfied with the treatment. 100% were very satisfied with the time taken for ozone treatment. 95% would recommend this treatment to family/ friends, whilst 80% would be happy to pay more for ozone treatment than conventional 'drill and fill'.	n/a	None
31.	Megighian GD, Dal Vera MV. Patients' Attitudes toward and Satisfaction with Managing Caries with Ozone as a Routine Treatment in Dental Private Practice J Dent Res, 82; B-269; 2003.	2003	250	n/a	n/a	O_3 treatment vs drill & fill	100% were happy or satisfied with the Ozone treatment, amount of time, would recommend this treatment to a friend or close relative and receive it once more. 85% considered the check up appointments to monitor the progression of clinical reversal of lesions a minor draw back. 55% were satisfied pay more than regular treatment, 80% of patients reported a reduction in anxiety ($p < 0.05$). In patients who received conventional but did not need ozone treatment, 100% were happy or satisfied with the treatment received, but 65% reported anxiety before and after the treatment.	immediate	None
32.	Domingo H, Abu-Naba'a L, Al Shorman H, Holmes J, Marshdeh MM, Abu-Salem AT, Smith C, Freeman R, Lynch E. Reducing Barriers to Care in Patients Managed with Ozone. AADR abstract no. 677; 2003.	2003	377	n/a	20 Seconds	All Lesions	99% happy with O_3 treatment, 97% happy with the time taken, 100% would like O_3 treatment agaian, 99% were not anxious after O_3 treatment	3 months	None
33.	Domingo H, Abu-Naba'a L, Al Shorman H, Holmes J, Marshdeh MM, Abu-Salem AT, Freeman R, Lynch E. Reducing Barriers to Care in Patients Managed with Ozone., IADR abstract no. 3473; 2004.	2004	20	n/a	n/a	n/a	83% – 99% Caries Reversal	n/a	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
34.	Domingo H. Steier L. Steier G. , Freeman R. & Lynch E. Patients Attitudes To Managing Caries With Ozone. IADR Abstract 2005.	2005	98	N/a	N/a	Caries & had a previous experience with conventional filling	100% satisfied with the O_3 treatment 100% satisfied with the amount of time the O_3 treatment required. 66% were satisfied to choose this treatment even if it cost more than regular conventional 100% would recommend it to a friend, receive this treatment again, not anxious. 86% of patients reported a reduction in anxiety ($p<0.05$).	Same visit	None
Treatment of Primary Root Carious lesions									
35.	Baysan A. Management of Primary Root Caries using Ozone Therapies. PhD Thesis, University of London, 2002.								
36.	Baysan A, Whiley R, Lynch E. Ozone effect on microflora from primary root caries ex-vivo. J Dent Res, 77: 1213; 1998.	1998		20	10 seconds in water	Root caries	O_3 reduced CFU from (log10 5. 91±0.15) ($p<0.001$) to (log10 3.57±0.37)	N/a	None
37.	Baysan A, Whiley R, Lynch E. The effect of a novel anti-bacterial Ozone-generating device on microflora from primary root caries ex-vivo. Caries Res, 32: 300; 1998.	1998	20	20	20 seconds ozonized water	Root caries	ozonised water group (log10 3.77±0.42) compared with the control group (log10 6.18±0.21) ($p<0.001$).		
38.	Baysan A, Whiley R, Lynch E The effect of Ozone on Streptoccoccus mutans in-vitro. Caries Res, 33: 291; 1998.	1998			10 seconds	*S. mutans* (NCTC 10449)	($p<0.0001$) difference (Mean±SE) between the control samples (log10 3.93±0.07) & O_3 treated samples (log10 1.01±0.27).		
39.	Baysan A, Whiley R, Lynch E. The effect of Ozone on Streptoccoccus sobrinus in-vitro. J Dent Res, 78: 1047; 1999.	1999			10 seconds	*S. sobrinus* (TH 21)	control samples (log10 4.61±0.13) & O_3 treated samples (log10 1.09±0.36).		
40.	Baysan A, Whiley R, Lynch E. Anti-microbial effects of a novel Ozone generating device on micro-organisms associated with primary root carious lesions in-vitro. Caries Res, 34: 498–501; 2000.	2000		40	10 or 20	Soft root caries	10 seconds O_3 Reduced CFU from (log(10) 5.91±0.15 to (log(10) 3.57±0.37) or 20-second from (log(10) 6.18±0.21 to log(10) 3.77±0.42) O_3 Invitro S. mutans (log(10) 1.01±0.27) & S. sobrinus (log(10) 1.09±0.36) compared with the control samples (log(10) 3.93±0.07 & log(10) 4.61±0.13, respectively).	None	
41.	Baysan A & Lynch E. Treatment of Primary Root Caious Lesions using Ozone for either 10 or 20 Seconds In Vivo IADR Abstract 2001.	2001	26	70	10 or 20 Seconds	Sec-Root Caries	10 seconds reduced CFU's from 7.0 to 4.35 Log10. 20 seconds reduced 6.0 to 0.46 Log10	n/a	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
42.	Baysan A, Lynch E. Management of root caries using Ozone in-vivo. J Dent Res, 80: 37; 2001.	2001	48	48	10 or 20	Root caries	Reduction (mean±SE) 10 s O_3 (log10 3.36±0.48) or 20 s (log10 1.17±0.62) compared with the control samples (log10 6.73±0.27) & (log10 6.30±0.28)	Same visit	None
43.	Baysan A, Lynch E. Clinical reversal of root caries using Ozone. J Dent Res, 81: A-343; 2002.	2002	80	214	10 Seconds	Root Caries	30.9% of PRCL reversed from severity index 2 to 0 (i.e., hard) in the ozone group, whilst none of the lesions reversed in the control group ($p<0.001$) and 34% of lesions reversed from severity index 2 to 1 in the ozone group compared to only 7.5% in the control group ($p<0.001$). Modified USPHS criteria revealed 61.5% of intact sealants in the ozone and sealant group, whilst 38.5% of intact sealants in the sealant only group ($p<0.05$). The ECM and DIAGNOdent readings showed improvements in the ozone group compared to the control group ($p<0.001$). The ozone and sealant group also had greater improvements in the ECM and DIAGNOdent values compared to the sealant only group ($p<0.05$).	3 months	None
44.	Baysan A, Lynch E. Management of Primary Root Caries using Ozone The First Pan European Festival of Oral Sciences, Abstract no. 195; Cardiff, UK. 2002	2002	79	220	10 seconds	Root caries	45% PRCLs reversed from severity index 2 to 0 (i. e. , hard) in the O_3 only group, 0% in the control group ($p<0.001$) 51% of lesions reversed from severity index 2 to 1 in the O_3 group, 8% in the control group ($p<0.001$). 6 & 9 months, the ECM & DIAGNOdent readings improved in the O_3 only group when compared to the control group ($p<0.001$	9 months	None
45.	Baysan A, Lynch E. 12-month Assessment of Ozone on Root Caries J Dent Res, 82: B-311; 2003.	2003	79	220	10 seconds	Root caries	47% of PRCLs reversed from severity index 1 to 0 (i.e., hard) in the ozone only group, whilst none of the lesions became hard in the control group ($p<0.001$) and 52% of lesions reversed from severity index 2 to 1 in the ozone group compared to only 11.6% in the control group ($p<0.001$) After 1, 3, 6, 9 and 12 months, the ECM and DIAGNOdent readings showed improvements in the ozone only group when compared to the control group ($p<0.001$).	12 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
46.	Holmes J. Clinical reversal of root caries using Ozone, double-blind, randomised, controlled 18-month trial. Gerodontology 2003: 20 (2): 106–14	2003	89	178	40 seconds	Root caries	At 18 months, 87 (100%) of O3-treated PRCL's had arrested, whilst in the control group, 32 lesions (37%) of the PRCL's had worsened from leathery to soft ($p<0.01$), 54 (62%) PRCL's remained leathery & only one of the control PRCL's had reversed ($p<0.01$).	18 months	None
47.	Holmes J. Ozone Treatment of Root Caries after 18-Months IADR Abstract no. 2881; 2004.	2004	89	178	40 seconds	Root caries	After 18 months, 81 patients completed the study. There were no observed adverse events. At 18 months, 100% of ozone treated PRCL's had reversed, whilst only 8% of the control lesions reversed ($p<0.001$). At 18 months, in the control group, 12% of the PRCL's had progressed from severity index 2 to 3 ($p<0.001$); i.e. they had become more severe.	18 months	None
48.	Holmes J. Ozone Treatment of Root Caries after 21-Months. IADR Abstract no. 117; 2004.	2004	89	178	40 Seconds and	Root Caries also treated by Remineralising pastes, mouthrinses and sprays were also dispensed	After 21 months, 81 patients completed the study. There were no observed adverse events. At 21 months, 100% of ozone treated PRCL's had reversed, whilst only 8% of the control lesions reversed ($p<0.001$). At 21 months, in the control group,12% of the PRCL's had progressed from severity index 2 to 3 ($p<0.001$); i.e. they had become more severe.	21 months	None
49.	Baysan A, Lynch E. Clinical Assessment of Ozone on Root Caries. IADR Abstract no. 80; 2004.	2004	80	226	20 Seconds	Root Caries	47% of PRCLs reversed from severity index 1 to 0 (i.e., hard) in the ozone only group, whilst none of the lesions became hard in the control group ($p<0.001$) and 52% of lesions reversed from severity index 2 to 1 in the ozone group compared to only 11.6% in the control group ($p<0.001$). Modified USPHS criteria revealed that there were 61% of intact sealants in the ozone and sealant group and 26.1% of intact sealants in the sealant only group ($p<0.05$). After 1, 3, 6, 9 and 12 months, the ECM and DIAGNOdent readings showed improvements in the ozone only group when compared to the control group ($p<0.001$). The ozone and sealant group also had greater improvements in the ECM and DIAGNOdent values when compared to the sealant only group ($p<0.05$).	12 months	None

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Table 1: Cont.

Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
Treatment of Pit & Fissure Carious Lesions								
50. Abu-Naba,a Management of Primary Occlusal Pit and Fissure Caries Using Ozone. PhD Thesis, Queens University Belfast, 2004.								
51. Reaney D. Management of Occlusal Caries Using Ozone. M Clin Dent , London University, 2003.								
52. Abu-Naba'a L, Al Shorman H, Lynch E. Ozone Efficacy in the Treatment of Pit & Fissure Caries. AADR abstract no. 2002.	2002	47	210	10 Seconds	Primary Pit & Fissure Carious Lesion	Lesions were divided by severity, 10s O_3 significantly remineralised lesions with DIAGNOdent readings below 40 at baseline	3 months	None
53. Megighiam GD, Bertolini L, De Pieri A., Lynch E. In-Vivo Treatment of Occlusal Caries with Ozone. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 434; 2002 And J Dent Res, 82: C-535; 2003	2002	60	200	10 seconds	Primary Occlusal Fissure Carious Lesions	DIAGNOdent readings correlated significantly with the clinical classification. A significant overall reduction of DIAGNOdent readings was produced immediately after Ozone treatment. The percentage of teeth which produced this reduction was over 50%. After one month DIAGNOdent readings showing reduction was over 90%.	1 month	None
54. Holmes J. Clinical Reversal of Occlusal Pit & Fissure Caries Using Ozone The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 431; 2002 and J Dent Res, 82: C-535; 2003	2003	193	579	10, 20, 30 or 40 seconds	Primary Pit & Fissure Carious Lesion	99% of the O_3 treated primary occlusal fissure carious lesions had clinically reversed based on the DIAGNOdent readings ($P<0.001$). The control primary occlusal fissure carious lesions, which had not received any ozone treatment, did not significantly change	4 months	None
55. Abu-Naba'a L, Al Shorman H, Stevenson M, Lynch E. Ozone Treatment of Pit & Fissure Caries: 6-month Results. AADR Abstract no. 765; 2003.	2003	78	240	10 Seconds	Primary Pit & Fissure Carious Lesion	mean ECM change was 1. 5 times better than baseline for the treatment group while for the control it was −1. 1 times deterioration of lesions. These improvements were regardless of tooth number, position, lesion's type or severity.	6 months	None
56. Abu-Naba'a L, Al Shorman H, Lynch E. Ozone Treatment of Primary Occlusal Pit & Fissure Caries (POPFC): 12-Months Clinical Severity Changes. J Caries Res, 37: 272; 2003.	2003	90	258	10 Seconds	Primary Pit & Fissure Carious Lesion	10% more lesions had their clinical scores reduced after O_3 treatment than control lesions, 5% more of teeth had clinical severity scores increase in the control group. A detailed clinical criteria should be used for monitoring O_3 treated lesions	12 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
57.	Holmes J, Lynch E. Arresting Occlusal Fissure Caries Using Ozone. AADR Abstract no. 678; 2003.	2003	278	1275	10, 20, 30 or 40 Seconds	Primary Pit & Fissure Carious Lesion	93% of the ozone treated primary occlusal fissure carious lesions had clinically reversed based on the reversal of the clinical severity index to lesions which were deemed to be reversing or to have reversed, whilst the control lesions were deemed not to have clinically changed ($P<0.01$). In addition, this clinical reversal was supported by DIAGNOdent readings, which showed a significant reduction in the test lesions compared to the control lesions ($P<0.001$).	2 months	None
58.	Abu-Naba'a L, Al Shorman H, Lynch E. Ozone Treatment of Primary Occlusal Pit & Fissure Caries: 12-month ECM results & Cinical implications. Caries Res, 37: 272; 2003.	2003	90	258	10 seconds	Primary Pit & Fissure Carious Lesion	treatment group's ECM change ranged from 0. 02 to 0. 62 (average 0.30 ± 0.009) & Control ranged from 0.07 to -0.27 (-0.13 ± 0.009). Difference between groups ranged from 0.22 to 0.56 ($p<0.05$ at 4 recalls). ECM showed significa+K56ntly a better change for the treatment group from the first recall at one month	12 Months	None
59.	Reaney D, Lynch E. Clinical Reversal of Pit & Fissure Caries After Using Ozone. AADR Abstract no. 674; 2003.	2003	22	78	30 Seconds	Primary Pit & Fissure Carious Lesion	74. 4% O_3 treated teeth clinically reversed 100% stable or reversed. 82% control lesions got worse	1 Month	None
60.	Daly T, Lynch E. Reversal of Occlusal Pit & Fissure Caries by Ozone. AADR Abstract n. 682; 2003.	2003	58	58	30 Seconds	Primary Pit & Fissure Carious Lesion	18 of the ozone treated primary pit and fissure carious lesions had clinically reversed based on the clinical measurement of lesion severity whilst 7 other lesions remained stable and 4 became worse ($P<0.05$). The control lesions did not significantly change clinically.	10 Weeks	None
61.	Stinson P, Abu-Naba'a L, Al Shorman H, Lynch E. Clinical Reversal of Occlusal Pit & Fissure Caries after Using Ozone AADR abstract no. 681; 2003 & J Dent Res 82: B-355 2003	2003	98	279	30 Seconds	Primary Pit & Fissure Carious Lesion	To date, 32 test subjects with 69 test lesions have attended the recall visit. There were no observed adverse events. 58 of the ozone treated primary pit and fissure carious lesions had clinically reversed based on the clinical measurement of lesion severity whilst the other 11 test lesions remained stable or progressed ($P<0.05$). The control lesions did not significantly change clinically.	3 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
62.	Holmes J. Clinical Reversal of Occlusal Pit & Fissure Caries Using Ozone. J Dent Res, 82: B- 354; 2003	2003	376	2364	10,20,30, 40 seconds	orPrimary Occlusal Fissure Carious Lesions	315 patients had been recalled for re-evaluation. There were no observed adverse events. 99% of the ozone treated primary occlusal fissure carious lesions (1918 lesions) had clinically reversed and this correlated with the improvement in the DIAGNOdent readings ($P<0.01$). The control carious lesions, which had not received any ozone treatment, did not significantly change in the study period.	12 months	None
63.	Jackson P, Lynch E. Healing of Pit & Fissure Caries after Using Ozone. AADR Abstract no. 1174; 2003.	2003	78	139	30 Seconds	Primary Occlusal Fissure Carious Lesions	After 3 months, 36 test subjects with 88 lesions, and 16 control subjects with 33 lesions reattended for re-evaluation. There were no observed adverse events. 70 of the ozone treated lesions had reversed based on the DIAGNOdent, which has been shown to correlate with clinical severity of primary fissure caries. 9 of the Ozone treated lesions maintained a stable DIAGNOdent reading and a further 9 had an increased DIAGNOdent reading ($P<0.05$). The DIAGNOdent readings for the control lesions did not significantly change.	3 months	None
64.	Cronshaw MA Treatment of Primary Occlusal Pit and Fissure Caries with Ozone: Six-month Results IADR Abstract no. 2750; 2003.	2003	18	49	30 Seconds	Primary Occlusal Fissure Carious Lesions	Of the 31 teeth treated, 25 showed improvements in DIAGNOdent measurements whilst 6 remained the same ($P<0.05$). 25 out of the 31 Ozone treated lesions had clinically reversed ($P<0.05$). In this test group there was an average reduction in Diagnodent readings of 49% ($P<0.05$). The control pit and fissure lesions, which had not received any ozone treatment, did not significantly change clinically and had deterioration in their DIAGNOdent readings.	6 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
65.	Johnson N, Johnson J, Johnson K, Lynch E. Effective Treatment of Occlusal Fissure Caries Using Ozone AADR Abstract no. 676; 2003.	2003	35	90	20 Seconds	Primary Occlusal Fissure Carious Lesions	After 1 month, 35 patients (90 lesions) were recalled for re-evaluation. There were no observed adverse events. Based on the clinical measurement of lesion severity, 59% of the ozone treated lesions showed visible signs of reversal, whilst 41% had remained stable ($P<0.05$). 100% of lesions had been stabilised with no progression. When measured using the DIAGNOdent, 79% of the ozone treated had reversed and 18% remained stable. The control primary occlusal fissure carious lesions, which had not received any ozone treatment, did not significantly change clinically	1 month	None
66.	Johnson N, Johnson J, Johnson K, Lynch E. Effective Treatment of Occlusal Fissure Caries Using Ozone. J Dent Res, 82: B-354; 2003.	2003	105	300	20 Seconds	Occlusal Fissure Caries	Based on the clinical measurement of lesion severity, the ozone treated lesions showed significant signs of reversal ($P<0.05$). When measured using the DIAGNOdent, 81% of the ozone treated primary occlusal fissure carious lesions had reversed and 17% remained stable. The control primary occlusal fissure carious lesions, which had not received any ozone treatment, did not significantly change clinically.	1 month	None
67.	Abu-Naba'a L, Al Shorman H, Lynch E. Clinical Indices Changes after-Treatment of Pit & Fissure Caries (PFC). AADR Abstract no. 1173; 2003.	2003	8	34	40 seconds	Primary Occlusal Fissure Carious Lesions	since one month, lesions treated with O_3 lose frostiness are judged as lesions that require more conservative treatment needs. Stains on the surface or undermining enamel of fissures didn't reduce in length	3 months	None
68.	Abu-Naba'a L, Al Shorman H, Lynch E. 6-month Clinical Indices Changes after Ozone Treatment of Pit & Fissure Caries (PFC). J Dent Res, 82: B-135; 2003.	2003	8	34	40 seconds	Primary Occlusal Fissure Carious Lesions	At six months, lesions treated with O3 lose frostiness, became smooth judged as more arrested lesions that require more conservative treatment needs. There was a trend for lesions to become darker & the stains on the surface or undermining enamel of fissures didn't reduce in length	6 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
69.	Morrison R, Lynch E. Efficacy of Ozone to Reverse Occlusal Caries J Dent Res: 82, B-354; 2003	2003	145	240	40 seconds	Primary Occlusal Fissure Carious Lesions	123 of the ozone treated primary pit and fissure carious lesions had clinically reversed based on the clinical measurement of lesion severity whilst the other 18 lesions remained stable and none became worse ($P<0.05$). The control lesions did not significantly change clinically	13-weeks	None
70.	Morrison R, Lynch E. Remineralization of Occlusal Pit & Fissure Caries After Using Ozone. AADR Abstract no. 680; 2003.	2003	108	186	40 Seconds	Primary Occlusal Fissure Carious Lesions	80.5% lesion reversal, 100% stability, no progression in O_3 treated group	13 weeks	None
71.	Huth KC, Paschos E, &. Hickel R. The Effect of Ozone on Fissure Caries in Permanent Molars IADR Abstract no. 2466; 2004	2004	41	114	40 Seconds	Primary Occlusal Fissure Carious Lesions	Immediately after the treatment the test teeth showed a significant improvement of DD- and ECM-values compared to the control (Wilcoxon-Test, $p=0.001$ for DD and 0.008 for ECM). When selecting patients with deterioration of the DD-values of their control teeth over the 3-months-period, the positive effect of ozone on the DD-values compared to the controls became also significant after 1, 2 and 3 months (Wilcoxon-Test, $p<0.05$). It appears that these selected patients showed significant more caries experience in the past (dmfs-index), a significant higher caries prediction value (Dentoprog-value) and a significant worse oral hygiene than the non-selected patients.	3 months	None
72.	Hamid A. Clinical Reversal of Occlusal Pit & Fissure Caries Using Ozone. IADR Abstract no. 3470 2004.	2004	184	184	40 Seconds	Primary Occlusal Fissure Carious Lesions	There were no observed adverse events. 86.6% of the ozone treated primary pit and fissure carious lesions had clinically reversed based on the clinical measurement of lesion severity whilst the control lesions did not significantly change clinically. ($P<0.05$). The DIAGNOdent® values correlated with the clinical findings.	6 months	None
	Treatment of Primary/Deciduous Teeth								
73.	Abu-Salem OT. Management of Occlusal Caries in Primary Teeth Using Ozone. Mphil thesis, Queens University Belfast, 2004.								

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
74.	Abu-Salem OT, Marashdeh MM, Lynch E. Immediate Effect of Ozone on Occlusal Caries of Primary Teeth. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 432; 2002 and J Dent Res, 82: C-535; 2003	2002		12 in vitro	10 seconds	Occlusal Carious Lesions	DIAGNOdent and standard scale ECM readings correlated significantly with the clinical classification ($rs=0.675$, $p=0.016$ and $rs=-0.697$, $p=0.012$ respectively). Ozone application reduced the DIAGNOdent readings after 10s application ($t=2.891$, $p=0.015$), and after 20s ($t=5.313$, $p<0.001$).		
75.	Abu-Salem OT, Marashdeh MM, Lynch E. Ozone Efficacy in Treatment of Occlusal Caries in Primary Teeth. J Dent Res, 82: B-136; 2003	2003	16	42	10 Seconds	Occlusal Carious Lesions	At six months ECM readings were improved significantly ($p<0.05$) and DIAGNOdent readings also improved significantly ($p<0.05$) compared to the baseline readings. The carious lesions in the treatment group showed significant improvement in the texture and perceived treatment needs indices with no significant changes on Ekstrand clinical index, frosted enamel index and stained enamel length.	6 Months	None
76.	Abu-Salem OT, Marashdeh MM, Lynch E. Ozone Efficacy in Treatment of Occlusal Caries in Primary Teeth. AADR Abstract no. 685; 2003.	2003	17	50	10 Seconds	Occlusal Caries + Air Abrasion	ECM readings were increased significantly ($p<0.05$) and DIAGNOdent readings were reduced significantly ($p<0.01$) in the test lesions compared to the control lesions.	3 Months	None
	Combining Ozone Treatment with Other Preparation Systems								
77.	Clifford C. Successful Use of Airbrasion in Conjunction with Ozone Treatment. J Dent Res, 82: B-2747; 2003.	2003	37	48	40 Seconds	Approximal lesion requiring drilling and filling airabrasion and Ozone with GI filling	All lesions were successfully exposed and a seal established for the delivery of ozone. Clinically acceptable seals have been achieved around all restorations. The airabrasion and Ozone technique was significantly faster than conventional drilling and filling ($P<0.05$).	3 months	None
78.	Clifford C. Reversal of Caries Using Airbrasion & Ozone- Nine Month Results. IADR Abstract no. 2467; 2004.	2004	34	68	40 Seconds	requiring drilling and filling airabrasion and Ozone with GI filling	All Ozone treated restorations were symptom less throughout the 9 months. All 34 Fuji 7 restorations, removed after 3 months recall, showed hard 'caries' to exploration suggesting remineralisation was successful. All lesions were successfully exposed and a seal established for the delivery of ozone at baseline. The airbrasion and Ozone technique was significantly faster (lesions were exposed, ozonated and sealed in under 7 minutes) than conventional drilling and filling ($P<0.05$).	9 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
79.	Holmes J & Lynch E. Reversal of Occlusal Caries using Air Abrasion, Ozone, & Sealing. IADR Abstract no. 3468; 2004.	2004	38	76	40 Seconds	Occlusal Caries	Group 1; air abrasion+O_3 40 seconds+ a mineral wash, then glass ionomer.After 3 months, a posterior composite; Group 2; drill and fill by posterior composite.3 5 patients attended all recall visits. 6 Subjects receiving the posterior composite restoration at baseline complained of some post-operative sensitivity whilst no sensitivity was reported associated with any of the Ozone treated teeth ($P<0.05$). At 3 months all Ozone-treated dentine caries was hard and required no additional removal	3 months	None
80.	Holmes J. Restoration of ART & Ozone treated primary root carious lesions. J Dent Res, IADR Abstract 2004.	2004	60	120	20 Seconds	Primary Root Caries	ART & O_3 lead to no puplp exposures, retained full vitality & strength of tooth. Drill&Fill lead to pulp exposures & further RCT was required before review period.	6 months	None
81.	Domingo H, Holmes J. Reduction in treatment time with combined air abrasion & Ozone compared to traditional 'Drill & Fill'. J Dent Res, IADR abstract 2004.	2004	64	128	40 Seconds	Primary Root Caries	AA & O_3 was faster than Drill&Fill. AA was more profitable than D&F	6 months	None
Cost Benefits of Ozone Treatment									
82.	Johnson N, Johnson J, Lynch E. Cost Benefit Assessment of a Novel Ozone Delivery System vs. Conventional Treatment. AADR Abstract no. 684; 2003.	2003	48	n/a	n/a	Occlusal & Root Carious Lesions	Conventional treatments (local analgesia, drilling and filling) necessitated a minimum of 20 minutes of chair time. Ozone therapy involved a minimum of 3 minutes. Typical UK costs for providing treatment were considered and compared with the costs of using ozone therapy. The time and cost of conventional treatment far outweighed the ozone therapy ($P<0.05$).	n/a	None
83.	Domingo H, Holmes J. Reduction in treatment time with combined air abrasion & Ozone compared to traditional 'Drill & Fill' & cost comparison. IADR abstract 2004.	2004	64	128	40 Seconds	Primary Root Caries	AA & Ozone was faster than Drill & Fill. AA was more profitable than D&F	6 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
Ozone Treatment of Endodontic Pathologies									
84.	Schwan L, Bamfaste M. [Experiences with the use of chlorine gas and ozone in the treatment of root gangrene and dental granuloma]. Dtsch Zahnarztl Z, 6: 301–308; 1951.	1951	n/a	n/a	n/a	n/a	n/a	n/a	n/a
85.	Brunel A, Vannier R, Archinet F. [Sterilization of minute endodontic material by the combination of ethylene oxide and ozone. Experimental evaluation of its effectiveness]. Acta Stomatol Belg 62: 355–359; 1965.	1965	n/a	n/a	n/a	n/a	n/a	n/a	n/a
86.	Sandhaus S. [Ozone therapy in odontostomatology, especially in treatments of infected root canals]. Rev Belge Med Dent, 20: 633–646; 1965.	1965	n/a	n/a	n/a	n/a	n/a	n/a	n/a
87.	Haimovici A, Lacatusu S, Irjicianu A, Joan E. [Ozone in endodontic therapy]. Stomatologia (Bucur),; 17: 303–307; 1970.	1970	n/a	n/a	n/a	n/a	n/a	n/a	n/a
88.	Chang H, Fulton C, Lynch E. Antimicrobial Efficacy of Ozone on Enterococcus faecalis. J Dent Res, 82: B-220; 2003.	2003	n/a	n/a	60, 30, 20, 10, 0 seconds	108 solution E Faecalis	60=0 cfu's, 30=0 cfu's, 20=0 cfu's, 10=0 cfu's at 106 & 105 concentrations. Higher concentrations were markedly reduced	n/a	None
89.	Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endodontics, 778–781; 2004. Part 2	2004				Enterococcus faecalis & Streptcoccus mutans infections in vitro in bovine dentin.	When the specimen was irrigated with sonication, ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite (NaOCl). After irrigation with ozonated water, the viability of E. faecalis and S. mutans invading dentinal tubules significantly decreased.		

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
Use of ozone in treatment associated with Dental and maxillofacial surgery									
90.	Minguez F, Gomez-Lus ML, Andre J, Cabronero MJ, Prieto J. [Antimicrobial activity of ozonized water in determined experimental conditions]. Rev Sanid Hig Publica (Madr), 64: 415–423; 1990.	1990				Disinfection of Buccal flora and hand flors	Antimicrobial activity in ozonized water on bacterial suspensions and contaminated materials was meaningful and depended fundamentally on concentration and time of exposure. On buccal flora, one rinse alone had no effect, but various successive rinses led to substantial reductions in the number of colonies of bacteria. Ozone had a similar effect, although more pronounced, on the flora of the hands. Ozonized water placed in an open dish kept up antimicrobial activity for the first 20 minutes, but after 30 minutes this activity decreased substantially		
91.	Kiniapina ID, Durnovo EA. [The efficacy of using ozone in the combined treatment of disseminated odontogenic phlegmons of the maxillofacial area]. Stomatologiia (Mosk), Spec No: 60–6; 1996.	1996	n/a	n/a	n/a	n/a	n/a	n/a	n/a
92.	Lazutikov OV, Lunev BV. [The use of ozonized solutions in the combined treatment of odontogenic putrefactive-necrotic phlegmons of the maxillofacial area and neck]. Stomatologiia (Mosk), Spec No: 64–65; 1996.	1996	n/a	n/a	n/a	n/a	n/a	n/a	n/a
93.	Malanchuk VA, Gorshevikova EV, Kopchak AV. [Antimicrobial action of ozone in the treatment of mandibular fracture]. Klin Khir, 3: 43–46; 2000.	2000	n/a	n/a	n/a	n/a	n/a	n/a	n/a
94.	Korotkikh NG, Lazutikov OV, Dmitriev VV. [The effect of ozone on the microbiological characteristics of the oral fluid in patients with mandibular fractures]. Stomatologiia (Mosk), 79: 20–21; 2000.	2000	n/a	n/a	n/a	n/a	n/a	n/a	n/a
95.	Korzhachkina NB, Radzievskii SA, Olesova VN. [Preventive use of ozone, short waves, and laser therapy alone and in combination in early postoperative period after dental implantation]. Vopr Kurortol Fizioter Lech Fiz Kult,6: 17–19; 2002.	2002	n/a	n/a	n/a	n/a	n/a	n/a	n/a

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
Ozone treatment for Gingivitis and Periodontitis									
96.	Brauner A. [Clinical studies of therapeutic results from ozonized water for gingivitis and periodontitis]. Zahnarztl Prax 42: 48–50; 1991.	1991	n/a	n/a	n/a	n/a	n/a	n/a	n/a
OHManagement Software for Patient Management									
97.	Scholz V. OHManagement Software for quality management in an Ozone treatment practice. IADR Absrtact no. 715; 2004.	2004	n/a	n/a	n/a	10 clinics operating with new OHManagement Software, compared to no OHM	Patient recall attendence (53% v 44%) & compliance (84% v 75%) were better with the new OHM	n/a	None
Ozone Treatment of Dentinal sensitivities									
98.	Ciriello G. [Ozone and dentinal sensitivity.]. Riv Ital Stomatol, 10: 159–164; 1955.	1955	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Ozone treatment of Pulpal pathologies									
99.	Dechaume M. [The use of ozone in the local treatment of caries, pulpitis and periapical osteitis.]. Suom Hammaslaak Toim, 48: 61–66; 1952.	1952	n/a	n/a	n/a	n/a	n/a	n/a	n/a
1H NMR Studies on Tooth Whitening									
100.	Holmes J, Grootveld M, Smith C, Claxson AWD, Lynch E. Bleaching of Components Responsible for Extrinsic Tooth Discoloration by Ozone. AADR Abstract no. 615; 2003.	2003	n/a	n/a	5,10,15, seconds	20tooth stain removal (Melanoidins) Stains were generated via the reaction of L-lysine (1.25–250.0$\times 10^{-3}$ mol. dm^{-3}) with an equivalent concentration of alpha-D-glucose in phosphate buffer (pH 7.00) at 80°C for a period of 240 hr.	Substantial bleaching of melanoidins following treatment with O3 [e.g., at an O3 delivery level of 4.48 mmol, the decrease in absorbance at 366 nm was 90±4% (mean±s.e.) for the 1.25×10^{-3} mol.dm^{-3} reaction mixture, and 28±3% for that initially containing 250.0×10^{-3} mol.dm^{-3} reactants]. The extent of the bleaching process observed increased with increasing levels of O3 treatment The mechanism of this process may involve the ozonation of (>C=C<) bond systems which contribute to the chromophoric properties of such "browning products".	n/a	None
Effects of Ozone on Dental Materials									
101.	Murakami H, Sakuma S, Nakamura K, Ito Y, Hattori M, Asai A, Noguchi T, Maeda H, Kameyama Y, Kimura Y, Nagao T, Kawai T, Hasegawa J. Disinfection of removable dentures using Ozone. Dent Mater J, 15: 220–225; 1996.	1996			O_3 in water 10 ppm	Removable partial dentures	C. albicans decreased to about 1/10 after 30 min & to 1/10(3) after 60 min.		

To be continues next page

Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
102.	Oizumi M, Suzuki T, Uchida M, Furuya J, Okamoto Y. In vitro testing of a denture cleaning method using Ozone. J Med Dent Sci, 45: 135–139; 1998.	1998			700 mg/h gas for 1 & minutes ozonated water at ppm & 3 ppm	Streptococcus mutans 3(IID 973), Staphylococcus aureus (209-P), & Candida al-1bicans (LAM 14322).	direct exposure to gaseous O3 seems to be a more effective microbicide compared with ozonated water, & that gaseous O3 can be clinically useful for disinfection of dentures.	N/a	None
103.	Krozer A, Hall J, Ericsson I. Chemical treatment of machined titanium surfaces. An in vitro study. Clin Oral Implants Res, 10: 204–11; 1999.	1999			O_3 made by commercial mercury lamp in ambient air	Implant material rinsed with a disinfectant solution with amino-alcohol	1. Amino alcohols forms a stable & dense film in vitro. Which most likely prevents re-integration to occur at the implant-tissue interface in vivo 2 Rrinsing in water, saline solution, & 5% H2O2 did not remove the amino-alcohol from the surface. O_3 complete removal of the adsorbed amino-alcohol adherent film		
104.	Suzuki T, Oizumi M, Furuya J, Okamoto Y, Rosenstiel SF. Influence of Ozone on oxidation of dental alloys. Int J Prosthodont, 12: 179–83; 1999.	1999			O_3 gas 20 mg/h	dental alloys (Co-Cr, Au-Ag-Pt, & Au-Cu-Ag-Pd).	reflectance, surface roughness, & weight were measured O_3 caused a slight change in the Au-Cu-Ag-Pd alloy in terms of measured reflectance, but the changes were significantly less than those caused by acid-electrolyzed water & one of the commercial denture cleaners.	n/a	
105.	Zhao H, Zheng D, Hong L. The disinfection efficiency comparison of different treatments on dental impression & gypsum castsHua Xi Kou Qiang Yi Xue Za Zhi, 18: 332–335; 2000.	2000		Gypsum casts			O_3 treatment is an effective method in disinfecting the gypsum casts	n/a	
106.	Hussey D, Armstrong C,Lynch E. Bond strengths of composite to enamel /dentine treated with ozone. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 697; 2002.	2002	n/a	40 teeth enamel and dentine samples	10 Seconds	2.7min diameter tubes of composite resin (Esthet-X, Dentsply, &bonding agent (Prime and Bond NT, Dentsply)	The mean loads (Newtons) to debond the specimens were as follows: enamel without ozone 116.4 (sd 50. 1), enamel with ozone 128.6 (sd 49.4), dentine without ozone 54.7 (sd 23.6), dentine with ozone 51.6 (sd 15.6). Wilcoxon signed rank test revealed no statistically significant difference between the groups with and without the ozone treatment (Enamel $z=-1.05$, $p=0.29$, Dentine $z-0.15$, $p=0.88$).		
107.	Baysan A, Lynch E. Management of Primary Root Caries using Ozone The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no 195; 2002.	2002	79	220	10 seconds	Fissure sealant applied over Root caries	61% of intact sealants in the O_3 & sealant group & 42% of intact sealants in the sealant only group ($p<0.05$). After 3 6 & 9 months, O_3 & sealant group also had greater improvements in the ECM & DIAGNOdent values when compared to the sealant only group ($p<0.05$).	9 months	None

To be continues next page

Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
108.	Matsumura K, Ikumi K, Nakajima N, Peng C, Hyon SH, & Tsutsumi S, A Trial of Regeneration of Periodontal Ligament around Dental Implants J Dent Res, 81: A-101; 2002.	2002				titanium implant PDL cell taken from dog's	a. titanium implant was oxidized by 30%H2O2 b. poly(-ethylene-co-vinyl alcohol)EVA) was coated onto the implant & O_3 c. Third, the collagen sponge was immobilized around the implant by freeze-drying method.d.PDL cells were seeded onto the implant and then implanted into dog's alveolar bone. After 3 months of implantation, the implants and their surrounding tissues were removed. The specimens were stained with Masson Trichrome stain. From the results, the tissue around implant was observed to be similar to the one around natural tooth.		
109.	Murakami H, Mizuguchi M, Hattori M, Ito Y, Kawai T, Hasegawa J. Effect of denture cleaner using ozone against methicillin-resistant Staphylococcus aureus and E. coli T1 phage. Dent Mater J, 21: 53–60; 2002.	2002			10 ppm	Methicillin-resistant Staphylococcus aureus (MRSA) and T1 phag virus	bacteria was 3.1×10(3) CFU/mL at the beginning of the experiment, fell to 1.0×10(0) CFU/mL 10 min later, and was 1.0×10(0) CFU/mL or less afterwards. In contrast, when the ozone supply was cut off (air bubble only), the number of bacteria was 3.4×10(3) CFU/mL at the beginning of the experiment, and had fallen to 3.0×10(3) CFU/mL 60 min later (no statistically significant difference). In the virucidal activity test, the number of phages was 1.2×10(6) PFU/mL before ozone treatment, fell to about 1/10 of that number 10 min later, and was 6.1×10(0) PFU/mL 40 min later.		
110.	Campbell D, Hussey D, Cunningham L, Lynch E. Effect of Ozone on Surface Hardness of Restorative Materials. J Dent Res,82: B-262; 2003.	2003	n/a	18 x 3	10 Seconds	Restorative Materials	Statistical analysis using a 2-way ANOVA did not reveal any difference in surface hardness following the treatment with O_3 ($p>0.15$)	n/a	None
111.	Baysan A, Lynch E. 12-month Assessment of Ozone on Root Caries J Dent Res, 82; B-311; 2003.	2003	79	220	10 seconds	Fissure sealant applied over Root caries	Modified USPHS criteria revealed that there were 61% of intact sealants in the ozone and sealant group and 26.1% of intact sealants in the sealant only group ($p<0.05$). After 1, 3, 6, 9 and 12 months, the ozone and sealant group also had greater improvements in the ECM and DIAGNOdent values when compared to the sealant only group ($p<0.05$). Conclusions: Leathery root caries can be treated non-operatively with ozone	12 months	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
112.	Hiller Ka, Federlin M, Mackow A, Redlich M, And Schmalz G. Influence of ozone treatment on marginal adaptation of fissure sealing Continental NOF Divisions of the IADR Abstract no. 62; 2004.	2004		120 extracted human molars		Art + Group I: O3 40s, seal Tetric Flow, Helioseal Clear or Fuji VII Group II: restorations were placed immediately after preparation.	Before TC, the frequency of silver-staining was 26–50% (group I) and 23–50% (group II), TF revealing the lowest (26%) and FU the highest frequency (50%) (group I). After TC, the frequency of silver-staining was 8–46% (group I) and 10–50% (group II), TF revealing the lowest (8%/10%) and FU the highest frequency (46%/50%) in groups I and II. Before and after TC, microleakage was limited to enamel with TF and FU, whereas with HC silver-staining included enamel and dentin. The Error Rates Method revealed a significant influence of the sealing material upon the frequency of silver-staining but no difference was determined with respect to ozone treatment.	thermocycling 60 s/cycle), stored in saline at 37°C for one week.	
113.	Czarnecka B, Deegowska-Nosowicz P, Prylinski M, Limanowska-Shaw H. Bond strength of glass-ionomer's to dentine after Heal Ozone treatment Continental NOF Divisions of the IADR Abstract no. 63; 2004.	2004		60 extracted bovine teeth	n/a	group Ozone then conditioned with conditioner group B were treated with Ozone and Reductant then conditioned. Group C (controls) were conditioned.	samples of glass ionomer Fuji Fast (GC International-X) and 20 similar samples of Ketac Molar Aplicap (3M-ESPE, Seefeld, Germany-Y) were bonded to the prepared surfaces; Group A gave the lowest values of SBS in both cases though this was statistically significant ($p<0.05$) only for cement Y. There was no statistical difference between group B and the control group. Heal Ozone treatment alone thus has a tendency to weaken the SBS of glass-ionomers bonded to bovine enamel, but this is eliminated by the use of Ozone Reductant.		
114.	Abu-Naba'a L, Al Shorman H, Lynch E. 6-months Fissure Sealant Retention Over Ozone-treated Occlusal Caries. IADR Abstract no. 3472; 2004.	2004	53	132	10 Seconds	Fissure sealant applied over non-cavitated Occlusal Carious Lesions	At baseline, severity of lesions in both groups was similar($p>0.05$). At 6 months, there were no significant differences between the groups in terms of FS retention, marginal discolouration, FS colour and secondary caries at any of the recall visits ($p>0.05$).	6 months	None
115.	Steier L. , Lynch E. 15-months Sealant Retention Over Ozone- treated Occlusal Caries. J Dent Res, IADR Abstract 2005.	2005	73	146	40 seconds	Primary Occlusal Fissure Carious Lesions & flowable fissure sealant	O_3 treatment had not affected retention of flowable composite resin sealants occlusally in-vivo, nor marginal discoloration, FS color & secondary caries at any of the recall visits.	15	none

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
116.	Steier L , Steier G. Ozone & Sealant Treatment of Root Caries after 12-Months. J Dent Res, IADR Abstract 2005.	2005	62	124	40 seconds	flowable root sealant Primary Occlusal Fissure Carious Lesions and	60/61 arrested in treatment group, 24/61in control at one month. retention of sealants on these arrested lesions is very promising 2/55 Tt; 8/32 in control lost retention completely or partially	1 & 12 month.	
117.	Abu-Naba'a L, Al Shorman H, Lynch E. Fissure sealant retention over Ozone-treated occlusal pit & fissure caries: 12-months results. J Dent Res, IADR Abstract 2005.	2005	53	132	10 seconds	Primary Occlusal Fissure Carious Lesions & fissure sealant	O_3 treatment followed by immediate sealant placement was not detrimental to retention rate compaired to the control group. 0% secondery caries, 0% complete loss of fissure sealant retention after one year	12 months	none
118.	Abu-Naba'a L, Al Shorman H, Hayajneh R, Lynch E. Ozone effects on denture acrylic surface. J Dent Res, IADR Abstract 2005.	2005		40 samples	1 & 2 hours	Heat cured acrylic with a polished & glazed surface	O_3 didn't produce any change in the roughness of the surface of treated samples		
Treatment of Dental Unit Water Lines (DUWL)									
119.	Filippi A, Tilkes F, Beck EG, Kirschner H. [Water disinfection of dental treatment units using ozone]. Dtsch Zahnarztl Z, 46: 485–487; 1991.	1991				Dental unit	Under the precondition that the dental chair had been thoroughly sanitized, the system showed a good disinfecting effect. Finally, for reasons of practical medical treatment, the ozone concentration in air and, for reasons of hygiene in drinking water, the ozone concentrations in water were determined under various conditions. In addition, the influence of continuous-flow water heaters is discussed. The problem of continuous-flow water heaters regarding their effects on the colonisation of water by microbes proves not to be significant. The exposure of patients to disinfectants is discussed		
120.	Al Shorman, Coulter W, Lynch E Claxson AWD, Silwood CJL,Grootveld M. Use of Ozone to Treat Dental Unit Water Lines. BSDR Abstract no. 219; 2001.	2001	n/a	n/a	10 seconds	DUWL samples	the biomolecules in the DUW were acetate, proprionate, formate, the amino acid glycine, aromatic compounds & occasionally ethanol. O_3 treatment of the DUW gave rise to oxidation of ethanol & an increase in formate levels presumably due to oxidation of carbohydrate	none	

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
121.	Walker JT, Bradshaw DJ, Fulford MR, Martin MV, Marsh PD. Control of planktonic and biofilm contamination in a laboratory dental unit water system J Dent Res, 81: A-445; 2002.	2002			200mg/hour	Combizyme (1.25%) Tegodor (1%), Spor-Klenz (Neat), Dialox (Neat), Tegodor (1%), Bleach (0.5%), Ozone (200mg/hour), Chlorhexidine (0.2%), Ultrakleen (powder) and Betadene (10%) were tested overnight (16h).	Flushing did not reduce the viability or the extent of the biofilm on DUWS tubing. Only Combizyme did not completely reduce the total viable counts of planktonic or biofilms cells. However, the efficiency of biofilm removal was: Dialox > Bleach > Ultrakleen > Betadine > Spor-Klenz > Bio2000.		
122.	Cardon B, Eleazer P, Miller R, Staat R. Low concentration Ozone treatment insufficient to control DUWL biofilm. AADR Abstract no. 714; 2002.	2002	n/a	n/a	O_3 conc 05ppm	0.DUWL samples	The O3 concentrations at the handpiece during recirculation was 0.01 to 0.06 ppm; . Microbiological data indicated that water samples taken 30 or more minutes after the O3 treatment cycles all exceeded 10,000 CFU. After 10 weeks of O3treatment, microscopic analysis showed biofilm formation on the interior of all tubing sections.	10 weeks	None
123.	Al Shorman, Abu-Naba'a, Coulter W, Lynch E. Ozone efficacy in the treatment of Dental Unit Water Lines. J Dent Res, 81: B 299. 2002.	2002	2 units	dentaln/a	1 & 3 minutes/ unit/ days 1, 3, 4 & 7	DUWL samples after 2,flushing for 2 minutes	Reduction of bacteria 1000 fold on day 7 for the 1 min application. Sterile water on day 2 for the 3 min application, sterile water followed up till 5 weeks	5 weeks	None
124.	Al Shorman, Coulter W, Abu-Naba'a, Mohan G, Boyle C, Lynch E. Effect of Ozone on biofilms in Dental Unit Water Lines. AADR Abstract 2002.	2002	4 units	dentaln/a	1,2,3 minutes & 15 min	1,2,3 minutes application 2 min flushing, 15 min application & 15 min flushing	10 fold increase for 1 min, 10,000 fold decrease of bacterial counts for 2 & 3 min application times. Biofilm layer seen to be reduced at 15 min application time & 10 min flushing, but completely removed at 15 minutes application & flushing time	7 days for units 1,2 & 3	none
125.	Al Shorman, Abu-Naba'a, Coulter W, Lynch E. Ozone, An Effective Treatment For Dental Unit Water Lines. J Dent Res 81: A-112; 2002.	2002	1 units	dentaln/a	3 minutes, at 2100 ppm O_3, 615 ml/min	DUWL flushed for 2 minutes	O_3 treatment showed reduction from 5.2*103 CFU/ml to 300 CFU/ml after first application, then 0 CFU/ml at the second & subsequent (1000 fold reduction)	8 days	None
126.	Al Shorman, Abu-Naba'a, Coulter W, Lynch E. Ozone, An Effective Treatment For Dental Unit Water Lines. J Dent Res 81: A-112, 2002.	2002	1 units	dentaln/a	3 minutes, at 2100ppm O_3, 615 ml/min	DUWL flushed for 2 minutes	O_3 treatment showed reduction from 5.2*103 CFU/ml to 300 CFU/ml after first application, then 0 CFU/ml at the second & subsequent (1000 fold reduction)	8 days	None

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Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
127.	Smith C, Al Shorman H, Grootveld M, Silwood C, Lynch E, Mills B, Silwood C. Rapid Detection of Microbial-Derived Components in Dental Unit Water Lines by NMR Analysis. J Dent Res, 81: A-112, 2002.	2002	12 dental units			Multicomponent 1H NMR investigations of DUWLs	Results acquired revealed that many biomolecules were detectable in the samples examined, including a wide range of microbial fermentation products (MFPs). For example, the organic acid anions acetate, formate, lactate, propionate and succinate, and occasionally ethanol were present in the samples examined. Further components detectable included the amino acids alanine and glycine, and also a series of aromatic compounds. Treatment of DUWLs with the powerful microbicidal agent ozone gave rise to a substantial reduction in many of the MFPs detectable in samples collected 18 hr. after treatment ($p<0.01$).		
128.	Walker JT, Bradshaw DJ, Fulford MR, Marsh PD. Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. Appl Environ Microbiol, 69: 3327–32; 2003.	2003	A model in-vitro		O_3 was applied for 10 min	In-vitro model	Low concentration of O3 did not completely reduce the biofilm total bacterial count at this concentration nor reduce the percentage biofilm coverage	n/a	None
129.	Smith C, Al Shorman H, Abu-Naba'a L, Grootveld M, Silwood C and Lynch E Detection of Microbial-Derived Components in Dental Unit Water Lines using NMR. The First Pan European Festival of Oral Sciences, Cardiff, UK. Abstract no. 477; 2002 & J Dent Res, 82: C-542; 2003.	2003	16 dental units		10 seconds	Multicomponent 1H NMR investigations of DUWLs	signals presented in control samples by microbial-derived organic acid anionsused for chemotaxonomic 'markers' of, notably acetate, formate , lactate, propionate and succinate. Others are the amino acid glycine, a number of aromatic compounds and occasionally ethanol. O_3 caused reduction in many of the microbial fermentation products detectable in samples collected 18 hr. after treatment ($p<0.01$).	immediate	none

To be continues next page

Table 1: Cont.

	Study Title	Date	Subject number	Teeth	O_3 Time	Sample Type	% Success	Follow-up Period	Adverse Reactions
130.	Al Shorman, Abu-Naba'a, Coulter W, Lynch E. Primary Colonization of DUWL by P. aeruginosa & its Eradication by Ozone. J Dent Res, 82: B-284; 2003.	2003	n/a	n/a	5 minutes ozone then 10 minutes flushing	Dental Unit Water Lines treated either with continuous flu-H2O2 (Oxygenal, KaVo, Germany) or ozone	Oxygenal treatment continuously produced water with TVC of less than 100 CFU/mL. TVC of water from the control unit was 2.3×104 and 3.4×104 CFU/mL after 1 and 2 weeks of installation. The primary coloniser was identified (API 20 NE kit) as pure P.A. After the first O3 treatment the TVC was reduced to 60 CFU/mL and rose to 3.9×104 CFU/mL after a week with few Pseudomonas colonies. After two weeks, TVC was 2.8×103CFU/mL CFU/mL with no detected P.A. and became 0 CFU/mL after the treatment. Repeated sampling of the unit for 9 weeks showed no re-growth of P.A.	2 weeks	None
1H NMR Studies on Ozonated Oils									
131.	Lynch E, Grootveld M, Holmes J, Silwood CJ, Claxson AWD, Prinz J, Toms H. 1H NMR Analysis of Ozone-treated Grapeseed, Olive, & Sunflower Seed Oils. AADR Abstract no. 182; 2003.	2003	n/a	n/a	10 Minutes	Ozonated Oils Comparison	Treatment of each vegetable oil with O3 gave rise to the consumption of polyunsaturated fatty acids present (i.e. significant reductions in their mono- and bis-allylic-CH2 group resonances located at 2.06 and 2.76 ppm respectively, and also that of their vinylic protons at 5.38 ppm), consistent with their ozonation. Indeed, signals present in the 5.10–5.25 ppm regions of the ozonated GO and SO spectra are assignable to the ring protons of ozonides. Further O3-induced modifications to the oils included the production of aldehydes, i.e. -CH2CHO aldehydic group triplet resonances at 9.65 (ozonated GO and SO) and 9.74 ppm (all ozonated oils), terminal products arising from the decomposition of ozonides.	n/a	None
Other References									
132.	Baysan A, Lynch E, Grootveld M. The use of Ozone for the management of primary root carious lesions. Tissue Preservation & Caries Treatment. Quintessence Book 2001, Chapter 3, 49–67.	2001	50	100	10 Seconds	Primary Root Caries	None		

Table 2: General classification of reports included in the chapter

Analysis
Sources
National Library of medicine
Journal of Dental Research
IADR web site, Abstract search θOzoneö
Caries Research
Quintessence books
Thesis
Study types
In-vitro studies on extracted human and bovine teeth
In-vitro studies on controlled micro-organsim models
In-vitro studies using water line models
In-vivo studies on biomolecules
In-vivo studies on animal models
In-vivo studies performed on human subjects in controlled double blind clinical situations
In-vivo studies performed on patients attending general dental practices
Report Forms
Abstracts
Published papers
Published theses
Published book chapters
Researchers
University teachers
PhD and Master students
Full time researchers
General Dental Practitioners
Recognitions owed by studies
Aylin Baysan won First Prize at the 2001 International Association for Dental Research (IADR) meeting for her HealOzone research methodology.
Layla Abu-Naba'a won the prestigious "Basil Bibby" First Prize from the Cariology Group at the 2002 IADR Meeting for her research into the reversal of pit & fissure caries.
Julian Holmes won First Prize at the 2004 IADR Meeting for his HealOzone research into the reversal of root caries at 21 months follow-up.

Methods of application and safety

Ozone as a therapeutic agent is similar to many invaluable medicaments that are only effective if applied in the correct dose and for the validated procedures. The current literature addresses the potential of adverse events in the event of ozone exposure beyond the European Union (EU) and FDA's recommended levels. The only device used for treating dental caries in the published research is HealOzone. Other methods of ozone production were used within controlled in-vitro environments but the implications of operator and patient safety are greater. At the time of writing of this review HealOzone is the only approved device for intraoral applications. The main features which will improve its safety are as follows:

- The tight fitting design of the delivery device made by the cup and tooth should contain the ozone treatment.
- The dentist performing the treatment will be trained to prevent any large amounts of ozone escaping.
- The device operates by suction only, the pathway for ozone being under negative pressure. This means ozone should not leak out. In the event of a leak, only air should leak in–no ozone should leak out.
- If the seal is incomplete or if a leak arises, a flow sensor will shut down the ozone generator.
- After the delivery of ozone, automatic suction will remain on for an additional 10 seconds to purge away any remaining ozone.
- Ozone is stable for only a very brief time. It decomposes to form oxygen and hence disappears very quickly.
- A remineralizing solution applied in some of these studies contains xylitol, which is also a reductant.
- Saliva has strong anti-oxidant effect and will wash over treated lesions.

Another safety aspect of the treatment is consideration of the potential hazards of alternative treatments. Elimination of potentially toxic restorative materials as mercury in amalgam or uncured resins in all resin-containing fillings contributes to the salvage of the pulp from the irritants combined with invasive procedures as heat and trauma.

None of the clinical studies included reported on any adverse event. Study 1 found the intraoral concentration of ozone around the application cup (1–2 mm) is below the FDA and EU permissible levels in air (Baysan and Lynch, 2001d). Studies 2 and 3 used ozone dissolved in water in different concentrations. They found that ozonated water, not being isotonic, had no negative effect on periodontal cells (cementoblasts and fibroblasts) remaining on a freshly extracted tooth surface after irrigation for two minutes (Ebensberger et al, 2002). Mouse fibroblasts were also subjected to ozon-

ated water and their metabolic activity remained high when the cells were treated in contrast to what was found when they were rinsed with a 2.5% NaOCl solution (sodium hypochlorite, a widely used endodontic irrigant) (Nagayoshi et al, 2004). In implants treated with ozone there was regeneration of periodontal cells similar to that around natural teeth (Matsumura et al, 2002).These studies provide a good basis for claiming the safety of ozone applications on soft tissue, which has already been reported in many ealier studies treating gingivitis and periodontitis (Brauner, 1991).

The last study (Shargawi et al, 1999) used ozone in a gas form produced with variable emitter distances, exposure times, relative humidity and under aerobic and oxygen-free conditions. When utilized in a non-ventilated room, levels of ozone produced did not exceed recognized safety limits.

Mode of action of ozone

Microbicidal activity

Ozone's oxidative reactions renders it a very powerful biocide that destroys microorganisms such as bacteria by oxidizing bacterial cell walls and membranes and may destroy these microorganisms by rupturing their membranes (Yamayoshi and Tatsumi, 1993). This therapeutic potential of ozone, to inhibit bacterial proliferation, was realized in dentistry as long ago as 1930. HealOzone utilizes ozone to destroy bacteria, including all those associated with the development and progression of caries.

Microorganisms have been taken from actual clinical situations in studies; root carious lesions (Baysan et al, 1998a,c, 1999, 2000, 2001a,b), infected instruments (Brunel et al, 1965) or dental unit (Cardon et al, 2002; Al Shorman et al, 2002a,d; 2003; Walker et al, 2003); waterlines, or used microorganisms known to contribute to the problems addressed (Minguez et al, 1990; Murakami et al, 1996, 2002; Shargawi et al, 1999; Zhao et al, 2000; Chang et al, 2003; Nagayoshi et al, 2004). Other studies, for which the full text was not availabe on-line, reported the microbicidal effect of ozone as the main result presented in their paper as indicated from the titles. (Korotkikh et al, 2000; Malanchuk et al, 2000).

Bacterial reduction was expressed by the reduction of the total colony-forming units. Other methods included viewing under scanning electron microscopy (SEM), confocal microscopy with dead/live bacterial stains or under microscope viewing the biofilm distribution. The results showed the ability of ozone, in the correct dose and concentration, to reduce bacterial counts from 10–100,000-fold reaching in some cases to complete sterilization of the samples tested. Reductions correlated with ozone application periods and concentrations used and the gas form proved to be more effective. The cell walls of *Streptococcus mutans* were seen to be disintegrated under SEM. The bactericidal ability of ozonated water was reduced as the concentration of ozone gradually decreased if the water was left in open air.

Oxidation of biomolecules featuring in dental diseases

Ozone has the effect, through its powerful oxidizing properties, of not only removing the protein protection and being bactericidal, but also oxidizing the biomolecules that allow the niche to survive and expand (Smith et al 2001; Claxson et al, 2002; Lynch et al, 2002a,b, 2004). This has a severely disruptive effect on the bacterial population in the carious lesion and obliterates the cariogenic bacteria and their ecological niche, thereby swinging the equilibrium in favour of remineralization. No more acid can be produced within the lesion when the acid-producing bacteria are eliminated. For example, pyruvic acid, one of the strongest naturally occurring acids manufactured by bacteria, and implicated in the progression of caries, is oxidized by ozone to acetic acid and carbon dioxide. Acetic acid is less acidic than pyruvic acid, and this decarboxylation reaction leads to mineral uptake due to the more alkaline conditions in a carious lesion.

Oxidation of salivary biomolecules (Silwood et al, 2002; Turner et al, 2002a,b) that supply the plaque and carious lesions were also proven. Other oxidation reactions in saliva include the dissociation of volatile methionine products responsible for malodour (bad breath). Oxidation reactions are seen in plaque as well (Grootveld et al 2001, 2002).

Once the oxidation and the bactericidal effects occur, it could be proposed that barriers for the highly mineralized environment to finally reach demineralized tissue protected in the niche are broken down. The remineralizing potential of oxidizing agents has previously been shown for sodium hypochlorite. This has been shown to be effective in the removal of organic material,

such as proteins, from demineralized dentin, which improved the permeability of the lesion to remineralizing fluoride (Inaba et al, 1995, 1996). It has been hypothesized that ozone, a much more powerful oxidant than sodium hypochlorite, could remove proteins in carious lesions.

Clinical effectiveness results

Detection near the base of the caries iceberg: diagnosis of demineralization and not cavitation

Early diagnosis of primary pits and fissure caries is of great importance in children and adults because of the rise of a new model of carious lesion, which is difficult to diagnose with traditional methods as oral radiographs and probe. Low sensitivity to visual, probing and bitewing examination leads to a significant number of teeth with dentinal caries being undetected. Lesions have a natural history of deepening into dentine leaving a macroscopically undamaged enamel surface. Minimal mineral loss prevents radiographic evidence of caries, and absence of macroscopic cavitation means there is no probe stickiness. Systems using indirect light fluorescence have been demonstrated to be effective in the clinical diagnosis of caries in the permanent and deciduous dentitions.

Integration of the HealOzone treatment unit into the surgery environment will totally change the general dental practitioner's (GDP's) approach to the treatment of his or her patients. GDPs have to reassess their diagnostic criteria completely when these are applied to dental caries and potential treatment of the carious lesion. Caries can be considered to be a failure of prevention and maintenance. As such, looking for holes is a concept that cannot be part of modern dental care – where the dental profession is looking towards prevention. Thus, the detection of demineralization within the enamel layer is of paramount importance, before the carious process has an opportunity to penetrate through into the dentin below. The dental probe may be set aside while examination is based on the use of a digital intraoral camera combined with selective use of the DIAGNOdent or the Electric Caries Monitor (Lode, Belgium) in addition to the visual methods which are now validated (Ekstrand et al, 2001). It has become essential for other fine clinical changes to be recognized and recorded by the dentist. These tools have been used in many studies and correlate with each other at baseline (Abu-Naba'a, 2002, 2003).

Root caries

In all studies caries severity was clinically assessed using the clinical severity index (CSI) (Beighton et al, 1993), which classifies root caries as soft (4, most severe) through to hard (0) (Fig. 2). All studies reported that root caries treated with between 10 and 40 seconds ozone either stabilized or reversed and none of the ozone-treated root caries progressed (i.e., worsened). Ozone appeared to be effective on caries of varying severity, including the severest soft caries (CSI 4). In comparison, control caries generally did not change significantly or worsened.

Caries reversal was observed whether the lesion received a single ozone treatment or more than one over several months. Holmes (2003e) showed that three months after ozone treatment (40 seconds) 69% of caries had reversed to hard from CSI 2. Re-treatment at 3, 6, 12, and 18 months resulted in 100% of caries reversing to hard by the 18-month and 21-month reviews. It is difficult to hypothesize whether all caries would have reversed to hard without re-treatment, although Baysan (2002c, 2004b) showed that 81% of caries (CSI 1 or 2) had reversed to hard three months after a single 20-second ozone treatment.

The study of Baysan et al (2004b) further suggests that ozone treatment for 20 seconds was more efficacious than 10 seconds in terms of lesion severity. Seventy three per cent (24/33) of caries treated with 10 seconds of ozone reversed, including seven carious lesions, which reversed to hard (21%). In comparison, 100% (32/32) of caries showed some degree of reversal when treated for 20 seconds, including 26 that reversed to hard (81%).

The 12-month study of Baysan et al (2004a) showed improvements in both DIAGNOdent and Electronic Caries Monitor (ECM) readings for ozone-treated caries compared with the control group after 1, 3, 6, 9, and 12 months. Both techniques are employed as diagnostic tools, correlating with the clinical severity indices employed by dentists (Baysan 2004c) and providing information on changes in tooth substance and porosity. In this study, reduced DIAGNOdent and increased ECM values were indicative of a reduction in caries severity and tooth remineralization. It should be noted that during the remineralization process, some lesions might

take up deep stains, leading to a stable or increased DIAGNOdent reading in a few cases. Such 'false positives' need to be investigated and tested further, using a range of other diagnostic criteria well known to dental practitioners. In addition, DIAGNOdent and ECM readings suggest that treatment of root caries with ozone and root sealant is more beneficial than sealant alone, in terms of improvement in caries severity (Baysan, 2002b).

Other outcomes

One study assessed the retention of sealants on root caries and appeared to show a benefit of ozone treatment prior to sealant application. Sixty one percent of root caries treated with ozone and a root sealant had intact sealants after 12 months compared with 26.1% in those treated with sealant only ($p<0.05$) (Baysan, 2002b).

Non-cavitated pit and fissure caries

Caries severity

Four studies on patients with non-cavitated pit and fissure caries showed that ozone treatment resulted in caries reversal and remineralization in both permanent and deciduous teeth, as determined by DIAGNOdent and ECM measurements. These two diagnostic tools show significant correlation with visual clinical severity indices and are good indicators that remineralization or demineralization are occurring (Abu-Naba'a et al, 2002a).

A double-blind controlled clinical study used 10 seconds of treatment with ozone and these patients underwent up to five re-treatments over 12 months. Remineralization in the treatment group was detected as early as the first recall visit at one month in permanent teeth (Abu-Naba'a et al, 2003a,e). Fissure sealants applied over ozone-treated lesions had not affected short-term retention (Abu-Naba'a et al, 2003a, 2004a), nor 1-year retention rates (Abu-Naba'a et al, 2005).

Clinical indices showed a significant change that could be detected by the clinician in the general practice (Abu-Naba'a et al, 2003b,c). Significantly, more lesions became arrested in the treated teeth and were translated to less dental procedure requirements for the treated teeth. Huth et al (2004) and Holmes (2003d) treated their patients' caries with a single ozone dose of 40 seconds followed by review up to six months post treatment. In addition, Hamid (2004) showed that 86.6% of non-cavitated caries, deemed severe enough to require drilling and filling, were clinically reversed by two sessions of ozone treatment (40 seconds) three months apart (Table 4). Untreated control caries did not change significantly. Assessments of clinical severity were based on the Ekstrand Index, a visual assessment of clinical severity. A subanalysis of patients with high caries risk (Huth et al, 2004) found that 40 seconds of ozone treatment had a significant beneficial effect on uncavitated caries compared with controls whose DIAGNOdent readings deteriorated over three months.

In the study of Holmes (2004c), 40 seconds of ozone treatment was combined with sealing of the caries with a glass ionomer, and daily use of remineralizing toothpaste, spray and mouthwash. All caries initially extended 2–4 mm into the dentin and as such were deemed severe enough to require drilling and filling. By the 3-month follow-up 100% of caries had reversed to hard.

Other outcomes

The study of Holmes (2004c) also assessed the incidence of postoperative sensitivity associated with ozone treatment compared to conventional treatment. The treatment group was treated with ozone for 40 seconds and the lesion sealed with a glass ionomer, which was replaced with a composite filling after thee months. In comparison the control group underwent drilling and filling with a composite. There was no sensitivity reported by patients in the six months following treatment incorporating ozone, while six (17.1%) control patients complained of some sensitivity ($p<0.05$).

It may be that the proportion of patients suffering from postoperative sensitivity would require some additional treatment, or at least need to seek professional advice necessitating a follow-up dental appointment.

Cavitated pit and fissure caries

None of the available studies specifically describes the treatment of cavitated pit and fissure caries. In the absence of such detail, the studies presented in this section refer to carious lesions which are deemed to require drilling and filling. While non-cavitated caries may be treated in this way, drilling and filling is the conventional treatment for cavitated caries and it is therefore assumed that these studies included a proportion of cavitated caries.

Caries severity

Eight studies reported a beneficial effect of between 20 and 40 seconds of ozone treatment on pit and fissure caries (Table 5). The range of results across all studies was 59–99% of caries improving (reversal), based on either clinical severity ratings or DIAGNOdent measurements. Untreated controls generally showed no clinically significant change or progressed. Caries progression was reported at rates between 0 and 11%, although in the study of Daly et al the sample size was small, such that only thee patients suffered from caries progression (Daly et al, 2003).

Either specific doses (i.e., a set time) or a range of doses of ozone have been used. However, it is difficult to draw conclusions regarding a dose-dependent effect, particularly given the range of follow-up times from 1 month to 12 months. For example, 84% of caries were deemed to have reversed one month after treatment with 20 seconds of ozone (Johnson et al, 2003c). In other studies, 30 seconds of treatment lead to reversal of 59–81% of caries when assessed up to six months post treatment (Cronshaw, 2003; Daly et al, 2003; Reaney et al, 2003), while 87% had reversed 13 weeks after ozone treatment for 40 seconds (Morrison et al, 2003).

One study re-treated patients with ozone every three months if reversal had not occurred, up to the 12-month review appointment (Holmes, 2004c). In this case, 99% of caries had clinically reversed by 12 months post treatment.

Approximal caries

In a study applying the ozone on hidden approximal surfaces, lesions were assessed clinically and using radiographs as needing drilling and filling. Air abrasion was used to prepare minimal lesions and glass ionomer was used for fillings. These fillings were later replaced by permanent composite fillings. All lesions were successfully exposed and a seal established for the delivery of ozone. Clinically acceptable seals were achieved around all restorations. The air abrasion and ozone technique was significantly faster than conventional drilling and filling (Clifford, 2003). On recall, all ozone-treated restorations were symptomless throughout the nine months. All 34 Fuji 7 restorations, removed after thee months recall, showed hard 'caries' on exploration suggesting remineralization was successful. All lesions were successfully exposed and a seal established for the delivery of ozone at baseline. The air abrasion and ozone technique was significantly faster (lesions were exposed, ozonated and sealed in under seven minutes) than conventional drilling and filling (Clifford, 2004).

Treatment of early carious pit and fissure lesions in deciduous teeth

The management of early pit and fissure carious lesions has been extensively studied by Abu-Salem (2004). Non-cavitated occlusal carious lesions were treated in this study. The teeth were cleaned using an air-abrasive system, then the following were recorded: clinical severity index, DIAGNOdent and ECM IV readings. After randomization, one lesion was treated with ozone and another was reserved as a control in each child. No adverse effects were recorded. Ozone treatment significantly remineralized lesions ($p<0.01$) while lesions in the control group suffered from deterioration in their mineral content. Ozone treatment is an alternative treatment for non-cavitated occlusal carious lesions in primary teeth.

Where caries is found and the radiolucency is visible radiographically, but not exposing the pulp, this can extend more than half way into dentin. It is relatively simple to treat using a modified atraumatic restorative technique (ART) combined with ozone. The application of, for example, FujiVII (GC Japan) may supply long-term fluorine and mineral release, as well as preventing ingress of food debris and re-establishment of the acid-niche environment. Treatment is simple, fast (the average ozone application time for practitioners using the HealOzone is 30 seconds) and involves little preparatory work. The loose debris and outer necrotic carious dentin layer is first cleaned away, until a leathery base is reached. This can be done with hand instruments. Ozone is applied, the lesion wetted with the HealOzone (KaVo) remineralizing wash and then the glass ionomer restorative cement can be applied. This modified ART technique, combined with ozone, has been clinically proved by Holmes (2004b).

Allied to this, ozone has been used in the treatment of deciduous molar teeth with poor prognosis as a result of caries. In some parts of the UK and USA, it is upsetting to find many children at 3–4 years of age with gross decay. For these patients the usual outcome is a general anaesthetic and tooth extraction. These lesions are treated with ozone and it has been found that the majority of children are cooperative and actually enjoy

the experience (Holmes 2004). Of great interest is that the toothache in young children has been reduced and even abolished after ozone treatment, with much relief for the parents (Holmes 2004). Ozone treatment seems to be an excellent palliative treatment option for such youngsters.

An attempt has also been made to use ozone in the treatment of approximal deciduous caries where the adjacent two marginal ridges are intact. When treatment is required for these lesions then an operative approach gaining access to the caries is used combined with ozone, in a similar way to that described above for managing deciduous teeth lesions visible radiographically.

Figures 1–19 show examples of ozone-treated pit and fissure caries, deep occlusal lesions and root carious lesions.

Patient-centred outcomes

Studies were identified that assessed patients' attitudes towards treatment of caries with ozone, compared with conventional drilling and filling (Domingo et al, 2003b; Johnson 2003b; Megighian et al, 2003). A total of 727 patients who were to undergo ozone treatment for a carious lesion completed a questionnaire. They had all undergone conventional drilling and filling for a similar lesion either three (Megighian et al, 2003), six (Domingo et al, 2003b) or an unreported number of months previously (Johnson 2003b). In the study by Megighian et al (2003b) a further 45 patients were assessed who had undergone conventional minimally invasive treatment (drilling and/or air abrasion and drilling) in the past but did not require ozone treatment.

It is well recognized that some patients attending dental clinics show some level of anxiety, which may cause them not to attend the clinic. In these HealOzone studies, 65–83% of patients were anxious about their teeth being drilled and 80% nervous about local anaesthesia. In comparison, 33% of patients were slightly anxious about ozone treatment based on a verbal description of the treatment. After receiving ozone treatment 80–100% reported either a reduction or absence of anxiety about the treatment.

The majority of patients reported satisfaction with HealOzone treatment (99–100%). They were happy with the time it took (97–100%) and would have liked it used again (100%) or recommended it to family and friends (95–100%). Between 55 and 95% of patients would be satisfied to have ozone treatment again even if it cost more than current treatment.

Treating anxious children is a major challenge in dentistry, where treatment options may have to include use of sedatives or general anaesthesia. Dahnhardt et al (2003) evaluated parents' attitudes towards dental treatment in 20 anxious children. Seventy-five per cent of children were afraid of visiting the dentist prior to ozone treatment but following ozone treatment they lost some of their fear and all were happy to return for the follow-up appointment. The majority of parents would use ozone treatment again (75%) and were willing to pay more for ozone treatment than conventional treatment (80%).

These results have allowed GDPs to concentrate almost exclusively on the removal of carious tissue while retaining as much sound hard tissue as is possible, a first step towards the minimally invasive approach now advocated. However, this carious tissue still has to be removed, whether by use of the hand piece, air abrasion, or with hand instruments (ART) used in conjunction with caries-removing liquids and gels (e.g., Carisolv). HealOzone treatment of dental caries removes the requirement for physical removal of diseased tissue as it promotes remineralization and not amputation of carious dentin. GDPs can offer treatment for a wide variety of carious lesions where there is no need for local anaesthesia and drilling, and can treat many lesions in a very short space of time, painlessly and atraumatically (Al Shorman et al, 2002a; Dahnhart et al, 2003; Domingo et al, 2003a, b, 2004a,b, 2005). Patients are delighted after treatment and are particularly motivated towards oral hygiene and dietary control when they realize that in improving and concentrating on these areas they can effectively avoid the local anaesthesia/drill approach (Johnson et al 2003; Domingo 2003, 2004).

One area that has not been looked at in past papers was anxiety of parents and carers for those they brought for dental treatment and care. Ozone treatment has been shown that not only patients but also their parents and carers can benefit from this non-invasive treatment modality (Dahnhart et al, 2003).

Patient compliance

Many of the studies followed-up patients for long periods. Including these recalls into the protocols have resulted in increased compliance of the patients with different aspects of managing caries risk behaviours.

Scholz used a software in order to quantify if ozone-treatment protocols including the recalls improved compliance and if the software itself encouraging. It was used in 10 clinics offering treatment with ozone. Patient recall attendance (53% versus 44%) and compliance (84% versus 75%) were better with the new system (Scholz, 2004).

Root canal therapy

The aim of conventional root canal therapy is to provide a clean, shaped, root canal that facilitates the placement of root filling systems. Until recently, the dental profession relied on irrigants reaching these areas to disinfect and dissolve organic debris where it is impossible to instrument mechanically. In cases where previous root canal treatment has failed, *Enterococcus faecalis* seems particularly prominent and especially difficult to eradicate.

Ozone at application times of 10–60 seconds produced zero colony-forming units (CFU) in solutions containing 105 and 106 CFU (Chang et al 2003). Further studies are needed to endorse the claim that ozone will penetrate through the apical delta of accessory canals and if it reaches the foramen, and enter into the surrounding and supportive bone tissue. The effect of ozone on these tissues will be to encourage healing and regeneration.

In-vitro bovine dentine samples were impregnated with *E. faecalis* and *S. mutans* and then the specimen was irrigated with sonication; the ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl. After irrigation with ozonated water, the viability of *E. faecalis* and *S. mutans* invading dentinal tubules significantly decreased (Nagayoshi et al, 2004).

Other applications have been reported and these include the treatment of root gangrene and dental granuloma (Schwan et al, 1951). Sterilization of minute endodontic material (Brunel et al, 1956), in odontostomatology, (Sandhaus, 1965) and in endodontic therapy as general (Haimovici et al, 1970). As these references are general and may not apply the new instruments for endodontic treatments, but would provide guidelines for further studies.

Dental unit water lines

Dental unit water lines (DUWLs) have been shown to be heavily contaminated with biofilm and high bacterial counts have been recorded in the water from dental units. This may be worrying where immunocompromised patients are concerned. Biofilm contamination plays havoc with dental units, often causing annoying blockages in couplings, hand pieces and 3:1 syringes. These applications for ozone are discussed elsewhere in this book.

1H NMR studies on ozonated oils

Ozone gas is a lung irritant, and HealOzone has been manufactured to a design that makes it impossible for free ozone to be released into the oral cavity and oronasal complex. In the same way that some antibiotics have various delivery systems (for example a liquid, capsule, and cream presentation) practice has shown that an alternative delivery system for ozone would be an advantage. Ozonated oils are not new and have been manufactured for a number of years. A 2002/2003 study published in 2003 showed that the purest oil substrate that was cheap and easy to source was sunflower oil (Lynch et al, 2003). Unlike olive oils that can contain a number of impurities, depending on the country, region, locality, and chemicals used to recover the oils, sunflower oil was shown to be the purest, irrespective of source and re-seller. It is notable that sunflower oil has been the oil of choice for the Cuban National Ozone Clinic for the past 20 years.

Dentine hypersensitivity

Exposure of the dentinal tubules with related symptoms of sensitivity is an extremely common problem presenting to the GDP. All treatment methods are directed at sealing of these tubules and vary from the application of fluoride varnishes to the placement of bonding systems on the root surface. The 'hydrodynamic theory' proposed to explain dentin hypersensitivity has been around for some time. As well as fluid movements within the dentinal tubules, bacteria have also been shown to be associated with the tubules. This problem can be simply and immediately eliminated with the use of HealOzone treatment. Ozone penetrates the exposed tubules, eliminates bacterial contamination and effectively 'priming' tubules to allow mineral ingress and

subsequent sealing. It is vital that the seal obtained allows ozone delivery to the area being treated and in these cases a liquid rubber dam is a great help in achieving this seal around the marginal gingivae. Once a seal is obtained, an ozone delivery of 40 seconds is followed by painting the treated area with the supplied CurOzone remineralizing solutions. This protocol is usually sufficient to completely eliminate any symptoms arising from the area undergoing treatment. In more severe cases, a second 40-second application of ozone may be required. A final application of fluoride varnish may be done and the patient is given oral hygiene instructions and advice about erosion before leaving in order that future problems of this nature can be prevented. This procedure was reported in an early study in 1955, but there is need for a controlled clinical study to confirm this phenomenon (Ciriello, 1955).

1H NMR Studies on tooth whitening

Teeth may be whitened using ozone gas, due its strong oxidizing properties, and ozone oil has been used in some Cuban studies in root-filled teeth to lighten them. A study in 2003 by Holmes (2003a) showed that ozone could oxidize the components responsible for tooth discoloration. Studies conducted in Spain and the UK with a full mouth tray delivery system have shown spectacular results within a short treatment time. And, of course, the full mouth tray system would solve a number of issues for the treatment of interstitial caries and mesial or distal lesions where a seal with a round cup delivery system is difficult to use. It would make the treatment of the elderly, infirm, and disabled people potentially easy and predictable in terms of oral hygiene care and reduction in the incidence of caries in patients with physical impairments.

Integration of HealOzone in the dental surgery

Most preparation and restorative systems are not used in isolation, and are integrated into dental practice restorative routines. Filling materials fail at alarming rates. Costs can be measured in terms of pain, discomfort, and in financial terms such as lost productivity. In England and Wales, restorations carried out in NHS dentistry cost a total of &pound ;1.25 billion in 2001. This does not include private treatment, which is currently estimated to be 50% of dentists' income. The total costs of all dental treatment in England and Wales probably exceeded &pound ;3.26 billion in 2001 (General Dental Council UK, 2001).

Studies have shown that the use of ozone combined with air abrasion and ART (Johnson et al, 2003e; Domingo et al, 2004b) can have significant effects in reducing the duration of treatment, the cost, and preservation of natural tooth tissue when compared to traditional 'drilling'n'filling'. When ozone is integrated fully into treatment modalities, there are significant cost benefits; ozone usage combined with air abrasion or ART shows significant cost benefits in terms of reduction in treatment time, predictable outcome, and income into the practice. Nor are single teeth usually treated. The majority of the dental practitioners who first invested into the HealOzone technology now routinely treat lesions with ozone while other restorative treatment is being carried out. Thus the potential of the HealOzone to be a 'profit-centre' in a dental practice is unlimited. In other words, the way this technology is integrated into dental care is limited solely by the vision of the dental practitioner.

Ozone and dental materials

Dental prostheses are subject to the sorption of microbial biofilms on their surfaces, which could cause long-term infections. If immunity of the wearer is reduced, then these local infections may prove to be hazardous. Disinfection of denture material with ozone has been shown to be successful when tested in-vitro using either gaseous or water dissolved ozone (Murakami et al, 1996; Oizumi et al, 1998). Neither of these produced surface roughness change in wet acrylic samples either polished or glazed (Abu-Naba'a et al, 2005). Some loss of reflectance of dental alloys was noticed, but this was only a surface change and repolishing was suggested (Suzuki et al, 1999). Gypsum casts and impression materials were also disinfected by ozone but there is a need for controlled studies on the effects on dimensional stability of different methods of application of ozone (Zhao et al, 2000).

The bonding forces of bonding systems have not been shown to be affected by the application of ozone

nor the surface hardness of filling materials (Hussey et al, 2002; Campbell et al, 2003; Czarnecka et al, 2004).

Further research

The continued commitment to research into the use of HealOzone in dentistry is essential and a number of ongoing studies are due for completion and publication over the next 12 months. In addition, a 12-month, multicentre, double-blind randomized controlled clinical trial is planned by CurOzone, in direct support of their FDA submission, and is expected to run from September 2004 to September 2005; 360 patients will be recruited, each with two similar fissure caries in permanent teeth. Patients will be randomly assigned to receive ozone treatment with HealOzone for 30 seconds or a placebo with the device for the same period. The primary efficacy outcome will be the change in clinical severity of the caries after 12 months. Secondary outcomes will include change in severity and change in DIAGNOdent readings at follow-up intervals of 3, 6, 9, and 12 months.

Conclusions

The studies identified in this chapter have demonstrated the safety and effectiveness of HealOzone in treating dental caries, albeit various types of caries with different treatment durations and frequency. Treatment with ozone using HealOzone was shown to have a significant antimicrobial effect, destroying at least 99% of microorganisms found in root caries, with 20 seconds of treatment. It is well recognized that the interaction of microorganisms with plaque and the tooth structure is responsible for the initiation and progression of dental caries.

Detailed comparison between trials is problematic given the differences in treatment duration, treatment frequency, follow-up times and initial severity of caries and the lack of detailed published data. However, the common outcome of the majority of studies is clear. HealOzone was shown to have a stabilizing or reversing effect in both root and pit and fissure caries of varying severity in permanent teeth. One study showed an improvement in non-cavitated pit and fissure caries in deciduous teeth. The reversal process is associated with remineralization of the tooth and this was demonstrated using ECM and DIAGNOdent measurements in many of the studies. This makes it especially relevant to the younger patient, who may find conventional treatment unacceptable and also for the elderly, who may have medical problems that may complicate conventional dental treatment. The treatment is simple, completely safe to provide and often renders the need to introduce potentially toxic restorative materials unnecessary.

The use of HealOzone therefore not only preserves tooth structure by avoiding the use of invasive techniques to remove carious tissue, but also destroys the microorganisms responsible for the caries and promotes caries reversal and tooth remineralization. Patients treated with HealOzone revealed that they were satisfied with the treatment. The considerable levels of patient anxiety associated with 'drilling'n'filling' techniques were significantly reduced when they underwent ozone-based treatment.

In conclusion, ozone therapy provides a treatment modality with considerable benefits for dental patients of all ages. It is applicable to a wide range of conditions of the intraoral hard and soft tissues.

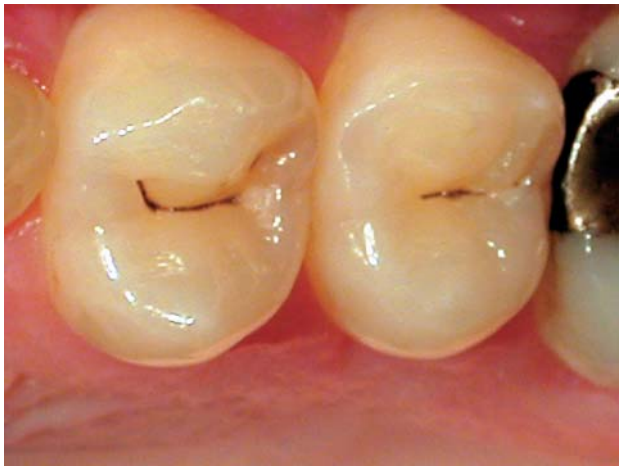

Figure 1: The occlusal fissure caries in these 2 premolars had presented with DIAGNOdent readings of 36 in the first premolar and 24 in the second premolar, four weeks previously. Both of these premolars received 40 seconds of HealOzone treatment and the Patient was prescribed a HealOzone toothpaste, mouthrinse and sprayon the first visit. On this recall visit as shown in this picture, 4 weeks later the caries was judged to be arrested, another 40 seconds of HealOzone treatment was administered and the fissures were then sealed. A liquid phosphoric acid etchant was applied for 30 seconds, washed and dried prior to the application of a dental adhesive, light polymerisation and the placement of a flowable composite resin.

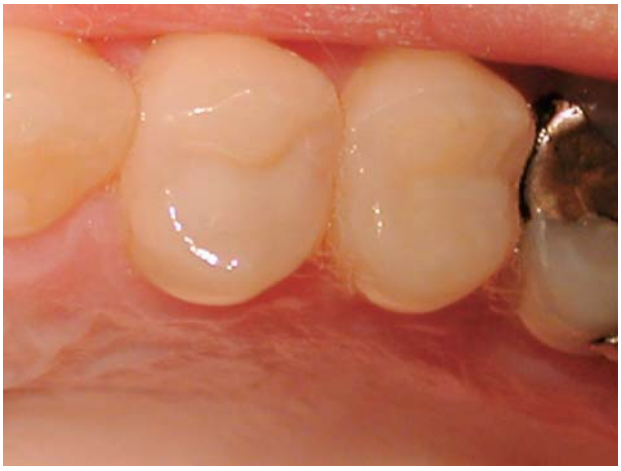

Figure 2: This picture shows the 3 year follow up recall assessment of the 2 sealed HealOzone treated occlusal fissure caries in the 2 premolar teeth.

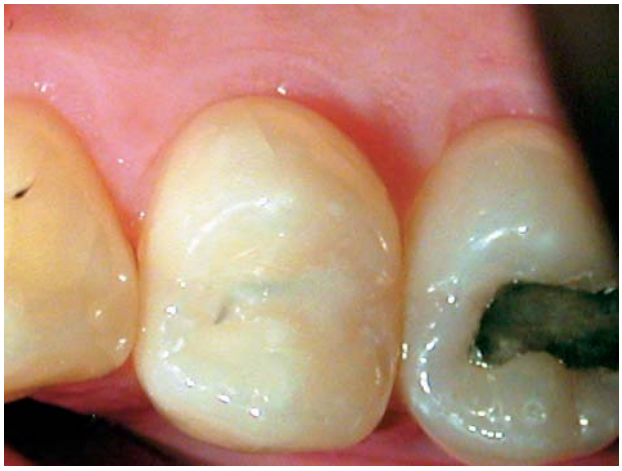

Figure 4: The flowable composite resin chosen in this case was an enamel shaded material. It would have been preferable to have choose a more opague flowable composite rsin such a dentine shaded composite resin which would have masked the underlying stained fissure. However the Patient was very happy with this 38 month recall result.

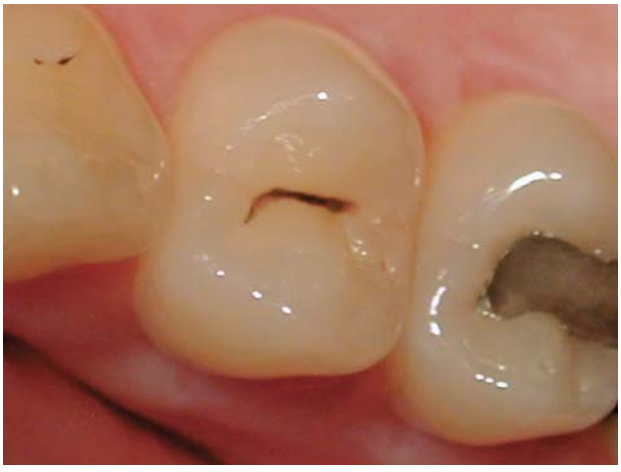

Figure 3: The occlusal fissure caries in this premolar had presented with a DIAGNOdent readings of 38 in this first premolar four weeks previously. The occlusal caries had received 40 seconds of HealOzone treatment and the Patient was prescribed a HealOzone toothpaste, mouthrinse and spray on the first visit. On this recall visit as shown in this picture, 4 weeks later, the caries was judged to be arrested, another 40 seconds of HealOzone treatment was administered and the fissure was then sealed.

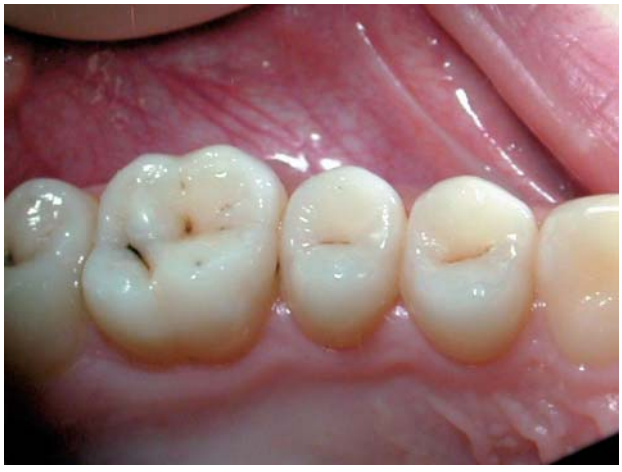

Figure 5: The occluso-palatal fissure caries in this upper first molar had presented with a DIAGNOdent reading of 37. This Patients tooth received 40 seconds of HealOzone treatment and the Patient was prescribed a HealOzone toothpaste, mouthrinse and spray on their first visit. On this 38 month recall visit the caries was judged to be arrested. The recommended protocol would have been to reapply another 40 seconds of HealOzone treatment 4 weeks after the first treatment followed by sealing of the fissures using a flowable composite resin. However in this case no sealant had been applied.

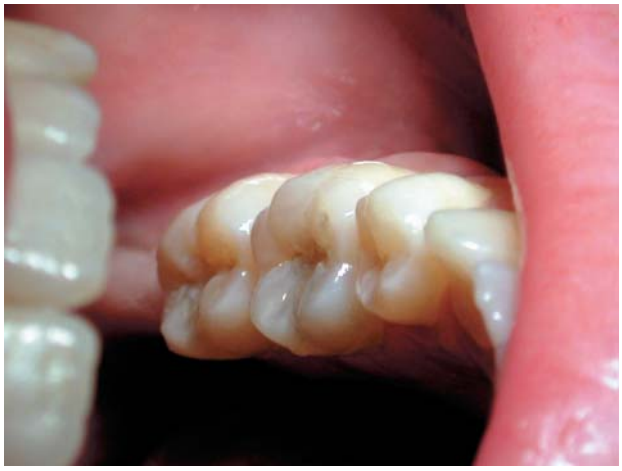

Figure 6: Occlusal pit and fissure caries on the lower left first molar which is too extensive for HealOzone treatment alone. This Patient presented complaining of sensitivity to cold.

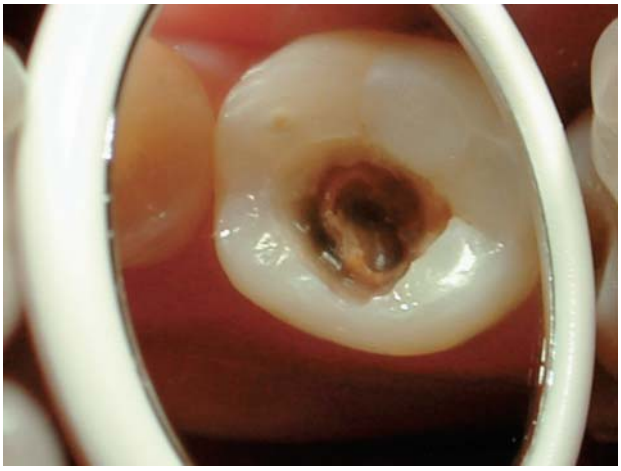

Figure 8: During the cavity preparation, the last 1 mm of leathery infected caries overlying the pulp was not removed. The tooth was then treated with the Healozone for 40 seconds and the HealOzone remineralising solution containing fluoride, calcium, phosphate, zinc and xylitol was placed into the cavity.

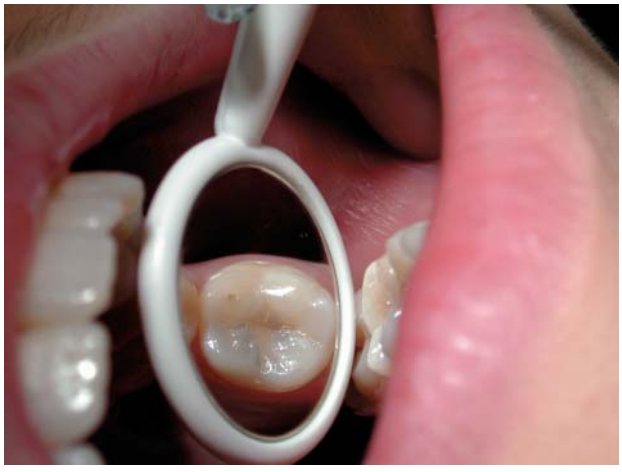

Figure 7: It is obvious that the placement of the fissure sealant 2 years previously has not been successful.

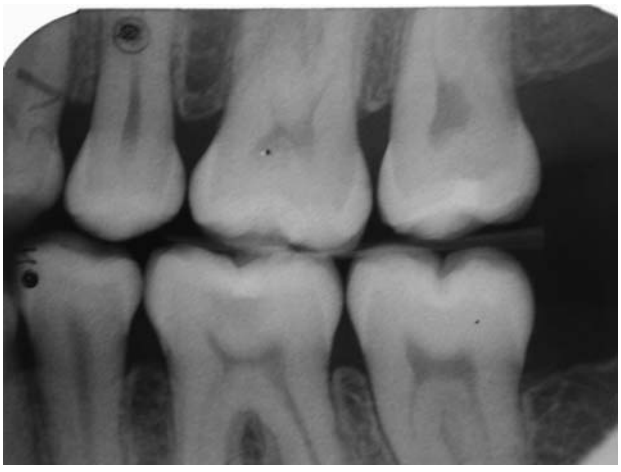

Figure 9: A previous radiograph revealed the extensive deep occlusal caries approaching the pulpal tissue.

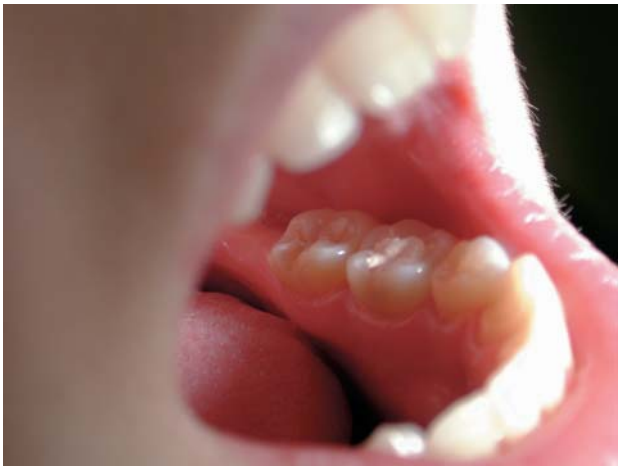

Figure 10: A simple zinc oxide eugenol temporary restoration was then placed. The authors would recommend that a glass ionomer cement restoration would be a more suitable material for this purpose. In this case the authors wanted to assess the result without the additional benefit of a glass ionomer cement restoration.

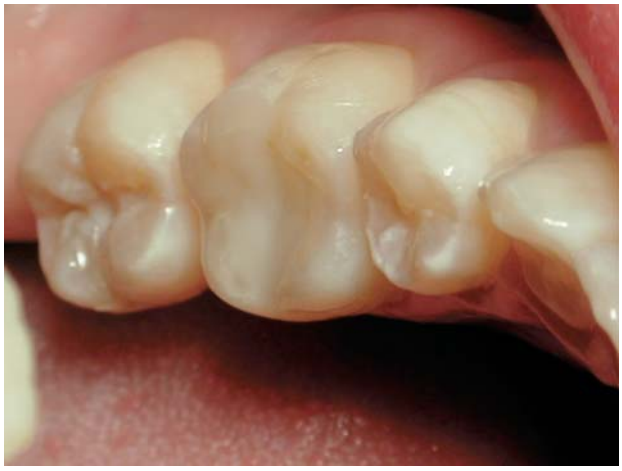

Figure 12: A simple posterior composite resin was placed. The aouthors recommend that a glass ionomer cement be used as the dentine replacement and the composite resin be used as the enamel replacement material.

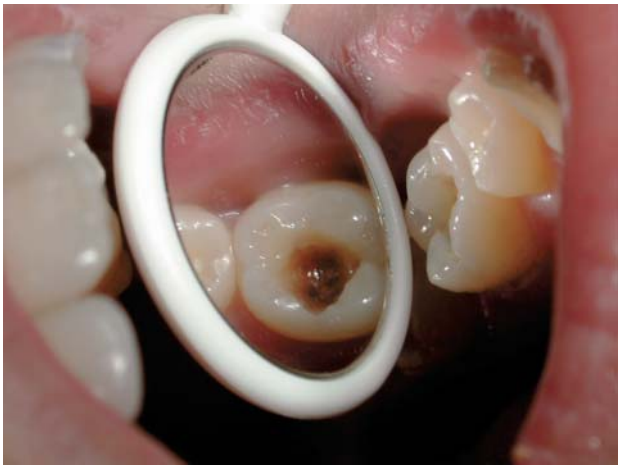

Figure 11: Three months later the Patient was recalled and the temporary cement was removed, taking great care not to remove any tooth tissue. The floor of the cavity was now hard and arrested.

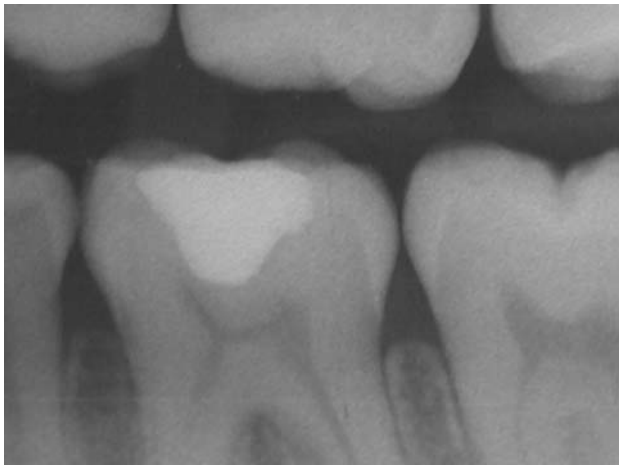

Figure 13: The postoperative radiograph revealed the extent of the depth of the cavity. The authors consider that if the operator had removed most of the infected caries on the floor of the cavity on the first visit that there would have been a high probability that a root canal therapy may have been required.

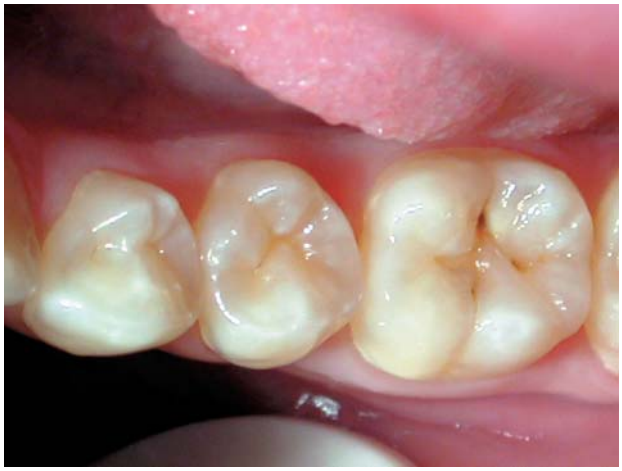

Figure 14: If it is deemed that the occlusal caries is extending more than 2mms into dentine beneath the pits and fissures then it is preferable to open up these lesions, remove all the peripheral caries, leave the last 1 mm of caries on the pulpal floor, HealOzone treat this cavity and then place a restoration

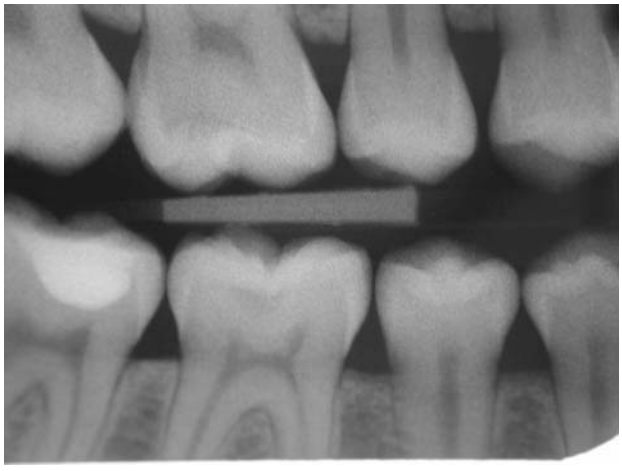

Figure 15: The radiograph of the first molar seen in fig 14 confirmed the clinical detection of caries expending more than 2 mms into dentine occlusally.

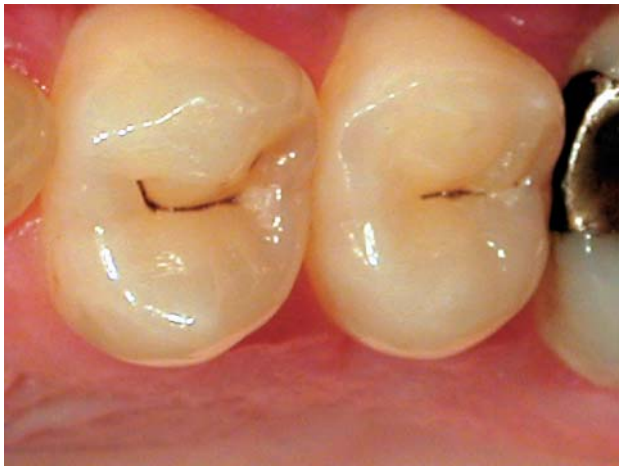

Figure 16: This picture was recorded during the recall of this Patient who had pit and fissure caries in both of these premolars, which had been HealOzone treated 38 months earlier. At baseline the DIAGNOdent readings had been 27 in the first premolar and 24 in the second premolar.

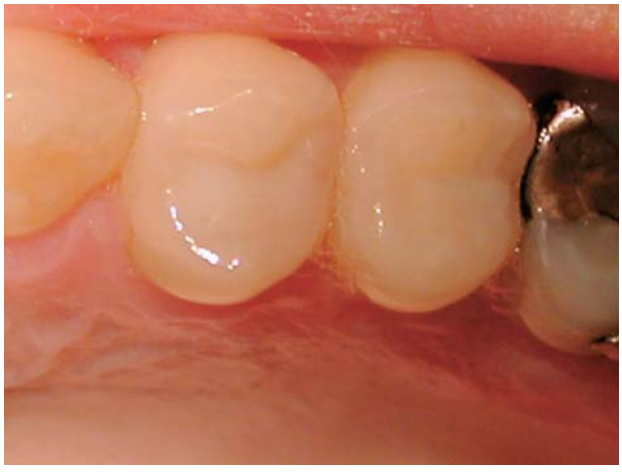

Figure 17: The lesions in the 2 premolars shown in figure 16 had been deemed to be arrested at the one month recall visit. They were acid etched with a liquid phosphoric acid, washed and dried, prior to the placement of a dental adhesive and dentine shaded flowable composite resin. This picture shows the 3 year recall result.

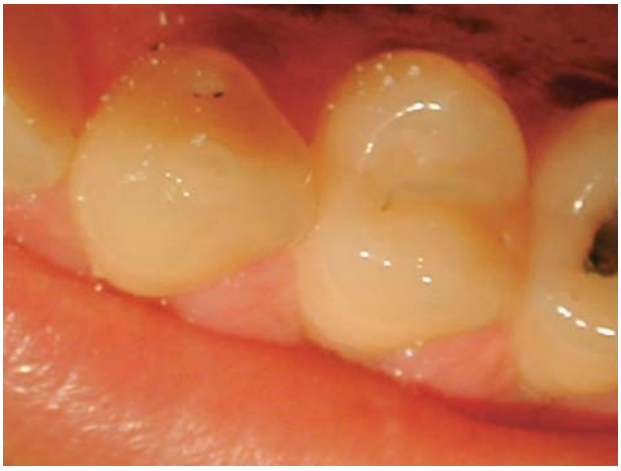

Figure 18: This slide shows another 3 year recall of a HealOzone treated occlusal pit and fissure carious lesion which had been deemed to have infected dentine at the baseline visit. Healozone had been applied at baseline and after 4 weeks and then the lesion had been sealed as recommended. However it is notable that an enamel shaded flowable composite resin had been used and therefore the remineralised lesion beneath is partly showing through.

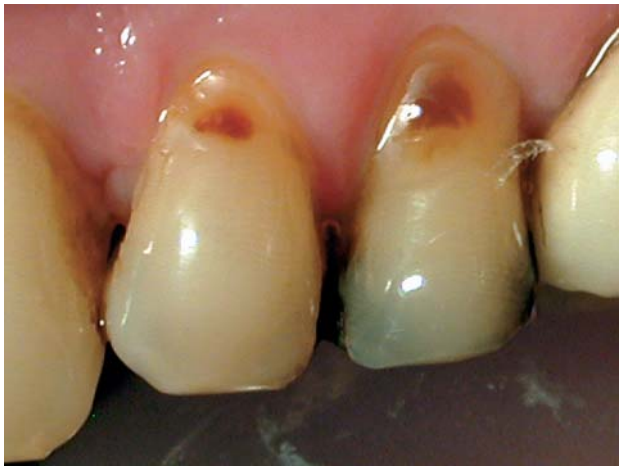

Figure 20: Three year recall of 2 Healozone treated root carious lesions. The hardness of the lesions now confirm their arrest. All the HealOzone treated Patients are informed that the HealOzone treatment is part of a preventive programme. This Patient was therefore prescribed the standard preventive advice. Firstly they were advised to reduce the frequency of consumption of fermentable carbohydrtaes to less than 6 times daily and secondly to also increase their exposure to fluoride containing toothpastes, mouthrinses and sprays. In this case this Patient had also been given the HealOzone toothpaste, mouthrinse and spray to use, incommon with the Patient in figure 19.

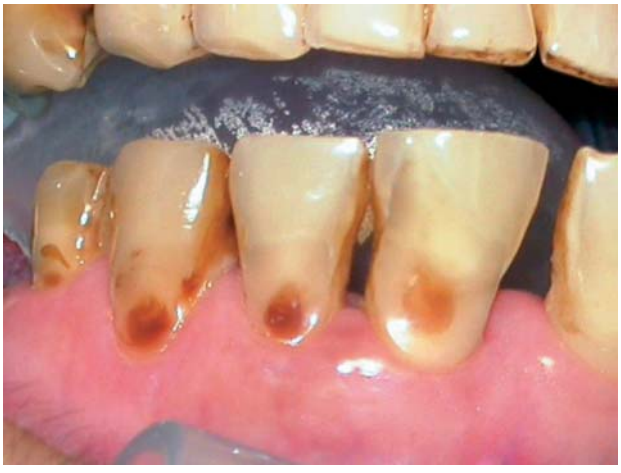

Figure 19: 38 month recall of root carious lesions which had been present in each of these 4 teeth. The hardness of the lesions now confirm their arrest. The Patient was not concerned about the appearance as she did not show these lesions during smiling. If the Patient had been concerned about the discoloured arrested lesions, then the use of a dental adhesive and composite resin would have easily solved this problem, without the need for any local analgesia, nor removal of any tooth structure.

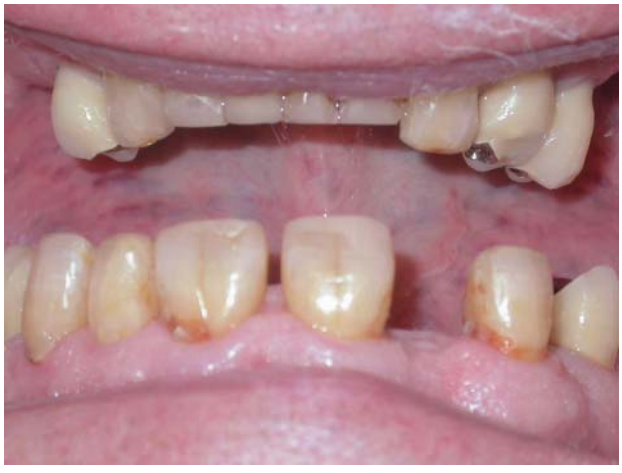

Figure 21: This Patient with xerostomia presented with active root caries in his upper right canine and soft caries on the labial of the upper left central incisor. Each carious lesion was treated for 40 seconds with the Healozone followed by the application of the HealOzone remineralising solution. In addition the Patient was given all the usual dietary and fluoride advice. The Patient was also provided with a HealOzone toothpaste, mouthrinse and spray.

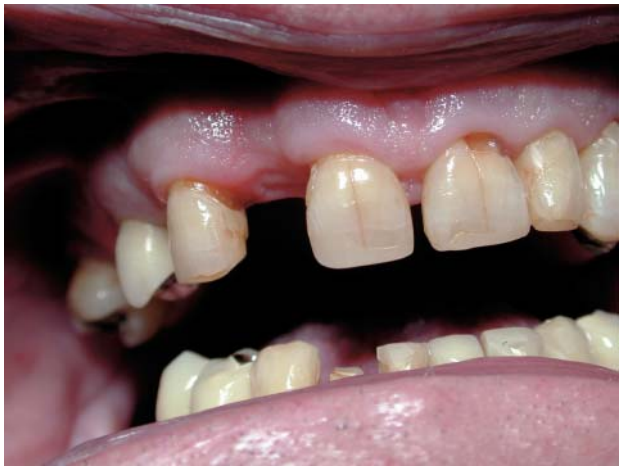

Figure 22: Four weeks later the patient was recalled and both the lesions were now hard and arrested. The central incisor was retreated with the HealOzone and then restored whilst the canine lesion was simply retreated with the Healozone and the Patient advised to continue their preventive program.

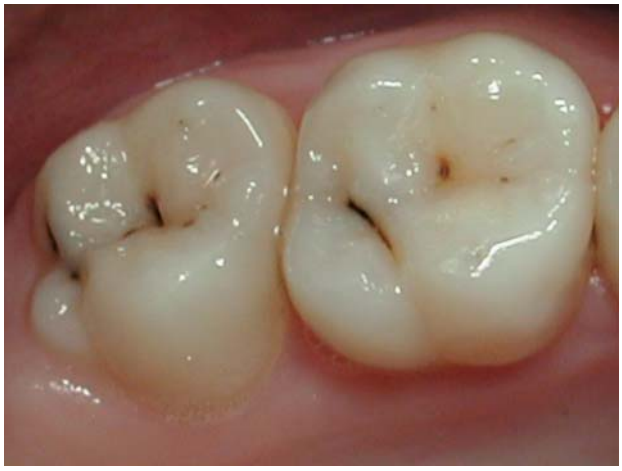

Figure 24: This photograph is the recall appearance of these 2 molar teeth. Both occlusal surfaces had been recorded as having infected dentinal caries, just extending beyond the ADJ, 3 years previously, when they both recorded DIAGNOdent values of 32. Although they are now arrested lesions, it is worth noting that that some Dentists might be confused with this discoloured appearance and mistakenly think this might need drilling and filling. Also some Patients would prefer not to see the discolouration. Therefore, it is recommended that each pit and fissure lesion with any dentinal involvement should be retreated with the HealOzone after 4 weeks, prior to the placement of a flowable composite resin. This also has the added advantage of reducing any chance of a reestablishment of an ecological niche cariogenic microflora.

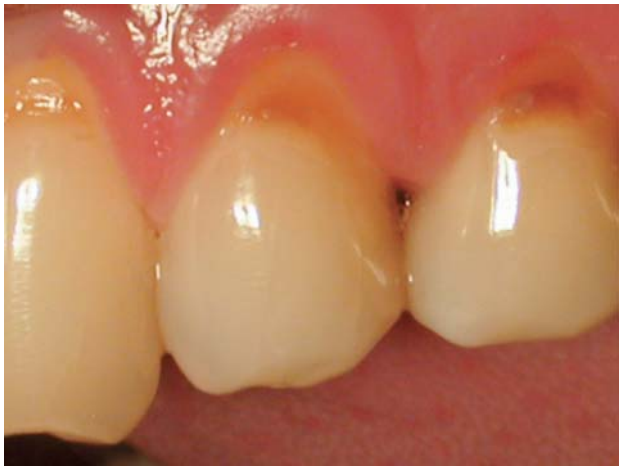

Figure 23: This picture shows the recall view of these 3 root carious lesions which were arrested by the use of a single HealOzone treatment 3 years previously. Of course the Patient was also advised about the standard dietary and fluoride advice.

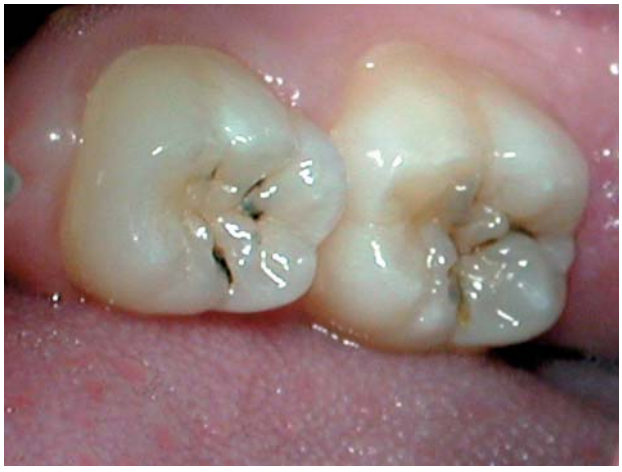

Figure 25: This second deciduous molar tooth shows the 38 month recall arrested result after Healozone treatment of the pit and fissure carious lesion. At baseline the cariuos process was active in this high caries risk child and there was definate infection of dentine occlusally.

Figure 26: It is recommended that during treatment the Ozone be delivered with the source of the Ozone contacting the carious lesion. In this case the metal tube delivering the Ozone inside the HealOzone cup is contacting the tooth in the center of the carious lesion.

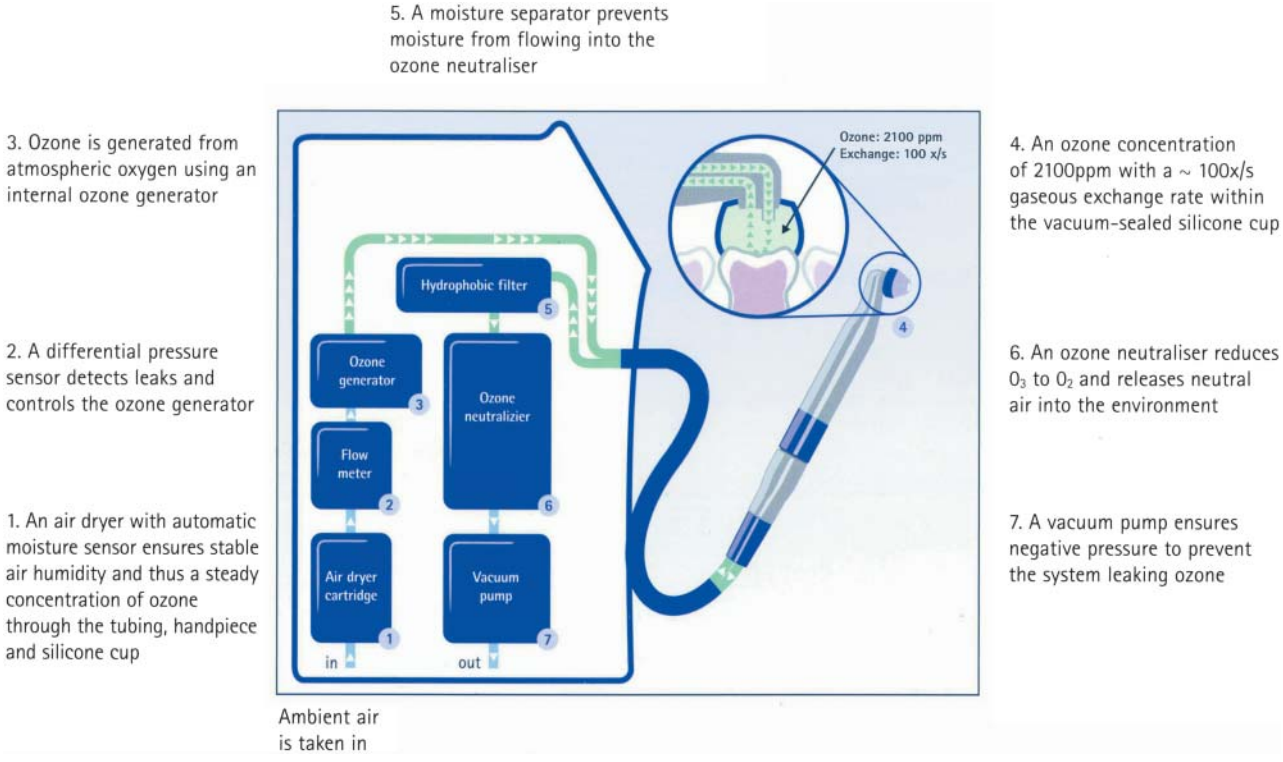

Figure 27: This outlines the method used by the HealOzone to deliver Ozone.

Figure 28: HealOzone Remineralizing Patient kit which contains a toothpaste mouthrinse and spray, with fluoride, calcium, phosphate, zinc and xylitol. It is recommended that Patients use this product at least for the first 4 weeks as part of their HealOzone oral health care program.

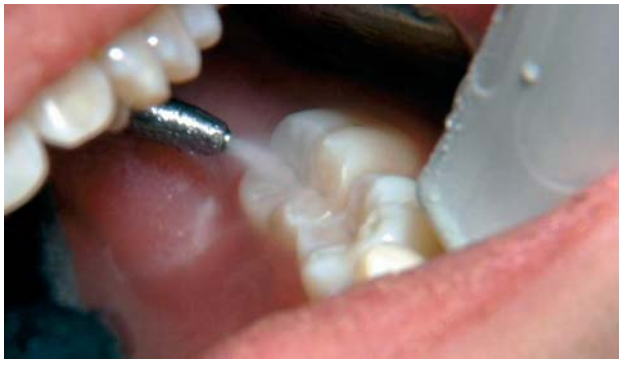

Figure 29: The use of the PROPHYflex (KaVo) is recommended prior to DIAGNOdent (KaVo) and HealOzone application. This thoroughly cleans the surface removing all extraneous plaque, calculus and extrinsic stain which will make the DIAGNOdent reading more reliable as well as allowing the Ozone to penetrate the lesion mre predictably.

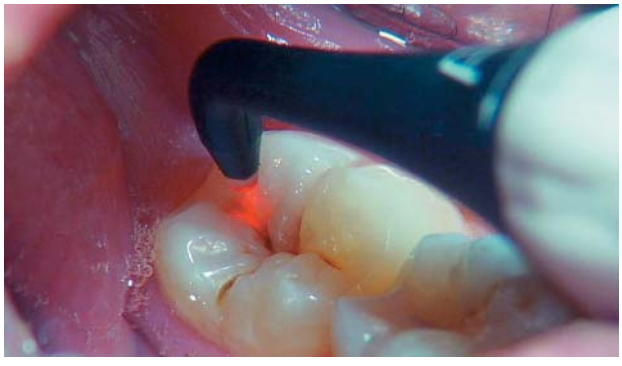

Figure 30: DIAGNOdent readings are recorded after the PROPHYflex cleaning.

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CHAPTER 3.2

The role of ozone in "Minimal Intervention Dentistry"

R. Hickel & K. C. Huth

"Minimal Intervention Dentistry" incorporates different fields of dental science and emphasises the understanding that caries is an infectious disease (Tyas *et al.*, 2000; Murdoch-Kinch and McLean, 2003). "Minimal Intervention Dentistry" is a contemporary philosophy of assessment and management of dental caries in an individual. Primarily, it focuses on the assessment of a patient's caries risk and utilises emerging technologies for early detection of caries and modern modalities of intercepting and treating dental caries. These modalities can be divided into the non-invasive interventions, selective invasive interventions and minimally invasive interventions (Table 1). The use of ozone in dentistry can be classed as a completely non-invasive intervention and its use can be considered either alone or in addition to other preventive or invasive interventions. The application of ozone in dentistry is an emerging technology, one that clinical trials results have proven remineralisation of demineralised and non-cavitated enamel and dentine.

The aim of this chapter is to describe the role of ozone in the non-invasive caries intervention and to report its use at the University of Munich in Germany. Other non-invasive strategies will also be shortly reviewed.

Table 1: Modern modalities of intercepting and treating dental caries within "Minimal Intervention Dentistry"

Intercepting and treating dental caries

1 Non-invasive interventions
- Oral health education / nutrition counsel
- Fluorides (topical)
- Chemical plaque control agents (e.g. CHX varnish)
- Preventive fissure sealing
- Ozone

2 Selective invasive interventions
- fissurotomy
- Chemical caries removal (e.g. enzymes)
- "selective drilling" (e.g. "smart prep" bur, laser)

3 Minimally invasive interventions
- Minimally invasive cavity design / adhesive materials
- Repair of restorations

Dental caries

Today there is an increasing understanding concerning the initiation and progression of caries and it is understood to be a multifactorial, infectious disease that is well-described (Featherstone, 2000). Acidogenic and aciduric, cariogenic bacteria (mutans streptococci and lactobacilli) located in plaque biofilms on tooth surfaces produce acids as metabolic by-products of carbohydrates fermentation (Loesche, 1986). These acids can demineralise the tooth's enamel and dentine. Conversely, the redeposition of mineral from saliva or additional remineralising solutions is also possible resulting in a process of continual demineralisation-remineralisation. This process is a balance between demineralising cariogenic challenges and neutralising, remineralising aspects, such as the salivary buffering and minerals (Featherstone, 1999). When this cariogenic challenge become too frequent or the equilibrium is shifted, especially in relation to the salivary flow, then the rate of demineralisation and subsequent tooth breakdown will increase.

Non-invasive interventions

The focus of all the non-invasive strategies within the philosophy of "Minimal Intervention Dentistry" is centred on the prevention of caries and on a maximum conservation of demineralised but non-cavitated enamel and dentine. In these cases, remineralisation and "restitutio ad integrum" seem to be possible (Silverstone, 1977; Schroeder, 1991) by elimination of bacteria and their by-products and by aiding the remineralising process, for example with fluorides. A non-invasive approach to these caries lesions is also justified on the basis that caries progression in enamel may be very slow. It has been reported to take from 2 to 4 years for a lesion to progress through enamel in freshly erupted first permanent molars and even longer in permanent molars of adolescents (Shwartz, 1984). Once a cavity has been cut, however, a tooth will require lifelong dental care, as usually no restoration will last a lifetime. All further restorative procedures must be carried out on the basis of well understood preventive techniques.

1 Oral health education

Oral health education modified to the individual patient's caries risk may help to prevent, to slow down or even reverse caries progression. Changes in dietary habits focus on limiting the frequency of fermentable carbohydrate intake and of erosive drinks, identification of hidden sugars and advice on positive alternatives (Burt and Pai, 2001). Effective plaque removal by toothbrushing and flossing is another foundation of oral health education.

2 Fluorides

The incorporation of systemically administered fluoride is now thought to have a minor role in increasing enamel resistance (ten Cate, 1991). Fluorides from topical sources affect the process of demineralising and remineralising in several ways, which have been reviewed in detail (Featherstone, 1999; Featherstone, 2000). Fluoride also inhibits bacterial metabolism after diffusing into the bacteria as the hydrogen fluoride when the plaque is acidified. Within the bacteria it acidifies the cell and inhibits essential enzyme activity (Van Louveren, 1990). It inhibits demineralisation of carbonated hydroxyapatite crystals from acid attack. Fluoride enhances remineralisation by adsorbing to the crystal surface and attracting calcium and phosphate ions resulting in new mineral formation on the partially demineralised subsurface crystals in the lesion. The mineral consists of hydroxyapatite and fluorapatite, which is highly resistant to dissolution by acid (ten Cate, 1991). Within the process of caries prevention and reversal saliva plays an essential role, which becomes evident in the case of salivary dysfunction (Mandel, 1974).

There is evidence that caries reduction is most effective when a low concentration of fluoride is maintained consistently in the oral environment (Silverstone, 1984). The main reason for the caries decline in the so called "developed countries" over the past decades has been attributed to water fluoridation and the use of fluoride toothpastes (Marthaler *et al.*, 1989). Many studies have shown a significant decrease in caries increment by the regular use of fluoride varnishes (Marinho *et al.*, 2002; Zimmer *et al.*, 2001). The potential risk of dental fluorosis must always be considered when using high levels of fluoride in children.

3 Chemical plaque control agents

The main role of chemical plaque control agents is the management of high caries individuals, especially for those who are medically compromised. An essential criterion for the efficacy of chemical plaque control agents is their clinical substantivity (Hickel, 1997). Chlorhexidine digluconate has proven to be the most effective of these agents (Matthijs and Adriaens, 2002). The drug has a strong affinity to oral structures and interferes with cell wall transportation and metabolic pathways of susceptible bacteria. Particularly sensitive is the *mutans streptococci* group. A synergistic effect of chlorhexidine and fluoride has been shown in clinical trials (Emilson, 1994). Several studies have shown a significant decrease in caries in permanent molars with the regular use of the 1% chlorhexidine varnish Cervitec® (Vivadent, Schaan, Liechtenstein) and a significant reduction of microorganisms in root carious lesions (Baca *et al.*, 2002; Lynch and Beighton, 1993). A significant reduction of *mutans streptococci* in saliva and plaque could be shown following the use of Chlorzoin® (ICI, Macclesfield, England), a 10 or 20% chlorhexidine acetate varnish (Sandham *et al.*, 1991). However, none of these varnishes could maintain a significant suppression of *mutans streptococci* for a period up to 6 months (Matthijs and Adriaens, 2002), requiring repeated applications and an appropriate use to avoid side effects.

The use of xylitol chewing gum has also demon-

Figure 1: Optical microscope image of a cross section through the main fissure of a molar with a probe at the fissure orifice (original magnification x1.5). This figure shows that caries diagnosis with a probe is impossible.

Figure 2: Optical microscope image of a cross section through the main fissure of a molar with a toothbrush at the occlusal surface (light microscope x2). The image shows that cleaning the depths of the fissures with a toothbrush is not possible.

strated anticariogenic effects as it cannot be metabolized by *mutans streptococci* (Makinen *et al.*, 1995; Tanzer, 1995). Phenols such as essential oils and thymol have also been used as plaque control agents (DePaola *et al.*, 1989). The long-term application of combinations of amine/stannous fluoride or triclosan/additive, for example triclosan copolymer, have been suggested (Zimmermann *et al.*, 1992; Conforti *et al.*, 1994; Hickel, 1997; Schiffner, 2000). Natriumlaurylsulfate and iodine containing antiseptics also have been considered as plaque control agents (Maruniak *et al.*, 1992) but controlled long-term results are necessary to support their use.

4 Preventive fissure sealing

Fissure carious lesions at permanent molars still account for over 80% of the total caries experience in children and adolescents (Anderson *et al.*, 2002). Teeth are especially at risk of developing caries in the fissures within the first 3 years after eruption (Carvalho *et al.*, 1989). Pits and fissures are highly susceptible to caries as they are havens for plaque retention and are almost impossible to clean when they have a narrow and deep morphology (Figures 1–3). In addition, the post-eruptive maturation of enamel as well as the caries protective effect of fluorides is limited in the depths of the fissures. In such hidden areas of the tooth surface, even islands of less mineralised prismless enamel remain behind (Kodaka *et al.*, 1991).

Epidemiological studies showed that the sealing of pits and fissures is an effective approach to prevent pit and fissure caries (Ismail and Gagnon, 1995; Heinrich-Weltzien *et al.*, 1998). To achieve an optimal caries protective effect, the whole fissure system must be sealed completely and without gaps. After application of a sealant a distinct decrease of cariogenic microorganisms within the fissure system was observed (Handelman *et al.*, 1976) and an arrest of dentinal lesions could be shown after fissure sealing (Mertz-Fairhurst *et al.*, 1998). There is still controversial discussion, however, as to the use of fissure sealing over active occlusal caries that might involve dentine (Weerheijm *et al.*, 1992). For many years, there has been a low acceptance of

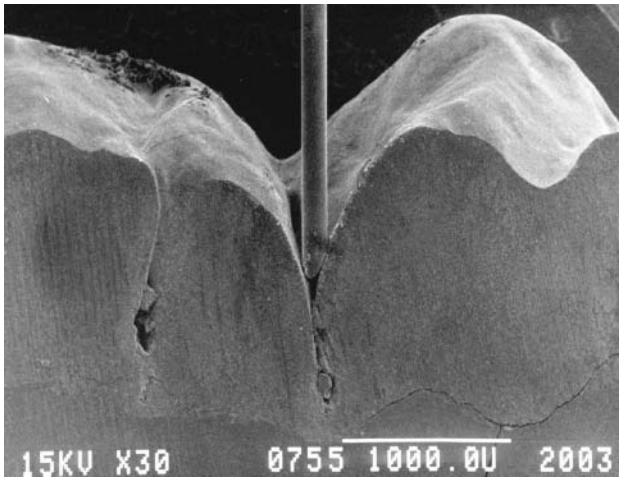


Figure 3: SEM photomicrograph of a section through the main fissure of a molar with a bristle of a toothbrush at the fissure orifice (original magnification x30). This figure demonstrates the relation between the size of the fissures and the size of the bristles of a toothbrush.

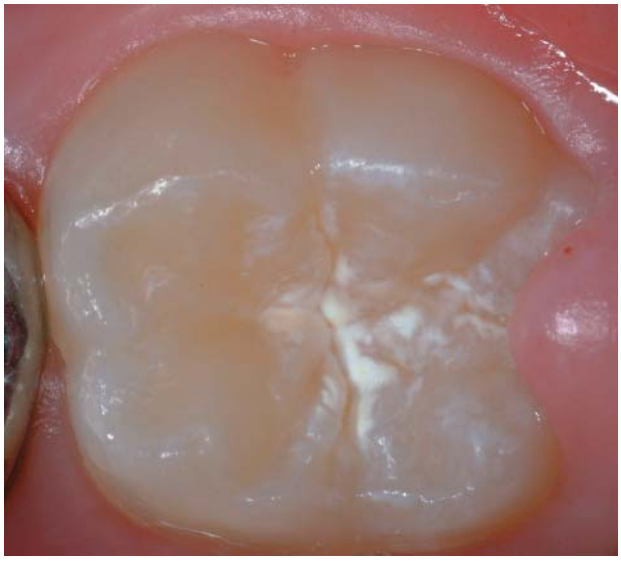


Figure 4: White discoloration of a fissure system

fissure sealing amongst practitioners, because there was a fear that caries could develop further beneath the sealant. Therefore, if there is a possibility to eliminate remaining bacteria or to give an initial carious lesion the opportunity to remineralise before fissure sealing then this may enable a tooth to remain caries free for a longer period of time. Ozone has been proposed as a promising tool to achieve this end.

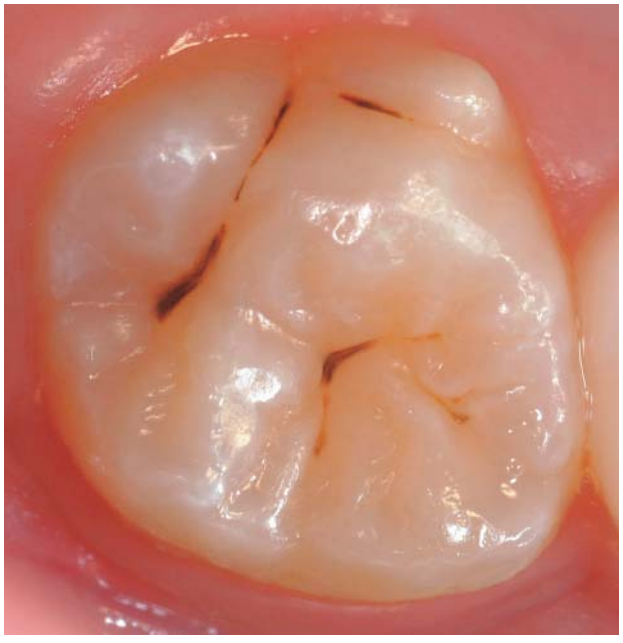


Figure 5: Brown discoloration of a fissure system

5 Ozone

The use of ozone in dentistry is not only considered to be caries preventive but additionally to be a non-invasive approach to treat already existing caries. A significant reduction of caries-associated pathogenic bacteria (*mutans streptococci*) was observed *in vitro* following the use of ozone. Further, there was a significant quantitative reduction in the total amount of microorganisms found in root caries lesions (Baysan *et al.*, 2000). It was also shown that ozone caused oxidation of carbohydrates and potentially demineralising acids within saliva and within carious tooth substance, which might have an additional caries protective effect (Claxon *et al.*, 2002; Mills *et al.*, 2002). Subsequently, the balance between de- and remineralisation within the caries process may be shifted to the promoted remineralisation, enabling healing to take place. Furthermore, such remineralised tooth surfaces are reported to be more resistant against new acid attacks (Featherstone, 2000).

Clinical study using ozone for the treatment of fissure carious lesions in Munich

Recent epidemiological oral health data in Germany has highlighted the incidence and importance of initial carious lesions without visible cavitation in the prevention

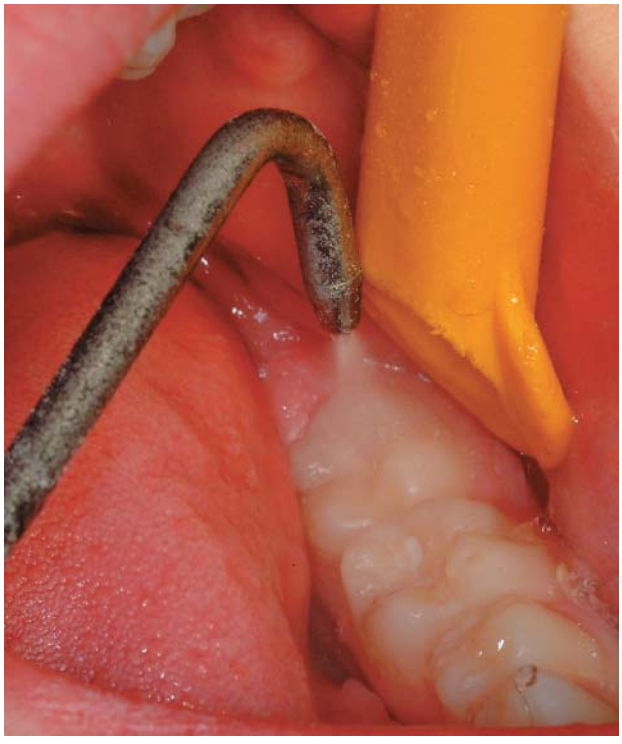


Figure 6: Cleaning the occlusal surface with an airflow

of future cavitated lesions (Micheelis and Reich, 1999). A controlled prospective clinical study using a split mouth design was undertaken. The aim of this study was to investigate the efficacy of ozone over time without using remineralising solutions. Patients were admitted to the study with 2 contra-lateral permanent molars showing non-cavitated occlusal fissure carious lesions within enamel or lesions that extended only to the outer half of the dentine at DIAGNOdent values between 10 and 30 (Figures 4 and 5). The site with the highest

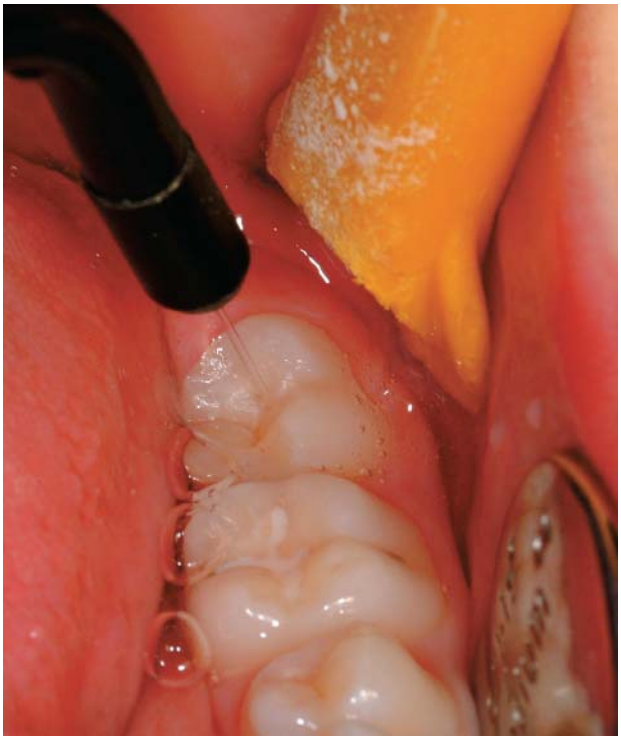


Figure 7: Cleaning the occlusal surface with a thorough water spray

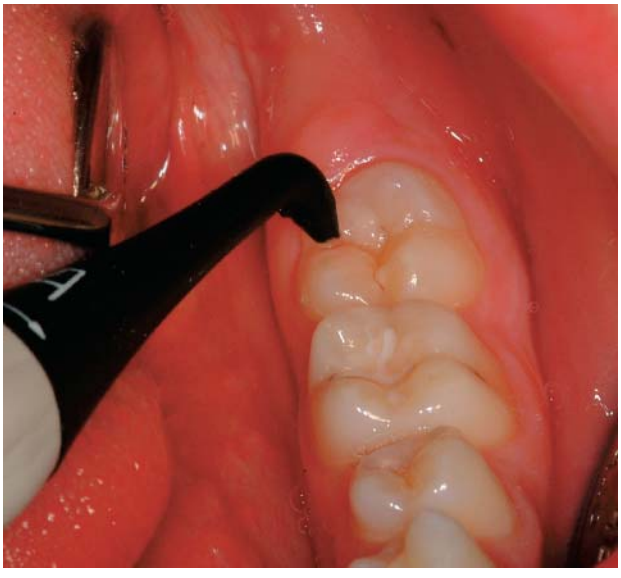


Figure 8: Site-specific recording of the DIAGNOdent reading

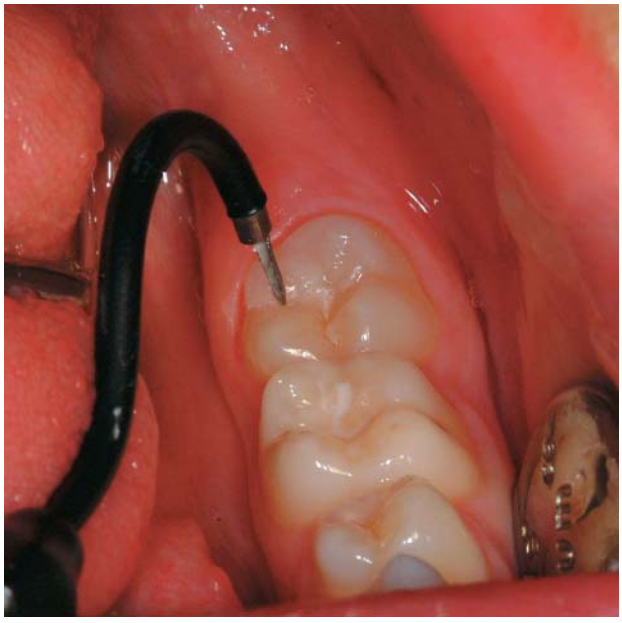


Figure 9: Site-specific recording of the ECM reading

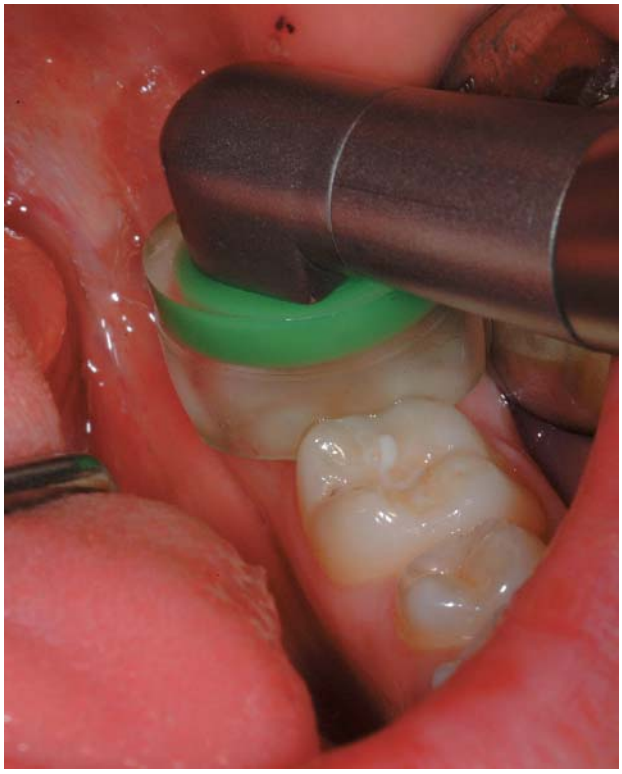
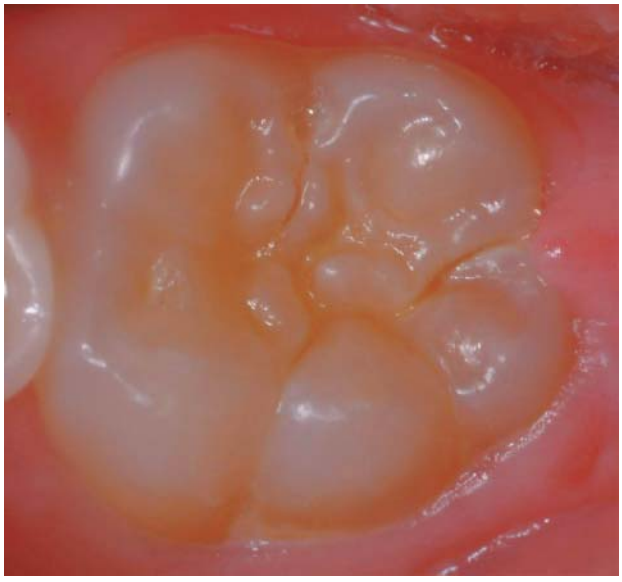


Figure 10: Application of ozone to the occlusal fissure carious lesion



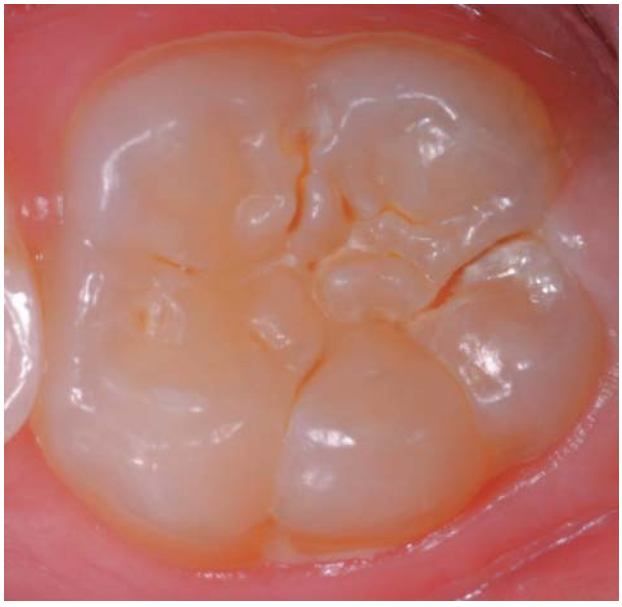


Figure 11 and 12: Visual deterioration of a fissure carious lesion within 2 months

DIAGNOdent value within the fissure system of each tooth was noted, recorded in a drawing and taken as the reference value. Forty-two patients with 57 pairs of lesions were enrolled in the study. Using a silicone-sealed cap via a hand piece connected to an ozone delivery system (HealOzone, KaVo, Biberach, Germany) ozone was applied to the test teeth once for 40 seconds, without additional remineralising solutions to maintain the effect of ozone as isolated as possible. The contralateral control teeth were left untreated. To quantify the effect of the treatment clinically the specific site of the occlusal surfaces was monitored by the laser fluorescence system DIAGNOdent (KaVo, Biberach, Germany) and the electrical conductance recording ECM (LODE Diagnostics, Groningen, The Netherlands). Before each measurement the occlusal surfaces were cleaned with an airflow and a thorough water spray. Figures 6 to 10 show the process of cleaning, monitoring and applying ozone to the teeth within the study. Measurements of test and control teeth were taken immediately after the treatment and after 1, 2, 3 and 6 months and then related to the pre-treatment reference values. Within the test group analysis revealed that there was a significant improvement immediately after the treatment and then after 1, 3 and 6 months. Within the control group there was no significant change in DIAGNOdent or ECM values. Figure 11 and 12 show an example of a visible deterioration of an occlusal caries lesion of a control tooth within 2 months. No adverse effects were recognised during the study. The detailed results of the study will be published soon (Huth *et al.*, 2003).

The outcome of this study is supported by many other clinical investigations that have been undertaken recently. The positive effect of ozone treatment on DIAGNOdent and ECM readings, as well as on the clinical severity index and the visual scoring system by Ekstrand (Ekstrand, 1998) has been reported when treating either fissure caries lesions or root caries lesions (Abu-Nabaa *et al.*, 2003; Holmes and Lynch, 2003; Abu-Nabaa *et al.*, 2003; Morrison and Lynch, 2003; Baysan and Lynch, 2003). These studies differ from the study described above either in the additional use of a remineralising solution directly after the ozone treatment, in the repetition rate of different times of ozone applications or in the observation times. The DIAGNOdent and ECM readings as well as the visual scoring system of Ekstrand have shown good correlations to the histological or clinical lesion depths (Lussi *et al.*, 2001; Ricketts *et al.*, 1996; Ricketts *et al.*, 2002). According to this preliminary data, the use of ozone may be considered an acceptable method of managing early carious lesions. The reported promising results offer encouragement to continue further investigations into the mechanisms of action and clinical applications of ozone in various fields of dentistry.

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CHAPTER 3.3

Clinical Management of Pit and Fissure Caries using Ozone

Layla Abu-Naba'a & Edward Lynch

It is no longer believed that tooth decay ultimately progresses to a frank cavity or a non-vital tooth. Understanding the basic interplay between diet, teeth, and microflora with the influence of other factors led to a fundamental reduction in the total caries numbers in the last two decades (Bratthall et al, 1996). Nevertheless, not all the population benefited equally from this general trend. Vulnerable groups that had not experienced the privilege included: racial and ethnic minorities (Dhawan et al, 2001), the socially deprived (Locker, 2000), the physically disabled (Gugushe, 1991), the medically compromised (Paunovich, 1994), the apprehensive (Klingberg et al, 1995), the psychologically disabled (Ulseth et al, 1991), and the elderly (Reich, 2001).

Within lesions' types, approximal surfaces were the main beneficiaries of the caries reduction in fluoridated communities, around 60%, while occlusal caries had a humble 10% reduction (Ripa, 1993) and thus topped the prevalence lists (Anderson, 2002).

Age is no longer a factor deterring any caries attack. Development of primary occlusal pit and fissure caries (POPFC) in sound occlusal surfaces occurred at a rate of 15% and 10% for ages 8 and 9 years, respectively. From ages 10 through 15 years, pit and fissure caries occurred in previously sound first permanent molars at a rate of 4.3%–6.8% per year. In contrast, approximal caries occurred at an annual rate of 0.3%–2.4%. Forty-two percent of the 17 to 20 year old patients developed occlusal caries (Ripa et al, 1988; Vehkalahti et al, 1991). These studies emphasised that although the majority of pit and fissure caries occur within the first 4 years following eruption, they may continue to occur throughout adolescence and well into young adulthood.

What is unique about the occlusal surface that might render it vulnerable to carious attacks?

Pathogenesis of dental decay on the occlusal surface is similar to any attack on other surfaces. The uniqueness of the occlusal morphology features affects the presentation of these lesions. Some of these features are:

- Enamel surface microanatomy: Surface texture variations from smooth to the most irregular normally occur so frequent in immature and mature specimens (Ekstrand et al, 1999; Galil et al, 1975).
- The narrowness of the fissures was suggested as another factor determining lesion progression. Tapered fissures (<25 degrees) do not develop caries faster than the wider fissures (26–75 degrees), but the distribution of lesions differ. Lesions in narrow fissures stretch to cover the walls from the entrance to the bottom, with the maximum penetration in more than two thirds of the cases at the entrance zone, followed by the middle part and few in the bottom. Wide fissure lesions are confined more to the base. Penetration depth in half of the cases in the bottom part and some at the entrance with no difference between them. The clinical implication of this observation is important. The extent of the lesion could be detected by careful vision of the surface, as the narrow fissures would have most of the lesion at the

entrance whilst the wide grooves are wide enough for the base to be seen. Furthermore, these lesions could be controlled by regular brushing (Bjorndal et al, 1995; Ekstrand et al, 1995, 1997).

- Surface porosity was suggested as a contributing factor. But no major differences were found between fissure and lingual enamel, neither with respect to mineralisation pattern during final stages of tooth development, nor to the degree of surface porosity prior to tooth emergence. Those two factors were dismissed from explaining why occlusal surfaces are the surfaces most prone to caries (Ekstrand et al, 1999).
- Plaque accumulates in the fissure's protected environment depending on the tooth morphology, functional use and stage of eruption. Thus, the carious process was described as an θunpreventable ubiquitous processö (Ekstrand et al, 2001). Permanent molars in full occlusion have less plaque than partially erupted teeth, most probably due to the functional usage of the occlusal surfaces. A relation was found between plaque and enamel demineralisations whilst none was found between the clinical accessibility of the fissures for cleaning and the demineralisation. Thus, clinical identification of caries susceptible areas on occlusal surfaces should be based on the actual plaque accumulation (Carvalho et al, 1992).
- Duration of tooth exposure to caries attack. Once in occlusion, another risk is present if. Total length of time since eruption in the wisdom teeth till full occlusion to occur, if ever, correlated with deeper (dentinal) lesions (Ekstrand et al, 1997). The rates at which dentinal lesions develop are faster in the occlusal caries than in the smooth surface caries. Lesions in dentine were seen in teeth just over one year after eruption (Ekstrand et al, 1997) whilst the smooth surface lesions were noticed after a range of two to four years post eruption (Pitts, 1983).
- The direction of the progression of the lesions follows the direction of the enamel rods and the lesions therefore usually had a broad base near the dentino-enamel junction (DEJ) (Thylstrup, 1987,1989; Bjorndal et al, 1995). Dentine demineralisation never occurs before established contact between the enamel lesion front and the DEJ. So dentine demineralisation is confined to the contact area between them and never spread along the junction laterally as was previously assumed (Silverstone et al, 1985; 1988). It is only in cavitated advanced lesions where the lateral spread occurs (Ekstrand et al, 1998). Lesions have a 'closed' nature due to their advance along the pattern of enamel rods which has this anatomical arrangement and configuration (Ekstrand et al, 1995; Thylstrup, 1989; Thylstrup, 1987). This lead early lesions to be missed clinically and radiographically, as these are small and surrounded by sound enamel.
- Even before caries reaches dentine or the DEJ, the reactions of the odontoblasts and mesenchymal undifferentiated cells begin to block off the carious attack. Evidence of this was even seen in active lesions which were not detectable clinically (Smith et al, 1994). Gradients of acidity, established in the lesion and the cariogenic plaque complex, may have played a role in this early dentinal reaction and sealing off either directly or indirectly (Bjorndal et al, 1998). In more advanced lesions, the pulp is blocked off by a layer of atubular dentine and reactionary dentine formation originated from the sub-odontoblastic region and which is known to have no regular tubules reaching to the pulp.

How does remineralisation occur?

The chemical change that takes place in enamel crystal structure during the caries attack may help explain how remineralisation occurs in lesions. In the active stage, acid cause ions to dissolve from the body of the enamel lesion and redistribute in the structure of other crystals. Adjacent crystals, unaffected by the acid, adsorb these dissolved ions and grow to accumulate a higher mineral content, hypermineralised. Surface crystals on the roof of the lesion take up some of those dissolved minerals. As the attack continues, dissolved calcium and phosphate ions diffuse out of the tooth structure, across the enamel and plaque layers. With continuous lesion progression, the hypermineralised roof of the expanding lesion becomes thinned to a point where it can't support the lesion beneath and collapses (Bjorndal et al, 1998). Probing a lesion at this stage might disturb the surface and significantly reduce its ability to remineralise (Robinson et al, 2000; Shore et al, 2000; Kirkham et al, 2000).

If the acidic attack is reduced, the minerals reshuffle

and the hypermineralised crystals leave their extra ions for the adjacent, hypomineralised crystals within the lesion. Further minerals diffuse from the saliva and plaque through the pores and channels in both the overlying plaque and lesions roof enamel into hypomineralised tissue. Fluoride is present in the oral fluid, plaque, and in higher concentrations in the outer 5 microns of the surface enamel. It decreases the total amount of acid that could cross the plaque-enamel interface. When the pH of the plaque drops fluoride is readily transported across the walls and into the cells of plaque bacteria. Fluoride ions in dissolved liquid phase within the hydration layer of surface enamel encourage remineralisation of partially demineralised crystals using mineral derived either from other crystals or from the plaque. The inclusion of fluoride into enamel crystals at the expense of other ions, such as carbonate, increases the crystallinity (Robinson et al, 2000).

Remineralisation is not only observed in enamel, but also in dentine. The pores within demineralised dentine lesions become supersaturated by apatite formation. The results suggest that dentine remineralisation, underneath enamel, can be achieved and could possibly be used in clinical treatment strategies (ten Cate, 2001; Arends et al, 1990).

What inhibits active lesions from remineralising in the hyper saturated oral environment?

The oral environment is rich with minerals needed for remineralisation, but inhibiting molecules protect active lesions from mineralisation of crystals.

Inhibition ions as Mg^{+2}, HCO^{-3} and proteins might be either endogenous (i.e. as a remnant of the developmental process) or exogenous, originating in the saliva or gingival crevicular fluid. Albumin and amylase are two inhabitant proteins found normally on the surface of enamel that prevent over growth of the tooth's crystals and protect from demineralisation (van der Linden et al, 1989; Arends et al, 1986). However, incorporation of these proteins into porous enamel occur at the surface of proximal and fissure white-spot caries, to a lesser extent in deeper regions (Robinson et al, 2000; Shore et al, 2000; Kirkham et al, 2000). Removal of these proteins increases the potential of tissue to remineralise (Inaba et al, 1995, 1996).

Other proteins and molecules are produced by the cariogenic micro-organisms to maintain their acidic environment. Amongst molecules are formic and pyruvic acids that contribute substantially to the decreased pH values associated with active caries lesions and inhibit the precipitation of the minerals (Silwood et al, 1999, 2002).

Levels of ozone activity on carious lesions

The aim of ozone is to shift the chemical balance promoting remineralisation of crystals of tooth structures. The following points are key effects through which ozone influence this activity (Fig. 1).

Anti-microbial effect

Ozone has long been used to reduce pathogenic micro-organisms (Bocci, 1999; Brauner, 1991). In dentistry, *C. albicans*, methicillin-resistant *Staphylococcus aureus* (MRSA) and T1 phage were significantly reduced after using 10 ppm ozonated water (Murakami et al, 1996). Cellulitis and gingivitis in adult subjects has been treated with an ozone solution (Lazutikov et al, 1996; Kiniapina et al, 1996; Brauner, 1991). Not only was there an antimicrobial effect, ozone produced a higher expression of cytokines that are important for wound healing, had a marked influence on cell proliferation,

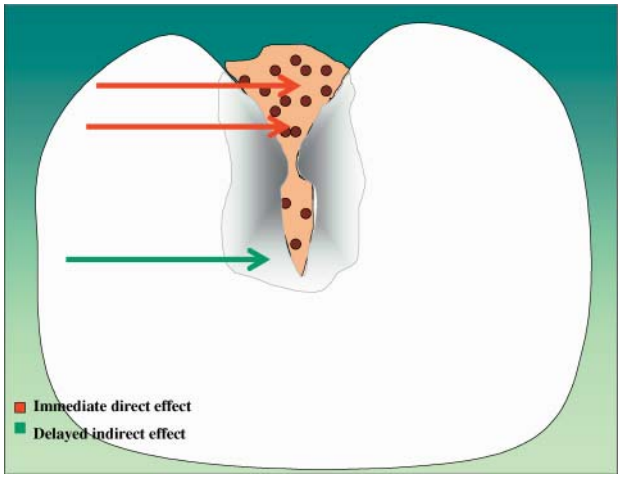

Figure 1: Levels of ozone effects on active occlusal carious lesions.

chemotaxis (monocytes and fibroblasts), angiogenesis, synthesis of extra cellular matrix and collagen synthesis. Ozonated water is effective in the dental surgery where it was reported to promote haemostasis, enhance local oxygen supply and inhibit bacterial proliferation. Ozone in 10 and 20-second gas exposure times and ozonised water reduced cariogenic bacteria (*S. mutans* and *S. sobrinus*) (Baysan et al, 2000).

Ozone effect on proteins

An important effect that ozone has on lesions is its strong oxidative effect on proteins. High Proton Nuclear Magnetic Resonance (^{1}H NMR) system is used for the analysis of biomolecules. Following 10-second ozone treatment, analysis of plaque, saliva and root carious biomolecules essential for the process of demineralisation of lesions to occur demonstrated their consumption by the powerful oxidative ability of ozone gas in the concentration proposed (Grootveld et al, 2001; Lynch et al, 2001, 2002). Amongst these cariogenic molecules are formic and pyruvic acids; that contribute substantially to the decreased pH values associated by inhibiting precipitation of the minerals (Silwood et al, 1999). Pyruvic acid oxidised by ozone forms acetate and carbon dioxide compounds associated with higher pH values (Lynch et al, 2002). The effect of ozone on the proteins of non-cavitated occlusal carious lesions was demonstrated by using DIAGNOdent readings, a laser quantification of fluorescent lesions' microbial proteins, as porphyrins. An immediate reduction in the readings was noticed in freshly extracted teeth and in clinical situation intraorally. Protein alteration most probably causes this immediate reduction in fluorescence. Sound surfaces had not changed DIAGNOdent readings after ozone treatment, while the larger the lesion, the larger was the reduction (Abu-Naba'a et al, 2002a, 2002b, 2004).

Table 1: Clinical severity index scores. (Ekstrand et al, 1998)

Score	Description
0	No or slight change in enamel translucency after prolonged air drying (>5s)
1	Opacity (white) hardly visible on the wet surface, but distinctly visible after air-drying (>5s).
1a	Opacity (brown) hardly visible on the wet surface, but distinctly visible after air-drying (>5s) .
2	Opacity (white) distinctly visible without air-drying.
2a	Opacity (brown) distinctly visible without air-drying.
3	Localised enamel breakdown in opaque or discoloured enamel and or greyish discolouration from the underlying dentine.
4	Cavitation in opaque or discoloured enamel exposing the dentine beneath.

Ozone effect on demineralised dental tissue

Once the above effects have been established, remineralisation becomes possible as an indirect delayed effect. Lesions were measured intra-orally using electric caries monitor (Abu-Naba'a, 2002a,2002b), that is dependant on the mineral content affecting tooth resistance. Before and after ozone treatment, no significant resistance change was present as no immediate mineral shift had occurred. The difference was present in one-month recall.

Effects of ozone on non-cavitated occlusal carious lesions

Study 1

The first study aimed to assess the ability of 10-second ozone application to remineralise lesions of varying clinical severity scores before frank cavitation. All lesions were cleaned using an air-polishing system then recorded using the following diagnostic tools (Abu-Naba'a, 2003):

Table 2: DIAGNOdent scores and possible clinical inferences (DIAGNOdent. manual 1999)

Score	D value	Description
0	<10	Sound
1	10–14	Enamel caries in the outer half
2	15–20	Caries in enamel up to DEJ
3	21–30	Caries in enamel reaching DEJ with some dentine demineralisation
4	≥31	Deep dentinal caries

Table 3: ECM scores, corresponding *end values* and possible clinical inferences. (ECM Manual, 2000)

ECM Score	Corresponding ECM *end value*	Possible clinical inferences
0	>10.00 MΩ	Sound enamel
1	10.00 to 2.50 MΩ	Lesions up to DEJ
2	2.5 to 0.151 MΩ	Lesions in dentine
3	Equal or less than 0.150 MΩ	Deep dentinal lesions

- The validated Ekstrand's clinical severity scores (Only scores 1–3; Table 1)
- DIAGNOdent readings and scores (Only scores 1–4; Table 2)
- ECM readings and score (All scores 0–4; Table 3).

Subjects were enrolled after signing informed consents.

Lesions meeting inclusion and exclusion criteria were entered in the study and randomised into groups 1 and 2.

Group 1: Teeth received the ozone treatment for 10 seconds.
Group 2: Teeth had only the cleaning.

The treatment and control lesions were designed to be in the same individual because changes in treated lesions are not expected to occur equally in different individuals. The reason for this is the variability of individual caries risk factors as sugar intake, oral hygiene habits, possible age related factors, salivary bacterial counts, social and racial variables may determine treatment outcomes. This subject variability in response to the treatment was significant when ECM results were further analysed.

Table 4: Clinical criteria used to assess all lesions within the treatment and control groups at the final recall visit

Plaque accumulation
- None seen after disclosing
- Seen after disclosing in the bases of pits and fissures
- Seen after disclosing over the pit and fissure walls
- Seen generalised on the occlusal surface

Eruption status
- Total occlusion of the tooth
- One side occlusion
- No occlusion but occlusal surface clear from soft tissue
- Soft tissue partially covering the occlusal surface

Surface destruction as seen after further plaque removal by the probe and drying
- No cavitation
- Microcavitation
- Frank cavitation

Texture of the lesion assessed by dragging the probe from sound to decayed walls
- Smooth
- Rough

Hardness of the base assessed by dragging a probe from sound fissures into lesion
- Hard base as adjacent sound groove or pit
- Leathery, different than sound bases
- Soft

Continue next column

Table 4: Cont.

Frostiness of enamel at the margins
- None seen after 5 seconds drying
- Seen after 5 seconds drying
- Seen with out drying and increase in severity when dried.

Undermining enamel
- None
- Confined to the fissure and pit margins
- Generalised under the walls of pits or fissures

Colour of the lesion
- Yellow
- Light brown
- Grey
- Dark brown
- Black

Clinical judgement
- Sound
- Carious
- Arrested

Depth of radiolucency from bitewings, if available within the last-month recall visit
- None
- In enamel outer half of enamel
- In enamel inner half of enamel up to the DEJ
- In outer 1/3 of dentine
- In middle 1/3 of dentine
- In inner 1/3 of dentine, approaching pulp

Table 5: Distribution of POPFC lesions within the treatment and control groups according to lesion location and baseline clinical severity scores

Clinical severity scores	Group 1 (Treatment, 10s O_3)		Group 2 (Control)	
	Number	%	Number	%
Score 1	63	49.6	79	62.2
Score 2	53	41.7	39	30.7
Score 3	11	8.7	9	7.1
Total	127	100.0	127	100.0
Mean (±SE)	1.591±0.056		1.449±0.056	
Mesial	12	9.5	21	16.4
Central	63	50.0	65	50.8
Distal	51	40.5	42	32.8
Total	126	100.0	128	100.0
Premolars	36	27.9	49	38.0
Molars	93	72.1	80	62.0
Total	129	100.0	129	100.0

Teeth were recalled and examined at 1, 3, 6, 9 and 12 month and only Group 1 received the ozone treatment at the first 4 recalls. Such repeated doses would not elicit any concern, as there were no reports of resistant micro-organisms to ozone gas. A final clinical assessment of the tooth's status was performed as in table 4 and to assess further treatment needs and disclosing tablets were used to assess plaque accumulation. Ninety subjects above 12 years old were enrolled into the study. Sixty percent were females and around 40% were males. The attendance afterwards was variable. Subjects who attended all 5 recall visits were 58 (104 lesions in each group).

The total number of lesions entered in both treatment and control groups (Groups 1 and 2) was 258 lesions. Lesions incorporated in the study, were various and covered the spectrum of all lesions presentation before frank cavitation (Table 5). Furthermore, using the entry criteria of the study, lesions were entered in age groups over 30 as well as younger groups and within both sexes, this might be a reflection that age was no longer a factor deterring any caries attack (Vehkalahti et al, 1991). Teeth were in the mouths of subjects for more than a year and had full eruption status. However, this didn't mean that all teeth were in full occlusion.

Ozone is delivered by a handpiece with a resin cup that seals over treated teeth. Accessibility of lesions to the ozone handpiece was not limited. Rotated teeth, inclined upper third molar teeth, teeth with close gingival margins, tight contacts, deep developmental grooves, orthodontic brackets and hyper active tongues were some of the clinical factors which were present in this study and did not interfere with the seal of the ozone delivering cup. Training improved the selection of cup sizes while attempts to seal the tooth didn't exceed 5seconds. Situations as a deep buccal or lingual vertical groove or even prominent cusp of Carabelli were solved by a gentle squeeze by the operator's fingers on the edges of the cup to produce the seal.

Slippage of the resin cup from the tooth occurred where teeth were not adequately dried from saliva. Once this happened the device immediately senses the loss of negative pressure and no ozone is delivered in the hand piece. The vacuum however stays working, taking up any remnants of ozone in the cup. There is no risk of recounting for a new 10-second ozone delivery cycle if it was interrupted as the device stops on the exact time point and continues from the same point after the seal is regained.

Diagnostic systems used in this study have many advantages. Validation studies were performed extensively and demonstrated the superiority of the three methods over conventional diagnostic techniques. At baseline, the outcomes of these tools correlated with each other and both groups had an equal share of lesion severity. Using these devices together helps integrate results obtained by each into a wider picture in attempt to overcome their individual limitations.

ECM VI has more than one method for drying and recording lesions. End-value readings, displayed by the ECM, were chosen for the various calculations as they represented the resistance status of the tooth where all clinical variables are reduced to the minimum. Although the clinical severity score and DIAGNOdent readings picked up some lesions to be carious, many lesions had an ECM reading comparable with sound teeth. This might have been by those lesions having a significant amount of regression or remineralisation even before the moment of diagnosis. This further demonstrates the limitations of the DIAGNOdent device and the clinical severity scores to detect carious activity rather than severity and emphasises the need to use more than one diagnostic tool to quantify lesion.

Results

Change in the ECM readings

A remineralisation difference between treatment and control croups was significant early at the 1-month recall visit. Later, the treatment group had a mean change that was significantly higher (better) than baseline at two recall visits, whilst the control group had its changes significantly lower (worse) than the baseline values on two recalls ($p<0.05$) out of the five recalls. The mean change throughout the whole year showed the treatment group being significantly higher than baseline and than control group. Mean ECM end-value readings change over this period was 0.337 for the treatment group and -0.065 in the control group in the overall recall visits ($p<0.05$). A repeated analysis of variance showed a mean difference of ECM readings for the treatment group 56% higher (better) than the control group in all recalls regardless to lesion location in tooth or jaw or lesion type.

The highest ECM change in the treatment group and control group were at the 3-month recall visit with the maximum number of lesions changing and the maximum mean ECM change. This effect might be because of the multiple doses at baseline and 1 month recall. When of the mean ECM change at 9 months were analysed for the number of treatment visits attended, more frequent ozone was given, then the higher the magnitude of remineralisation. The means showed that those attending all visits in the treatment group had the highest (best) ECM change (mean$\pm$SE= 0.28$\pm$0.13) followed by those in the treatment group who missed only one visit (0.06$\pm$0.2). Mean ECM change for control group who missed one visit was ($-$0.06$\pm$0.2) and finally by those in the control group who missed no visit was ($-$0.29$\pm$0.13). The 10-second application was not consistent in producing long-term shifts in ECM readings. If a sealant were considered, then it would be most appropriately applied after 3 months and up to 9 months after ozone treatment (where a significant difference between the treatment group and the control group were present).

The lowest ECM change in the treatment group was at the 12-month recall visit. This may have corresponded with the lowest dose of ozone delivered at 9 months. The device was checked for the ozone delivery at the 12-month and was found to be 30% of the maximum 2,100 ppm, i.e. 630 ppm. Possible reasons were possible need to change the internal desiccant change or possible electric voltage change as this device had been possibly handled roughly by being carried on 8 flights. There is a clear need for devices to be monitored for ozone concentrations.

The lesions in the control group also had a minimal but positive ECM change at the 3-month recall visit probably by having the lesions maximally benefiting from the cleaning. After those lesions that were able to be remineralised were achieved, then deep lesions lose minerals by further microbial activity, so the mean ECM change declined after this initial rise.

For the pooled data the following factors were tested for any contributing effects. Gender, age, tooth location within jaws (upper or lower) or within the jaw (tooth number) were not significant ($p>0.05$). For the lesion itself, its location (mesial, central or distal) or type (pit or fissure) was not significant ($p>0.05$). The only significant factors determining the mean ECM change in all recall visits were the baseline scores, which were derived from the readings, detected by the three diagnostic tools.

The possibility of including lesions from the 'hidden' carious type would be expected not to be a problem for ozone treatment. The variable micro-organisms involved in hidden lesions (Weerheijm et al, 1990) were proven to be sensitive to the ozone oxidative effect (Baysan et al 2000). Clinically, the access of the gas to those lesions proved to be adequate as there was a significant improvement in ECM change in lesions with DIAGNOdent readings below 40.). Evidence is that micro-organisms do not spread laterally in the DEJ before cavitation (Ekstrand et al, 1998), thus the penetration of the ozone such lesions is adequate. Those with larger lesions appearing on radiographs should be followed up carefully as there has been no research yet regarding the depth of penetration of ozone treatment in deep dentinal caries. Longer ozone application time and higher doses should be tested for efficacy. A combination of ozone with minimal invasive procedures should be assessed for this problem.

Benefits of localised ozone treatment confined to the tooth surface would overcome the limitation faced by other pharmaceutical managements of biofilms. Chlorehexidine for example that tends to have a generalised effect, possibility of developing micro-organisms' resistance, flourishing of opportunistic micro-organisms and other effects as staining of the tongue, teeth with

the development of calculus and altered taste (Addy et al, 1995). None of these effects were recorded in any subject who was treated with ozone and followed up for whole year.

Any concern that ozone could reach the pulp through dentine might be reduced by very early dentine reactions to early enamel lesions. Cellular changes occur before half of the enamel layer is involved in the caries. First signs of enhanced peritubular mineralisation and production of bundles of collagen fibres in the predentine are noted when the enamel lesions approach the DEJ with no dentine demineralisation. In more advanced stages of tertiary dentine formation, atubular dentine suggests a more complex response to early caries (Bjorndal et al, 1998).

There were no excavations or minimal preparations of the decayed tissue in the teeth included in this study so ozone was away from the pulp. However, a positive effect of the ozone on the pulp might be more likely as there have been studies done by Bocci and colleagues on effects of ozone on the blood. They found an up regulation of immune responses and antioxidants which overwhelm the initial ozone trigger and last for longer periods, and thus producing a net of positive effects on inflammatory and autoimmune diseases (Bocci et al, 1994).

Clinical severity index change

The clinical severity index used score features as present or not. Detecting the clinical change after 10 seconds of ozone treatment and using the total clinical severity index was not possible in the year recalls. However, an interesting finding was that the lesions in the treatment group were scored different from those in the control group. The changes occurred from baseline up to the recall visit at 6-month and then halted. Moreover, where changes in the control group were a steady deterioration towards a higher clinical severity scores, changes kept taking the means of both groups apart where there was a tendency to higher score changes after one year occurring in the control group than the treatment group. The percentage of teeth where the clinical severity score decreased was 10% more in the treatment group, whilst those that increased was 5% more in the control group. Further recall visits might be needed to monitor and prove if this trend was continuing as expected while using the same diagnostic method.

Mechanism for the previous change in the clinical severity index score is different for both groups. As there was no significant interfering factor in the control group lesions, then it might be natural to propose that the change was a steady deterioration of the health status of the lesions if left untreated. Changes in the treatment group should be further analysed considering the findings of the operator in this study where not all clinical changes could be detected or quantified using this 4 score system. Examples for this is a lesion with score 1 might incorporate more stain while remineralising (better ECM change is accompanied). This darker stain is picked as a higher clinical score. Differentiation between various tactile sensation data of roughness and hardness changes that could be picked up by a probe dragged in the lesion. Furthermore, it was not possible to quantify minor changes of single features as reduction of frostiness rather than recording only its total disappearance.

Using the incorporation of stain into lesions in the treatment group as an explanation for lesions getting higher clinical severity scores while having the ECM give signs of remineralisation was consistent with other clinical findings in the course of the study. Although colour was not recorded at baseline, lesions within the treatment group became darker with an increase in the smoothness of the walls and hardening of the base. This was consistent with the review of lesion presentations (Nyvad and Fejerskov, 1997). Another change was noticed at the borders of lesions within the treatment group. The borders of lesions, as seen clinically, were used in the protocol to mark locating the ECM probe in the 4 subsequent readings within their margins. The operator found the range of readings at the borders became higher than that for the deep area as if the lesion was narrowing. This might indicate more remineralisation at the borders.

DIAGNOdent change

The mean change over the 9 months recall visit was significantly higher for the control group from baseline ($p<0.05$) but not significantly higher (worse) than the treatment group ($p>0.05$). Results from the last visit

were dismissed after interference of plaque disclosing tablets with lesions records.

Lesions within both groups behaved similar to their response as detected by the clinical severity score system. Explanations could be comparable, taken that DIAGNOdent readings are sensitive to stains, giving a higher (worse) reading than without staining. Furthermore, there is the immediate in readings by the mere effect of ozone regardless to changes due to lesions activity. These interfering factors should be considered when implementing DIAGNOdent readings clinically. At the first recall visit, the number of treatment group lesions with no DIAGNOdent score changes was 61.7%. These reduced slightly at the 3-month recall visit and remained stable up to the 9-month recall visit at 57.1%. The number of teeth with decreased scores was gradually increasing up to the 9-month recall visit (from 12.5 to 25%) at the expense of those with increasing DIAGNOdent scores (from 24.3% to 17.9%). There was only a trend for teeth within the treatment group to have better DIAGNOdent score change than for the control group ($p=0.097$). For the control group, no significant changes occurred.

Baseline scores obtained by the diagnostic tools were the only significant co-factors when the mean change of the ECM (end value) readings was tested. They will be analysed separately, to detect how various methods of categorising lesions at baseline affect the way the tools pick up the change.

Lesions categorised by baseline clinical severity score

There was a significant difference between treatment and control groups mean ECM change over all recall visits due to the ozone treatment. A totally different picture emerges when these results are divided by baseline clinical severity score. Those in the baseline clinical severity score of 1 had a mean improvement in ECM readings for the treatment group. This suggested higher ECM resistance measurements most probably by more incorporated minerals. This same group had a mean increase in clinical severity scores and DIAGNOdent readings. So lesions that were seen to get darker clinically and thus changing to a higher clinical score were actually incorporating more stains into them due to remineralisation. This stain was also picked by DIAGNOdent higher (worse) change. The strong positive increase in the DIAGNOdent readings by these stains overwhelmed two negative changes expected to reduce them. First is the immediate reduction expected by ozone, applied at each visit. The second is the reduction in lesion activity, as found by analyses of the ECM readings. For the control group, with no interfering ozone treatment, all readings from the three diagnostic tools used, were heading in the same direction, i.e. getting worse. Score 3 had only 8 lesions and results could not be drawn for this category.

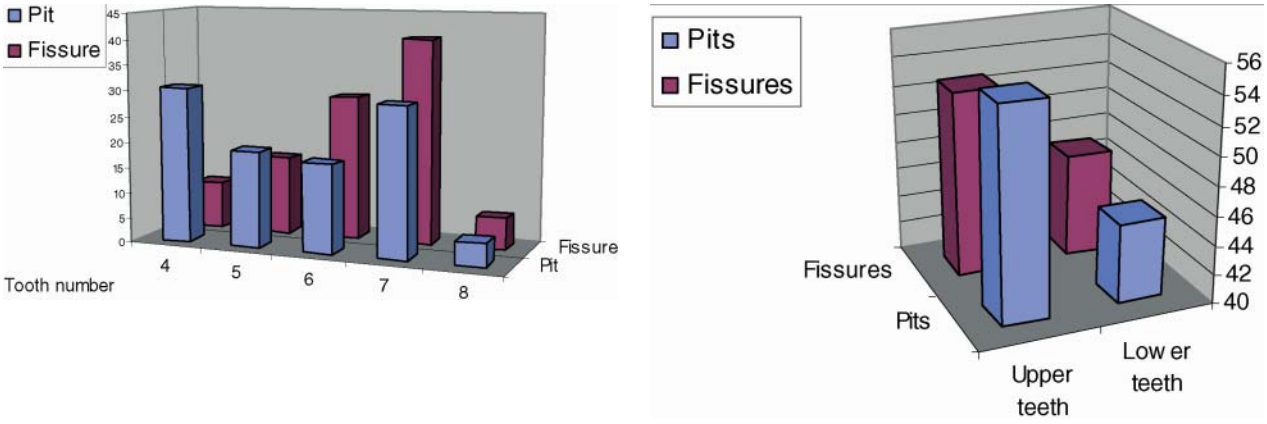

Figure 2–3:
Distribution of lesions enrolled in the main study according to surface type and teeth numbers and according to surface type and location within the jaws.

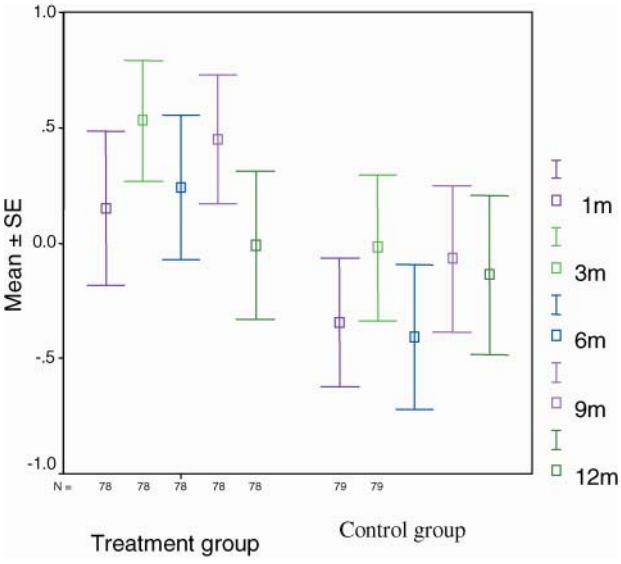

Figure 4: Mean Log_e ECM (end value) changes in each recall visits from baseline (m: month of recall). N: Number of lesions.

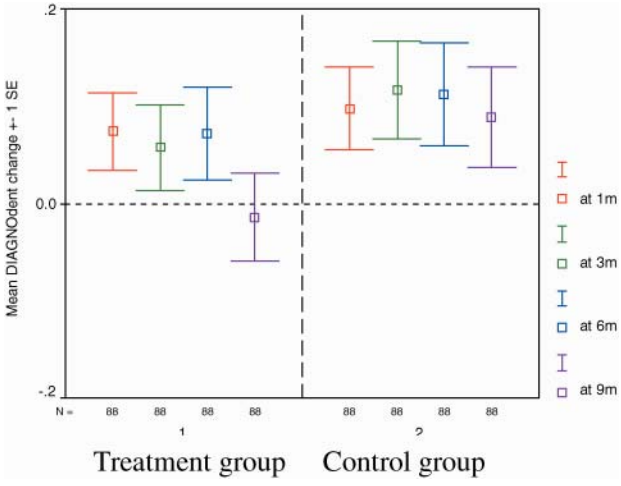

Figure 5: Mean (±SE) change in the Log_e DIAGNOdent readings from baseline to the 1, 3, 6 and 9-month recall visits (m: month of recall). N: Number of lesions. Note, a lower change is better for DIAGNOdent readings.

Lesions categorised by baseline DIAGNOdent score

Categorisation of lesions by the Baseline DIAGNOdent reading is better for comparisons as it had proved to have better performance than conventional and clinical diagnosis within validation studies. As the control group was not affected by the ozone and related changes discussed earlier, it is still reliable in detecting lesions

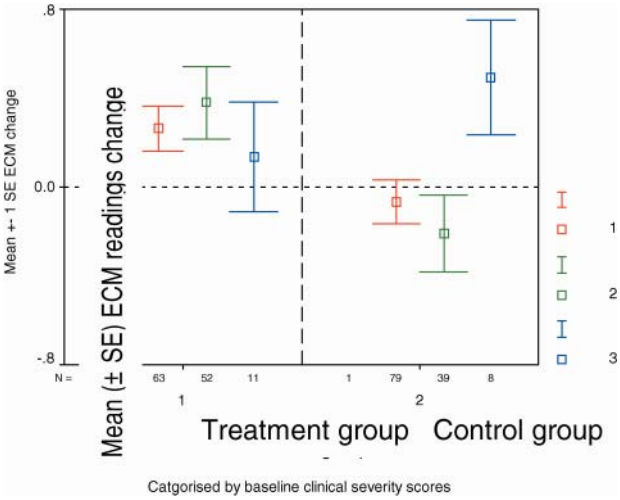

Figure 6: Mean (±SE) change of ECM readings over the 12-month period categorised by baseline clinical severity score. N: number of lesions.

depth within it. It is only in the recalls after ozone treatment where DIAGNOdent becomes limited. Using this tool also aided in the prediction of lesions, which could benefit from cleaning only (initial enamel lesions within the control group) and those that would benefit from ozone (all none-cavitated lesions, where even the most severe benefit by being stabilised).

Only enamel lesions up to the DEJ in the control group benefited from the cleaning by having the net ECM readings change to positive (DIAGNOdent baseline scores 1 and 2). However, most lesions benefited from the ozone (DIAGNOdent scores 1 to 4). The worst result for the treatment group was stabilisation of deep lesions as detected by the ECM readings change in comparison with severe deterioration in their counterparts in the control group. There was a trend of deep lesions (DIAGNOdent baseline score 4) to be less affected by remineralisation induced by ozone. This might be for more than one reason. As lesions in baseline score 4 are more likely to have dentinal lesions, it would not be possible to assess the penetration depth of ozone in this tissue without microbiological testing. An access cavity might be beneficial to increase surface area contact of the ozone with the lesion. Secondly, as these lesions tend to have either micro cavitations, or demineralisations in deep fissures within thin enamel, these anatomical features have more water content and are less resistant to electrical currents than full enamel thickness. ECM changes due to remineralisation would tend to produce less noticeable changes in the ECM

readings, as the enamel do. So it would be more beneficial to compare deep lesions' responses within similar groups not with enamel lesions groups and add larger number of lesions.

Lesions categorised by baseline ECM score

Please note from table 3 that ECM *readings* relate inversely to lesion severity while ECM *scores* relate directly to them. Categorising lesions by their ECM baseline scores was beneficial in spotting the mineral status of the tooth at the moment of the diagnosis rather than past lesion depth prior to the first examination. Many lesions were entered into the study where the clinical severity score and DIAGNOdent score systems detected a previous carious attack, but lesions had shifted in activity towards remineralisation long before the moment of lesion enrolment in the study. These teeth had a mineral content compatible with sound teeth (ECM baseline score 0). Entering those lesions in the study, unintentionally, gave a chance to study the effect or stability of those lesions over a year's time of continuous cariogenic challenges. The regression analyses showed that there was a trend for a better chance for the ECM scores 0 at baseline, as well as the other recall visits, to remain 0 in the treatment group and an 18% increased chance of those scores regressing in the control group.

There were significant differences between ozone treated lesions and those that received cleaning only. Where in the treatment group there was a slight rise (better) in the overall ECM readings change over a year for baseline scores 0 and 1, those in the control group had significant changes where there was a massive drop in the ECM readings and increase in ECM scores in the baseline score 0 group ($p<0.05$) and some increase in the score 1. So these lesions were still prone to demineralisation and could not be protected by the cleaning alone.

For baseline ECM scores 3 and 4 (demineralisations up to the DEJ and beyond), both ozone and cleaning helped to put back in some of the minerals into lesions but with a higher magnitude for the ozone treated carious lesions. So adding in minerals in the control group (which was not significant) doesn't mean a halt of further activity in the depth of lesions. This obliged the analyses to go back to the categorisation done by baseline DIAGNOdent scores, where deeper lesion showed a mean a decrease in ECM readings in the control group.

The last results show the indispensable need to look at lesions from more than one angle to try to get a clear picture. Deep lesions need an active approach as ozone for treatment rather than passive cleaning. Demineralisation is an unnatural situation in the hyper saturated environment of the oral fluids and tends to put the balance back into that unnatural situation, once the activity of microflora shifted (Robinson et al, 2000).

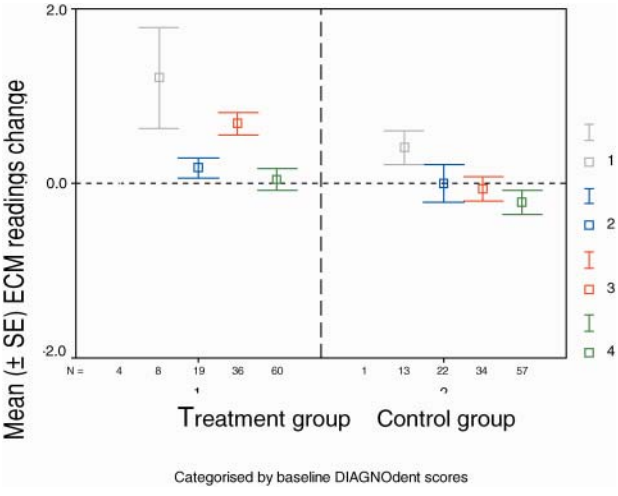

Figure 7: Mean (±SE) change of ECM readings over the 12-month period categorised by baseline DIAGNOdent scores. N: number of lesions.

Analysis at the last recall visit

In the treatment group, plaque tended to correlate with occlusion as expected (Carvalho et al, 1992). In the control group, the correlation between plaque and ECM change tended to be a negative one. This meant the more plaque there was then a tendency was for fewer minerals to be incorporated in the lesion in the past year, as expected. This supports the theory of the active biofilm and its role in the carious process, as well as the inability of the cleaning system applied once every 3 months to remove the risk of an active biofilm.

Surprisingly, there was a significant correlation between plaque and remineralisation following ozone treatment; the more plaque there was, the more remineralisation that tooth had had in the previous year after ozone treatment. It was interesting as this result sup-

ported the theory of plaque being one of the major sources of regulation and provision of minerals to lesions where the pH was higher than the critical demineralising pH through active pathways allowing diffusion of minerals from saliva and preventing and buffering external acid sources (Arends and Christoffersen, 1986; Robinson et al, 2000). This result would have an important clinical implication where plaque accumulation over treated lesions would be beneficial in providing remineralisation. Thus, sealing immediately after ozone would be preferably deferred until a later stage where substantial amounts of minerals would be allowed in the tooth. Another advantage would be that there is no fear from accumulations of plaque from the normal flora after ozone treatments, taken that the caries risk factors were kept low. This result may prove to be beneficial in patients with special needs and reduced manual skills that could not control the plaque accumulations over teeth.

As the hand can detect roughness up to a range, which could be produced by frostiness, and frostiness was correlated with lesion activity (Ekstrand et al, 1995,1998), it was suggested as a measure of lesion activity. Where smooth lesions were more likely to be related with sound surfaces or arrested lesions, occurrence of roughness clearly affected the clinical judgement of lesions, as they were more likely to be judged as carious in the treatment group. (Nyvad, 1993), there was more confusion to how much was roughness linked with active biofilm. Furthermore, ozone had a fast remineralising within one month and the smoothness of the surface has not been yet achieved by the functional occlusion. Here was the chance to measure both and correlate roughness to the activity of the lesions towards remineralisation and demineralisation. Care must be taken as this sensation was lost with the advancement with age and older dentists tend to compensate for this loss of sensation by applying higher pressure on the tooth and thus all the hazards of probing increase.

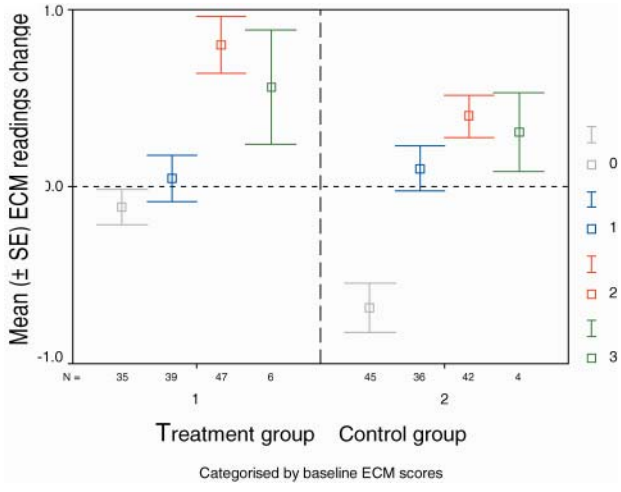

Figure 8: Mean (±SE) ECM readings over the 12-month period categorised by baseline ECM scores. N: number of lesions.

Lesions in the treatment group were significantly harder in one of the following cases, where they were judged clinically as arrested or when the ECM readings change in the whole 12-month period was positive. This feature of arrested lesions may be one of the main signs to record and compare after ozone treatment. More frostiness of enamel was present when signs of active lesions were present but in the control group it was further present when plaque was abundant.

Study 2

Management of dental subjects with a new treatment involves assessing their acceptability for it. Trait anxiety is defined as the relatively stable individual differences in anxiety proneness and inclination to perceive stressful situations as dangerous and threatening, then to respond to these situations with elevations in the intensity of their state anxiety (Spielberger, 1983). The term dental anxiety was first coined to describe the anxiety associated with dental treatment. The term dental anxiety was both descriptive and explanatory as it provides the dentist with the means of recognising and understanding the state of fear associated with dental treatment.

Anxiety is an individual characteristic, which could affect the perception of the subject of stressful situations and alter their behaviour before and during them. These behaviours also may be used to avoid getting into these situations. The dental clinic is one of these places, which could provoke such anxieties. The aim of this part of the study was to measure the effect of ozone treatment on state anxiety as subjects enrolled had completed a questionnaire designed to have the following parts:

Part one was presented to the subjects after signing their consent forms and consisted of general questions about various aspects of previous dental history. This

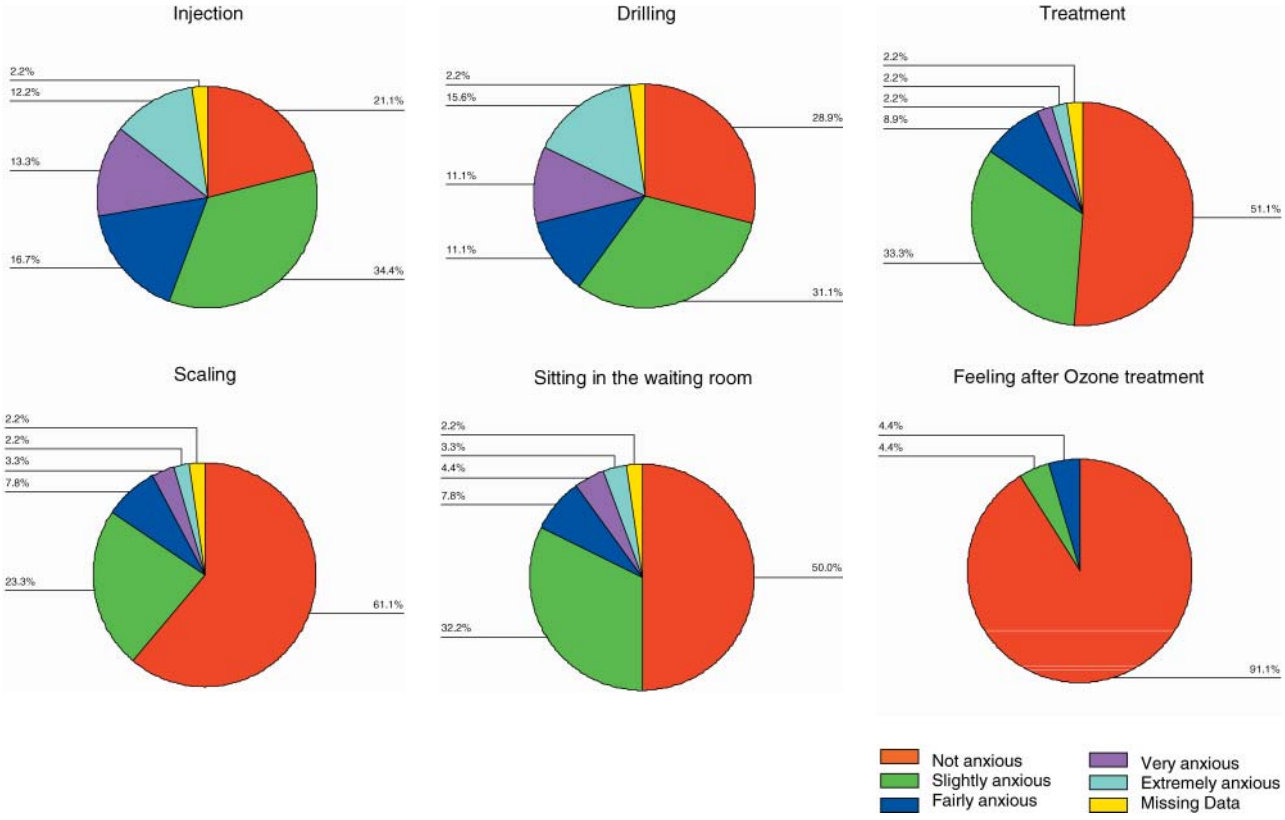

Figure 9: Distribution of anxiety scores for various dental procedures including ozone treatment.

was followed by a section of general questions to assess trait and state anxieties.

Part two was presented to the subject immediately prior to the ozone treatment but after all the procedure was explained.

Part three was presented immediately after the end of the treatment with the ozone for 10 seconds and assessed the state anxiety level and satisfaction from various aspects of the treatment.

In this study, all the subjects have attended appointments with their dentists and the majority had previous fillings procedures. Nevertheless, the number of subjects fearing these regular procedures was still high.

Trait anxiety scores distributed in this group as in the general population. Dental procedures, which had induced the most anxiety, were the injection and drilling of teeth. This was consistent with results from similar surveys. Here it was slightly lower than the percentages reported recently (64%) (Moore et al, 1996) most probably because our subjects were asked if they were going to receive it the next day, meaning that it was still a hypothetical situation for them. Scaling and

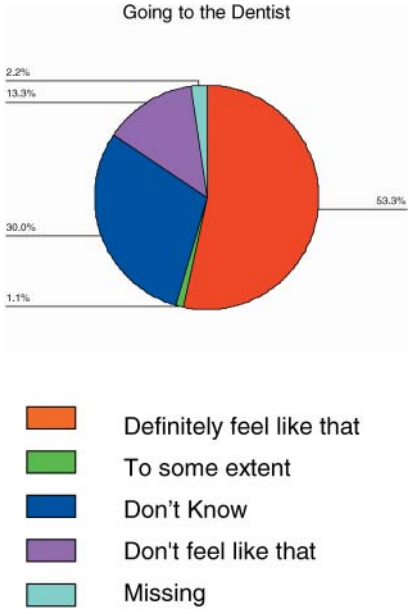

Figure 10: Possible behaviours related to dental anxieties.

polishing was the least procedure to induce fear as found in another study (Carson and Freeman, 2000).

Ozone treatment was the procedure to provoke the least state anxiety, and this anxiety further reduced after going through the procedure (Fig. 9). This would prove more valuable when treatment of apprehensive or vulnerable subjects who were in need of treatment the most as their caries prevalence rates remain high (Klingberg et al, 1995).

Decrease of anxiety levels might induce a long-term change in the behaviour of subject, which was probably directly related to higher anxiety levels (Fig. 10). In this study, these potentially harmful behaviours could be detected by the percentage of subjects preferring painkillers to avoid a visit to the dentist or even steer clear of attendance for regular checkups, where preventive procedures and early diagnosis would be most suitable. The change in behaviour was measured here by the subjects responding to questions about their conduct related to this treatment. The high percentage of those happy with the treatment was accompanied with satisfaction to receive the treatment again, paying for the costs, accepting time commitments, and even recommending it to a friend (Fig. 11). These behaviours might reflect on exposure to dental procedures, which might most likely improve the overall dental health.

Study 3

As was noticed in the main study, ozone treatment was accompanied with changes in the clinical presentation of the lesions that could not be picked up by the clinical severity score system used. If clinical changes were found accompanying treatment with ozone, then it would become credible for the dentist using a dental light and a probe to inspect a clean dried tooth for these changes and produce a clinically based judgement towards subsequent treatment needs. Furthermore, these changes should be recorded in a way that could be categorised and compared in the next visit.

The aim of this part of the study was to quantify the changes in the clinical indices from baseline with or without ozone treatment for 40 seconds. Eight new subjects who met the criteria were enrolled in this pilot study after signing consent forms. Where lesions were found, cleaning was performed then baseline information was recorded, and then these were randomised into only two groups:

Group 1: Teeth received ozone treatment for 40 seconds.
Group 2: Teeth had only baseline cleaning.

All subjects received preventive advice and were given a toothbrush and toothpaste (1,100 ppm F^-) to be used throughout the study and recall visits were set at 1,3 and 6 months. The readings were repeated for all cleaned teeth at each of the recall visits. Those within treatment group 1 then received another dose of ozone for 40 seconds.

Ozone treatment for 40 seconds was chosen here for more than one reason. First, lesions here were more severe than those involved in the previous main study (Table 7). Second, it seemed appropriate to measure the detailed clinical criteria changes when the recommendation from the first study were followed, using a higher dose of ozone for lesions that were more severe. As the 10-second ozone delivery cycle was repeated for 4 times, this would not be expected to increase any risk as there was no increase in the concentration of the gas, but only the time of exposure. The vacuum cycle was established at the end of the 4 cycles.

In this pilot study, lesions enrolled were more severe than the main study and the control group had its lesions slightly more severe than the treatment group. Whilst those in clinical severity score 1 in the main study were 49.6% and 62.2% respectively for the treatment and control groups, both here are only 21.1% are in this category. The same applied for the control group in the DIAGNOdent baseline scores 1 whilst the number in the treatment group was larger. ECM baseline score 0 had more lesions in this pilot study (42.1% and 36.8%) than those in the main study (28.1% and 35.2%) (Table 7).

The sample size here was smaller than detecting changes in the clinical severity scores, ECM and DIAGNOdent readings, but it was still adequate to measure detailed clinical criteria.

Frostiness significantly decreased from baseline for both treatment and control groups from the first recall. Thus the cleaning system which removed superficial active plaque over these lesions produced superficial enamel changes in the control group as it is expected to return to normal. This might have been by the combination of superficial remineralisation but not wear by

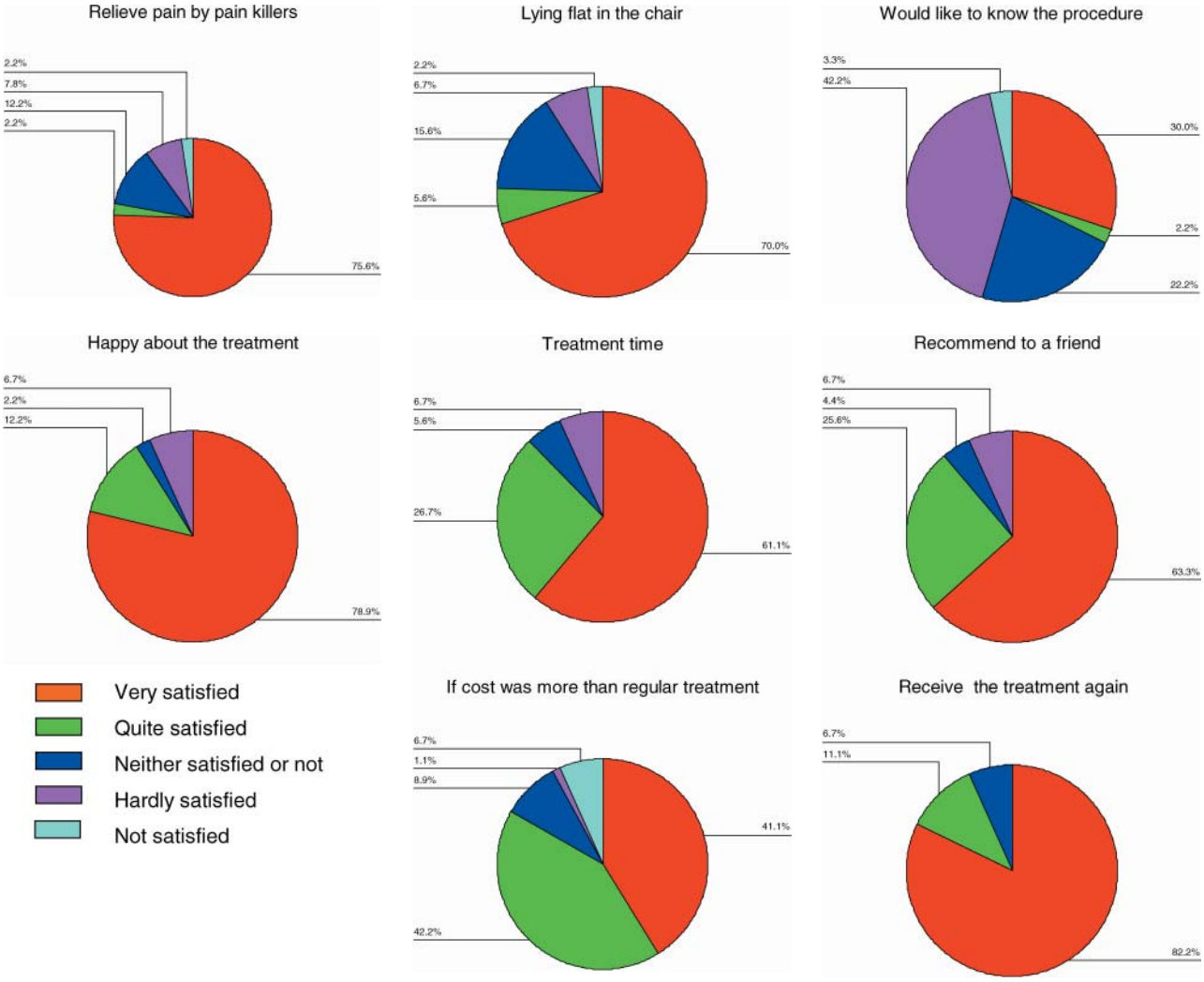

Figure 11: Distribution of satisfaction scores to variable aspects related to ozone treatment.

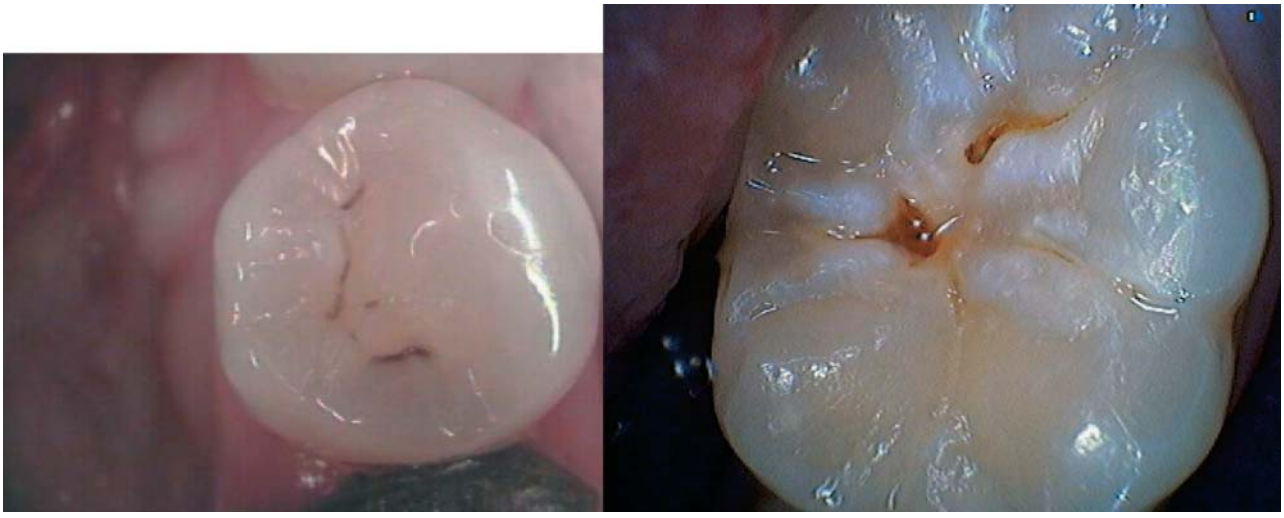

Figure 12: Samples of lesions assessed by the detailed clinical criteria.

Table 6: Detailed clinical criteria used to assess POPFC lesions

Surface destruction as seen after further plaque removal by the probe and drying
1. No cavitation
2. Microcavitation
3. Frank cavitation

Hardness of the base as assessed by dragging a probe from sound fissures into lesion
1. Hard as adjacent sound pit or fissure
2. Leathery
3. Soft

Visual index
1. Sound
2. Carious
3. Arrested

Colour of the lesion
1. Yellow
2. Light brown
3. Grey
4. Dark brown
5. Black

Perceived treatment need
1. Definitely requiring D&F
2. Possibly requiring drilling and filling (D&F) or preventive resin restoration (PRR)
3. Requiring a pharmaceutical approach but not D&F or PRR
4. Requiring no intervention

Frostiness of enamel at the margins in millimetres (mm)

Enamel Undermining shade length in millimetres (mm)

Table 7: Clinical severity scores of lesions enrolled in the pilot study at baseline

Control group		Treatment group		Score
%	Number	%	Number	Clinical
Clinical severity score				
21.1	4	21.1	4	1
42.1	8	57.9	11	2
36.8	7	21.1	4	3
DIAGNOdent				
5.3	1	21.1	4	1
15.8	3	5.3	1	2
31.6	6	31.6	6	3
47.4	9	42.1	8	4
ECM				
36.8	7	42.1	8	0
21.1	4	31.6	6	1
42.1	8	26.3	5	2
0.0	0	0.0	0	3
100.0	19	100.0	19	Total

functional occlusion as this was at one month after cleaning (Carvalho et al, 1992). If ozone was added on top of this careful cleaning procedure, these superficial changes would have been produced even without the ozone treatment, as found here. However, there was no reduction in the length of stain in either group, or the undermining shadow beneath enamel.

Treatment group had the colour of its lesions changing to darker shades at all recall visits from the control group. It was significant at the 3-month recall visit and stabilising since then. This even might be earlier than what we found for the lesions in the main study where their colour-related clinical severity scores stabilised at the 6-month recall visit. So as it supports what was found in the main study, here we have a clear earlier change, which might probably be by the longer ozone application time.

The main clinical change, which was distinctive for the treatment group, was the change in the hardness index scores. From the first recall visit, lesions were significantly harder than baseline and from control group since then. At the 1-month recall, the treatment groups had 9 teeth becoming harder ($p<0.01$). In the 6-month recall visit they increased to 11 teeth ($p<0.01$). In the control group 3 teeth became harder at the 1-month recall visit while only 4 in the last recall visit at 6 months. Two teeth became softer in the control group ($p>0.05$). The difference between the groups was significant from the 1-month recall visit ($p<0.05$).

Use of the probe for the hardness and roughness indices served a valuable tool that vision and diagnostic tools could not provide. There was a clear need to make sure that the fissures were thoroughly clean. Driving the probe in the fissures back and forth with light pressure, but enough to dislodge plaque or food particles performs this task. This could be described as a scratching motion rather than a probing one, where the probe is forced in the tissue. The amount of information gained by that simple scratching and cleaning test might be the main drive for most dentist to still use it. Failure to use it to clean the fissures could lead the dentist to subtract a step of cleaning that could prove very essen-

tial when a large amount of plaque is covering the lesions. Using the dragging motion as a recommendation rather than the indenting motion would minimise the chance of destroying the roof of an undermined lesion. This advice is clearly justifiable as the mean change in the cavitation index was a slight insignificant reduction rather than increasing as might be expected using the probing motion.

The combination of various results from the clinical indices was reflected on by the clinical conclusions as assessed by the visual index and treatment needs index. Significantly more lesions were judged as arrested in the treatment group than the control group and more lesion presentations were translated to less dental procedure requirements in the treatment group than the control group. Where 12 teeth in the treatment group changed their treatment needs to a more conservative approach, the percentage remained the same at the last recall visit ($p<0.001$). No tooth had its treatment needs assessed to be more aggressive. In the control group, 5 teeth had their needs reduced at the 1-month recall visit ($p>0.05$) to 9 teeth at the 6-month recall visit ($p<0.01$). 4 teeth required more aggressive interventions at the 1-month recall visit, these were assessed to be reduced to 1 tooth at the 6-month recall visit. The difference between the groups was significant at the 1-month recall visit but not significant at the 6-month recall visits ($p=0.058$ and 0.160, respectively).

More studies are ongoing to assess ozone penetration in cavitated lesions, enhanced by its small molecular weight and high reactivity. How deep could these effects reach were not studied here. Serial radiographs could effectively detect such changes in deep layers. Sampling of dentinal tissue would validate different combinations of ozone and temporary and permanent restorations.

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CHAPTER 3.4

Use of Fissure Sealants over Ozone Treated Occlusal Surfaces

Layla Abu-Naba'a, Hisham Al Shorman & Edward Lynch

Why are sealants an essential corner stone in caries management protocols?

From the wealth of long-term studies, the risk of caries development is still true for the overall population and the prevalence is still high (ten Cate, 2001) and thus there continues to be a need for site-specific *prevention.* With the occlusal lesions developing in around half of a population of young adults (Ripa et al, 1988), the *therapeutic* role expected from sealants becomes clear in reducing the need for invasive dental treatments (Soderholm, 1995).

How can sealants perform its role in prevention of dental decay?

The complex pit and fissure system allows a protected environment for the plaque to mature and may wrap up damage inflicted, away from the diagnostic tool. Thus, the carious process has been described as an θunpreventable ubiquitous processö (Ekstrand et al, 2001b).

Although different sealing materials have been developed (Simonsen, 1996), they still share the common feature of being technique sensitive (McConnachie, 1992). Microleakage resistance and retention are the physical properties most at risk. When jeopardised, these were proven to correlate with secondary caries incidence (Romcke et al, 1990). Many studies were conducted on a wide variety of materials as well as their application techniques in order to improve these physical properties and results have been incorporated into the curriculum of dental schools (Waggoner et al, 1996; Walker et al, 1990). Some of the steps affecting these two critical features are:

- Clean tooth
- Isolation during application

The use of the air polishing technique was found to improve the performance of fissure sealants as it produced less debris, greater sealant penetration, greater number of resin tags and higher tensile bonding strength than fissures cleaned with pumice (Brocklehurst et al, 1992; Brockmann et al, 1990).

Retention of fissure sealants are reduced when etched enamel is contaminated by any of the following; saliva, water, acid etchant remnants, plaque and prophypaste (Burrow et al, 2003; Duangthip et al, 2003; Fuks et al, 2002). Nevertheless, some were less affected by the contamination than others (Duangthip et al, 2003). Cleaning teeth and drying should be regularly checked for the use of clean water and moist-free air syringes. Eruption status of the teeth produced salivary contamination when close to gingival margins (Dennison et al, 1990). This limited the use of sealants in the most critical times where the caries risk was high as these emerging teeth were not fully mature mineral wise (Schulte, 1999; ten Bosch et al, 2000).

Isolation methods used with sealants include:

- Rubber dam, the best isolation
- Cotton rolls

- Dri aids/Dri tips
- Denta Pop.

- *Choice of sealant material*

Today we have a number of products that we can use as sealant materials:

- Unfilled sealants
- Filled sealants
- Light activated
- Auto-polymerizing
- Fluoride releasing.

First generation sealants utilized an ultraviolet light to cure the materials. Second and third generation sealants utilize an auto-polymerizing reaction or a visible light to cure the materials. Selection of a specific product depends on whether the practitioner feels more comfortable with an opaque, clear or tinted material that can be filled or unfilled, and light cured or auto-polymerizing. All these materials give comparable clinical results. It is recommended that product has been accepted by an approval body.

Sealants accepted by the ADA include:

- Alpha-Dent Chemical Cure Pit and Fissure Sealant
- Alpha-Dent Light Cure Pit and Fissure Sealant
- Baritone L3
- Helioseal F
- Helioseal
- Prisma Shield Compule Tips Tinted Pit and Fissure Sealant
- Prisma Shield VLC Filled Pit and Fissure Sealant
- Seal-Rite
- Seal-Rite Low Viscosity

- *Acid etching*

To optimally increase enamel surface area, different etching times, material and concentrations have been recommended (Gungor et al, 2002) if these differ for each manufactured product. The tooth should be etched with a 37% concentration of orthophosphoric acid for 15–30 seconds. This can be in the form of liquid or a gel. All pits and fissure surfaces are covered by the etchant and beyond to adjacent enamel on cusps.

- *Rinsing and drying the tooth*

Rinsing time varies but should remove all the etchant material from the tooth. Compressed air is used to dry the tooth using the three-way syringe, free of oil and water. The tooth is dried until it has a chalky, frosted appearance. Salivary contamination would necessitate re-etching.

- *Filling level and volume of the sealant*

Although sealing all the fissures is the prime aim, over filled fissures were inferior in quality. Problems were noticed in clinical studies: e.g. entrapment of air bubbles, extension of material to non-etched enamel, pooling of material in distal pits (by inclination of the occlusal surface and capillary action driving the sealant distally) (Geiger et al, 2000), altering the subject's occlusion, increasing the finishing procedures (Abu-Naba'a, 2003) as well as creation of ledges on the borders where further stains and plaque could accumulate. When fissures are over filled, the penetration depth was unaffected, and the retention and microleakage resistance was reduced. Setting shrinkage can cause sealant displacement from the enamel surface. Secondly, the formation of shoulders (raised boundaries) makes borders more exposed to the higher light intensity that may cause higher shrinkage. Retention by means of enamel tags is insufficient when a large amount of material is present. Finally, the concentrated forces of occlusion on high spots over the over-filled material increase these displacing forces. Retention can be increased with the use of adhesive (Perez-Lajarin et al, 2003).

- *Post insertion evaluation*

Sealants are evaluated conventionally using vision and the probe. Material deficiencies are re-applied and occlusion is adjusted.

- *Combinations of fissure sealants with other preventive materials*

Groups High-risk subjects for caries development were a special group where intensive measures for prevention may be appropriate. Chlorhexidine varnishes were recommended twice a year (Matthijs et al, 2002). Combination of the previous measures may even be more effective. The most effective combination was the use of fluoride with fissure sealants (Whelton et al, 2001). Fluoride was added to the unpolymerized resin in the

form of a soluble fluoride salt that releases fluoride ions by dissolution, following sealant application or as an organic fluoride compound, chemically bound to the resin and released by exchange with other ions (anion exchange system). Laboratory models gave evidence for equal retention rates to conventional sealants, fluoride release and reduced enamel demineralization. Studies concerning their long-term effect on caries and retention are needed (Ripa, 1993; Morphis et al, 2000).

Ozone has the potential to replace, mend or combine with fissure sealants for caries prevention

Plaque mechanisms for producing caries graduate from the initial accumulation of normal flora to the transformation towards more anaerobic conditions and carbohydrate-metabolising acidogenic species. Products of such activity are maintained by further fabrication of proteins that prevent remineralisation that might be logically assumed in a mineral rich environment (Marsh, 1999). Caries prevention enrols the intrusion of this harmful plaque activity on the tooth surface. Where fissure sealants provide a physical barrier between the microflora and their nutrient source, ozone interferes with this activity at more than one level. The microbiocidal effect of ozone has long been recognised (Bocci, 1999; Brauner, 1991). The effect of ozone on plaque biomolecules has been recently discovered. High Proton Nuclear Magnetic Resonance (^{1}H NMR) is a system for the analysis of components of biomolecules. Ozone has a powerful oxidative ability that consumed plaque and saliva biomolecules essential for the process of demineralisation of lesions to occur (Grootveld et al, 2001; Lynch E et al, 2001). Amongst these molecules are formic and pyruvic acids that contribute substantially to the decreased pH values associated with active caries lesions and inhibit the precipitation of the minerals (Silwood et al, 1999; Silwood et al, 2002). Pyruvic acid was oxidised by ozone to form acetate and carbon dioxide compounds associated with higher pH values (Lynch et al, 2002).

Although sealants proved to be effective in preventing caries, fissure sealants use on a large scale in all children was not adopted. It is only cost-beneficial

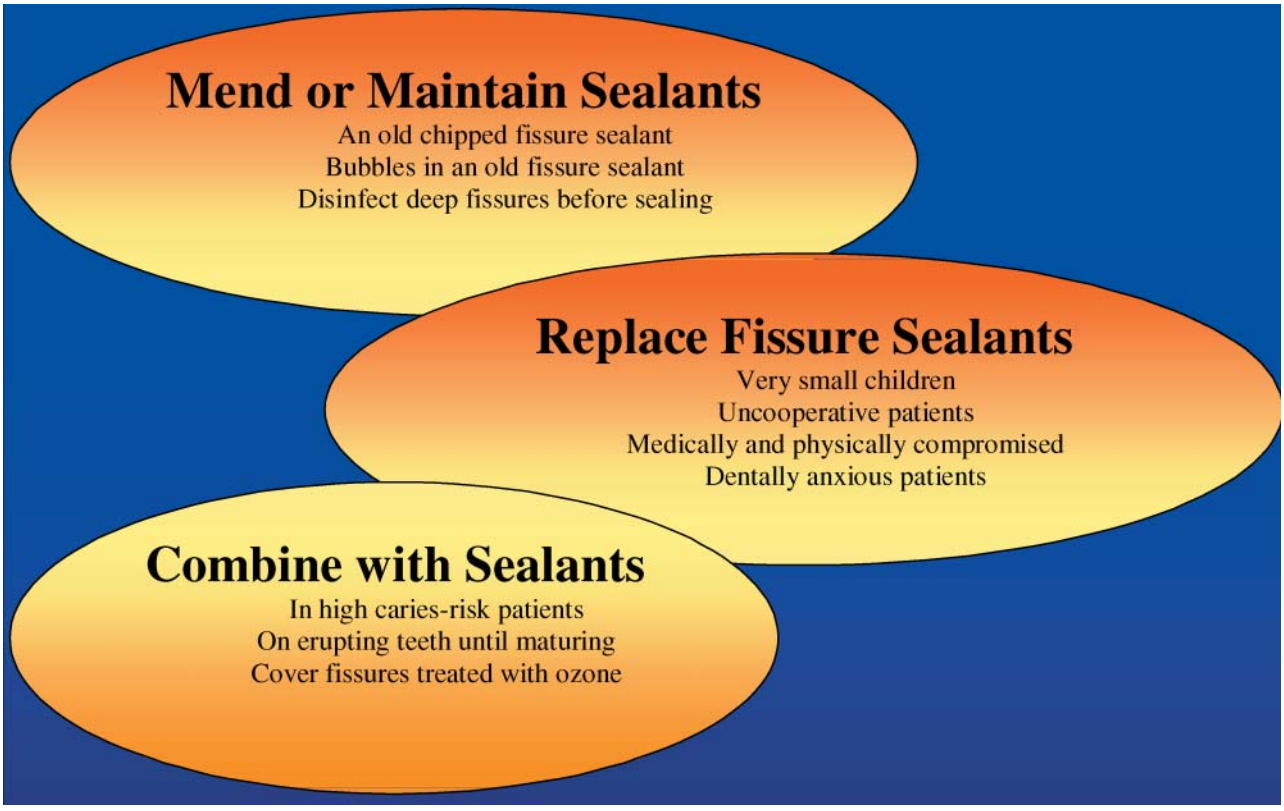

Figure 1: The potential role of ozone in caries prevention.

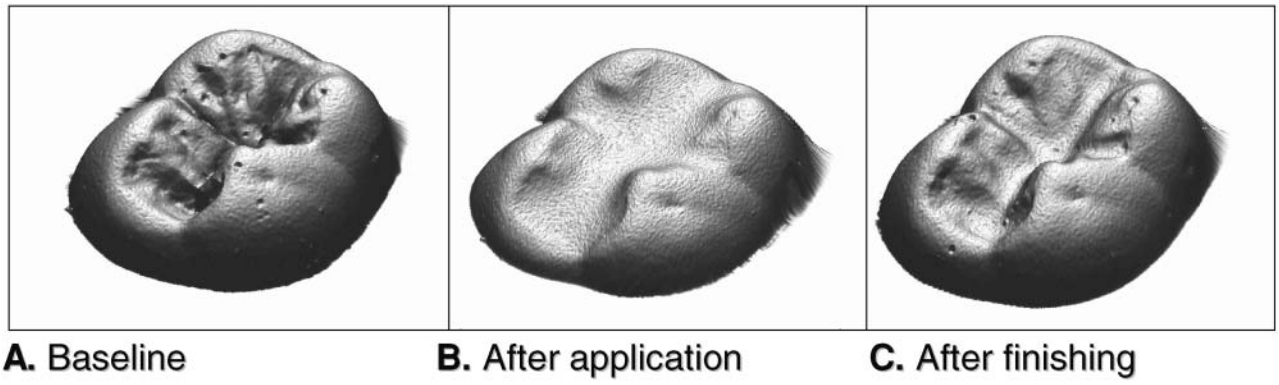

Figure 2: Scanned images: Steps of sealant application to the tooth.

when directed to caries risk patients (Charbeneau, 1982; Deery, 1999; Locker et al, 2003; Weintraub, 2001a). Ozone's minimal running cost (Ozone being produced from air), and minimal treatment time could be recommended for larger preventive schemes run by lower costs (Domingo et al, 2004). Furthermore, ozone can be proposed to be applied by dental auxiliaries as it is non-invasive, further reducing costs of large schemes and clinical settings.

Being technique sensitive, fissure sealants are further limited in their ability to be applied in subjects who can complete the procedure without saliva contamination or loss of cooperation. Where patients are medically, physically compromised, dentally anxious, very young or uncooperative, baring technique sensitive requirements might be impossible. Short application time and reduction of dental anxiety with minimal cooperation requirements and high satisfaction rates (Abu-Naba'a, 2003; Al Shorman et al, 2002; Domingo et al, 2004), ozone could replace fissure sealing as the first option for prevention.

For the caries-high-risk patients, ozone can be combined to fissure sealants to increase the preventive outcome. Erupting teeth are a temporary limitation as isolation from saliva is difficult (Feigal, 1998). Ozone is not impeded by the gum's margin and maintains the seal over these teeth while emerging from the gingiva. Their healthy status may be maintained until proper isolation procedures could be applied or till full maturation is reached (about 15 months in premolars and 36 months in molars) (Schulte et al, 1999; ten Bosch et al, 2000).

As a step prior to sealant application, ozone can penetrate and disinfect deep fissures. The integrity of fissure sealants must be maintained to perform its function. Once intact margins are lost, lesions beneath these sealants would rapidly deteriorate, especially in high caries risk patients (Bravo et al, 1996) and is considered by

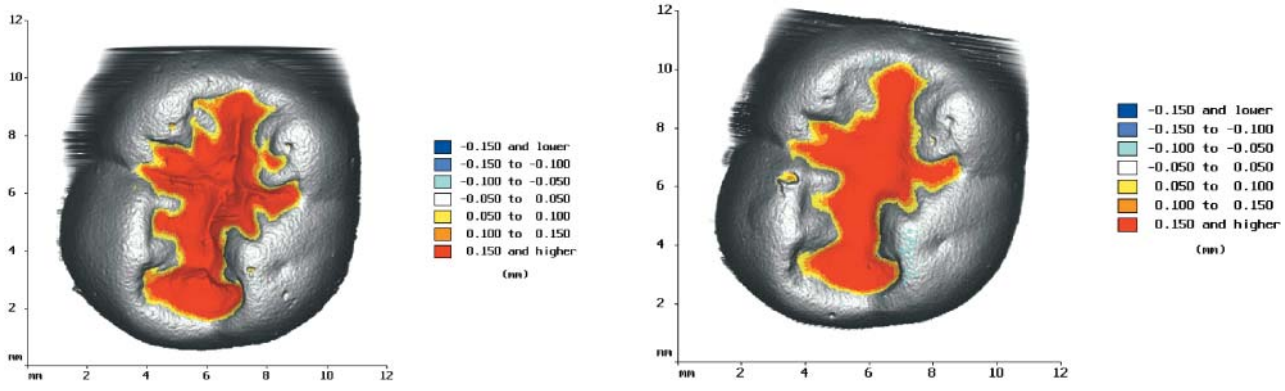

Figure 3: Difference images produced by superposition of pairs of images. Depth ranges of added material may be seen and this allows for objective selection of the region of interest (Molar A and molar B in Fig. 5.1, Molar B and molar C in Fig. 2).

many researchers as a definite requirement for sealant replacement. Loss of retention occurred in sealants in two phases. An initial loss was most likely due to faulty technique followed by a second loss by material wear from occlusion and other reasons (Weintraub, 2001). The highest rate of sealant reapplication at 3–6 months (Manton et al, 1995). In the long term, where a chipping or bubble appears in the sealants, ozone has the potential to be used for maintenance of defective sealants with no need to replace it. Further clinical studies should be performed to prove effectiveness of such ozone combinations with fissure sealants.

Fissure sealants role as a therapeutic measure for carious lesions

Drawing a line between prevention and pharmaceutical management of lesions is extremely difficult. Lesions on the microscopic level happen in advance way ahead of any detection possible. The low sensitivity of vision to detect signs of early disease often lead to many decayed teeth to be left untreated (Wenzel et al, 1991), underestimated in caries prevalence rates (Lussi, 1996) and over-treated with fissure sealants (Deery et al, 2000). Of some concern was the significant number of 17- and 20-year-old patients who had received sealants but in whom later radiography revealed underlying radiolucencies; these findings suggest that the sealants were placed without prior diagnostic radiography (Poorterman et al, 2000). In application, dentists are using fissure sealants for its therapeutic affect on dental caries either deliberately or by chance (Gonzalez et al, 1995; Handelman, 1991).

Histologically, initial non-cavitated lesions represent a range of clinical severities from early enamel lesions, reaching the dentino-enamel junction or up to the outer third of dentine (Ekstrand et al, 1995). These are clinical scores 1 to 3 in the Ekstrand scale (Table 1) (Ekstrand et al, 2001). When diagnosed by DIAGNOdent, a laser fluorescent diagnostic device, these lesions ranged had DIAGNOdent readings of 10 (Abu-Naba'a, 2003). Using these criteria helps to diagnose lesions clinically better than conventional radiographs.

Management of non-cavitated lesions by fissure sealants is an area of debate (Kidd et al, 1994; Manton et al, 1995). Sealing initial lesions up to the DEJ is considered as one of the therapeutic procedures recommended (Florio et al, 2001; Ricketts et al, 1997; Weerheijm et al, 1992). Radiographic and bacteriologic studies of sealed carious teeth have tried to calm the dentists' concerns about their use of sealants over minimal, non-cavitated occlusal lesions. What helped was the fact that dentine demineralisation is confined to the contact area between the two and never spread along the junction laterally as was previously assumed (Ekstrand et al, 1998). It is only in cavitated advanced lesions where the lateral spread was seen (Bjorndal et al, 1999). These studies have demonstrated that initial non-cavitated caries is inhibited and may in fact regress under intact sealants. Undamaged sealants isolate cariogenic micro-organisms from their source of nutrients and prevent further colonisation by other oral micro-organisms (Handelman, 1991b; Mertz-Fairhurst et al, 1998). Retention over carious pits and fissures was not different than sealing sound teeth (Handelman, 1991), or slightly less (Feigal, 1998). The cost-effectiveness indicated that sealing incipient or early carious lesions might be an alternative to restoration with amalgam (Adair, 2003).

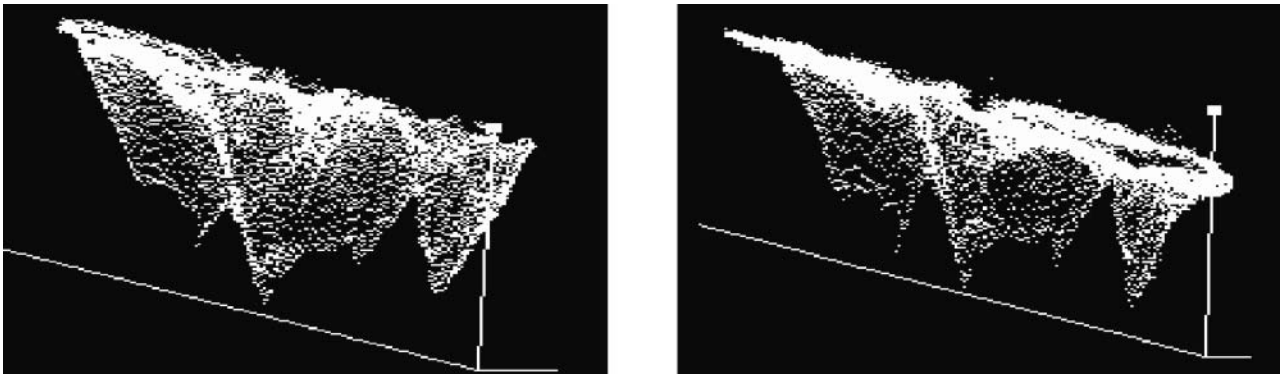

Figure 4: 3-D images of the area of interest (fissure area covered by fissure sealants) produced from difference images.

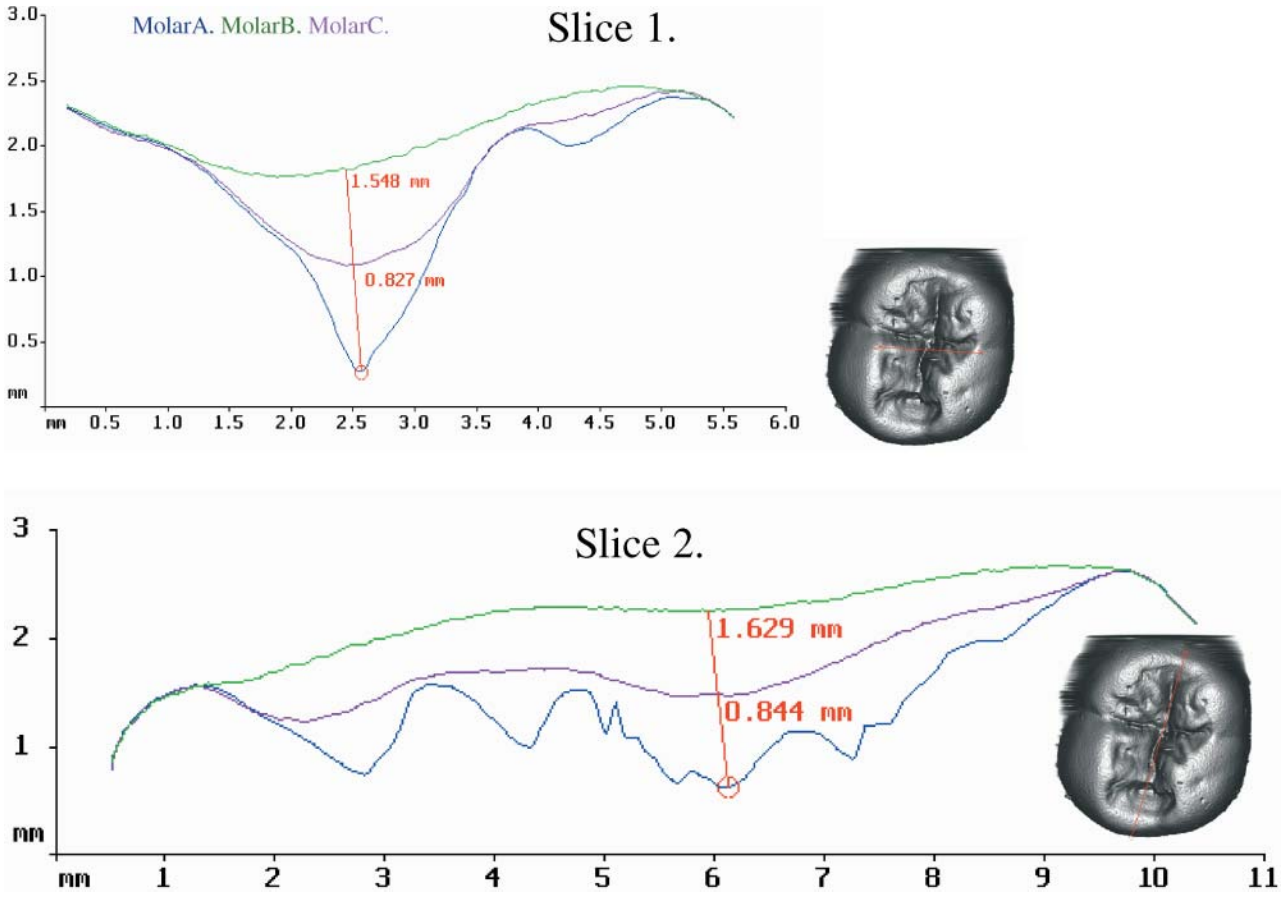

Figure 5: Depth changes in vertical and horizontal slices. Margins of the fissure sealant can be visualised for the presence or absence of ledges (Molars A,B and C in Fig. 2).

Table 1: Clinical severity index scores

Score	Description	Histopathology
0	No or slight change in enamel translucency after prolonged air drying (>5s)	No enamel demineralisation or a narrow surface opacity
1	Opacity (white) hardly visible on the wet surface, but distinctly visible after air-drying (>5s).	(Active)
1a	Opacity (brown) hardly visible on the wet surface, but distinctly visible after air-drying (>5s).	Enamel demineralisation limited to the outer 50% of the enamel layer (Arrested)
2	Opacity (white) distinctly visible with out air-drying.	(Active)
2a	Opacity (brown) distinctly visible with out air-drying.	Enamel demineralisation (might not be infected) more than the outer 50% of the enamel layer up to the outer third of the dentine layer (Arrested)
3	Localised enamel breakdown in opaque or discoloured enamel and or greyish discolouration from the underlying dentine.	Dentine demineralisation (lightly infected) up to the middle third of the dentine layer
4	Cavitation in opaque or discoloured enamel exposing the dentine beneath.	Dentine demineralisation (Heavily infected) up to the inner third of the dentine layer

The other side of the debate was the finding that sealed carious lesions harboured bacteria that survived up to 3.4 years under intact fissure sealants and radiological evidence of dentinal caries was present in 53% of intact sealants (Weerheijm et al, 1992). Mechanisms of reducing the bacterial load were to minimally prepare these lesions and place a preventive resin restoration (Feigal, 1998; Hassall et al, 2001; White et al, 2000; Yip et al, 2002). Air-abrasion was used as a preparation technique but *not* without acid etchant due to high leakage (Hatibovic-Kofman et al, 2001).

Fissure sealant application over ozone-treated initial occlusal lesions

Ozone's remineralising effect has been discussed thoroughly in chapter 4.1.

Steps for fissure sealant application combined with ozone should depend on the following procedures:

Caries risk assessment

Caries risk varies throughout an individual's lifetime and can depend upon many factors such as diet and changes in lifestyle. A list of risk factors for children or adults at moderate or high risk for dental caries:

- Seek dental care on an irregular basis, have no dental insurance or no access to dental services
- Have active dental caries
- Have high levels of infection with cariogenic bacteria
- Have family members with a history of high caries prevalence
- Have impaired ability to maintain oral hygiene
- Children with a history of caries in their primary dentition
- Root surfaces exposed by gingival recession
- Malformed enamel or dentin
- Reduced salivary flow because of medications, radiation treatment or disease
- Low salivary buffering capacity
- Presence of orthodontic appliances, or dental prostheses
- Frequent intake of high carbohydrate and sugar containing foods
- Inadequate exposure to systemic and topical fluorides.

Clean Tooth

For the occlusal surface, cleaning should remove materia alba, plaque and stains. Cleaning using a brush, probe, (Ismail, 1997) air-abrasive systems (Banerjee et al, 2000) prophy cup and pumice, air slurry polisher are recommended. Not all stains could be removed from the occlusal surface due to the complexity of the topography and adhesion of the stains. Any debris that is not removed might interfere with the proper etching process and the sealant penetration into the fissures and pits. For the ozone, less debris mean less surface reactants so the as could penetrate to the deep layers.

Baseline diagnosis

At baseline, lesions should be fully recorded using more than one tool. Using Ekstrand's clinical criteria, the detailed clinical criteria and tactile perception are recommended in the conventional dental setting (See chapter (Detection Methods of Occlusal Caries for Use in Clinical Practice)) Such diagnostic outcomes would help analyse treatment outcomes for future auditing and analysis of efficacy. Radiographs must be available, not to exclude dentinal lesions, but to record radiolucencies extent for future comparisons. Digital radiography and DIAGNOdent are some of the advanced tools used in some clinics. Their use would be very helpful in baseline diagnosis and for further recalls.

Ozone treatment chosen

Ozone is applied to the tooth using a standard dose and flow rate (2100 ppm O_3, 615 ml/min) during a 10 seconds cycle. Ozone is delivered from the HealOzone unit (CurOzone and KaVo, USA and Germany) through a hand piece with a silicone cup. Once the tooth is sealed, ozone is automatically delivered for 10 seconds followed by 10 seconds vacuum. If the seal is broken, the vacuum continues to pull in any remnant ozone gas. Repetition of this cycle would not increase the hazard of the treatment, as it is only repetition of the standardised cycle. Where lesions appear in the radiographs, longer application time could be recommended up to 60 seconds.

Why might we need to apply sealants over remineralised lesions?

Remineralised lesions are more resistant to future acid attack and less likely to regress (Robinson et al, 2000). Remineralisation of lesions in dentine is possible (Ar-

ends et al, 1990; ten Cate, 2001a). However, remineralisation is time dependant; although the oxidative effect of ozone is immediate, the remineralisation process needs the time for the mineral reshuffling that was inhibited by the previous biomolecules (Abu-Naba'a et al, 2003b).

Sources for remineralisation within the lesion can be from the structure adjacent (hyper-mineralised) crystals, surface hyper-mineralised crystals, (Robinson et al, 2000; Shore et al, 2000) dentinal tubules that had sclerosed with larger crystals (Arends et al, 1990; ten Cate, 2001a). The immediate application of fissure sealants over ozone treated lesions can be achieved with retention rates similar to non-treated lesions (Abu-Naba'a, 2003; Abu-Naba'a et al, 2004).

Remineralisation sources from outside the tooth are saliva, plaque, and ion rich layer between plaque and tooth surface (Robinson et al, 2000). It might be proposed that fissure sealants can prevent tooth remineralisation by creating a barrier for minerals to enter lesions. Leaving lesions without fissure sealants can be recommended for the time of maximum remineralisation; 3 months for enamel lesions and 6–9 months for lesions reaching DEJ or outer third of dentine (Abu-Naba'a et al, 2002; Abu-Naba'a et al, 2003c,2003b).

Leaving remineralised non-cavitated lesions without fissure sealant can also be a treatment option. There are no cavitations that might provide a retention niche for food accumulation and further occlusal wear would smooth the surface (Abu-Naba'a et al, 2003a; Carvalho et al, 1992). But with ozone, remineralised lesions tended to become darker at six months. Add to that, lesions were diagnosed having a baseline stain as well. Covering these lesions for esthetic reasons are justified, and if lesions could be cover immediately after ozone treatment or after remineralisation period.

Sealant choice of products with ozone

Regular techniques for cleaning, etching and curing are applied. The choice remains weather to choose between a radiolucent sealant or an opaque one. Opaque sealants are easier to detect marginal integrity. Staining of fissures at baseline does not go away at this dose of ozone treatment opaque fissure sealant could help obscure the stain for more aesthetic results. Clear sealants allow for seeing and monitoring lesions as they are apparent through the transparent sealant. Comparisons with visual photographs and intra oral images are possible. DIAGNOdent can also be used to monitor caries activity beneath lesions (Takamori et al, 2001).

How to monitor sealants over ozone remineralised lesions

Clinical assessments

Sealants are effective caries-preventive agents to the extent they remain bonded to teeth. Preventive resin restorations are also susceptible to failure as the overlying sealant fails. Careful analyses of studies reveal a measurable failure rate of sealants (5% to 10% per year) that must be addressed. Using a light and probe, sealants could be checked in the regular clinical situation. Further systems were used to put the clinical criteria of sealants into categories to facilitate future comparisons.

One of these systems is the "Modified US Public Health Service" (USPHS) criteria rating system for evaluating restorations (Table 2) (Jackson, 2001; Rix et al, 1994). A similar clinical assessment used only three criteria, colour, coverage, caries (Deery et al, 2001).

Using these criteria, ozone treated lesions that were covered immediately by an opaque fissure sealant had retention rates that were comparable with only sealed lesions. They also were comparable with other clinical studies (Feigal, 1998). Caries rate in this study (3.7%) was about equal to the 4% reported in another study at the end of a years follow up (Romcke et al, 1990). But that study applied fissure sealants over sound teeth. So the apparent caries on the margins of fissures here might be those that became uncovered by the sealant's chipping. Assuring this would be by carefully recording on photographs. There was no complete loss of sealants in any of the teeth while chipping occurred in 67.3 and 71.2 for the treatment and control groups, respectively (75% to 97% (Simonsen, 1991)).

Colour at the margins and within sealant material is dependant on the sealant transparency itself, volume and coverage area as well as lesion staining. Sealed lesions might shadows showing through the edges of the lesions. These differences at enrolment time must have been taken in account when results were analysed.

Radiographic assessment

Baseline radiographs should be present. These are justified by the clinical protocols for caries radiography as high risk patients (with one or more active lesions) may

Table 2: Modified US Public Health Service (USPHS) criteria rating system for evaluation

Category and Score		Criteria
Colour	0	Good colour
	1	Mismatch in colour, shade or translucency but within normal range of adjacent tooth structure
	2	Obvious mismatch outside of normal range
	3	Gross match
Margin Discoloration	0	No discoloration evident
	1	Slight staining; can be polished away
	2	Obvious staining; cannot be polished away
	3	Gross staining
Marginal Adaptation	0	No visible evidence of a crevice along the margin into which the explorer will penetrate
	1	Visible evidence of a crevice along the margin into which the explorer will penetrate of catch
	2	Restoration was mobile, fractured, or missing
Secondary Caries	0	No caries of evidence with the margin
	1	Evidence of caries at the margin of the restoration

have their teeth monitored by bitewings within 6 months intervals. For monitoring purposes and to reduce human error, quantification of this gradient of grey shade was possible by digital sensors produce images that are captured for computer analysis. Further contrast adjustments using specialised software allowed the enhancement of the resultant image improving the radiographic diagnosis and follow up of early lesions (Verdonschot et al, 1999; Wenzel et al, 1992).

Research assessment tools

Using a dental unit light and probe, much of this assessment is subjective, reliant on the clinician's experience, insensitive to small changes and produced few, if any, quantitative results. More discriminatory experimental investigations require an objective quantification of the retention or amount of material loss. Consequently, numerous such techniques have been developed and reviewed.

ECM

ECM was used not only to diagnose caries, but also to predict the need for a sealant or sealant restoration within 18 months after baseline (Verdonschot et al, 1993). The principle was further extended to measure marginal leakage around fissure sealants *in vitro* (Verdonschot et al, 1995). The fissure sealants were applied in three different ways to insure a non-leaking sealant, a leaking sealant and a leaking restoration. A gel was applied over lesions and readings were taken. The performance of that application was high but in the clinical situation, it was still to be considered.

DIAGNOdent

Not all sealant material can be monitored using DIAGNOdent. The fluorescence of the material might give a false positive reading. Instructions on its suitability with this diagnostic system should be consulted with the manufacturer. This laser diagnosis system was used for monitoring lesions under non-fluorescent clear sealant material and was recommended to be during the routine check-up of sealants (Takamori et al, 2001).

3D Scanner

Many laser scanners where used to scan surfaces. One of these is the Laserscan Pro system, designed specifically for dental applications. Being accurate, it could be used extra-orally to scan mounted replicas made of conventional die material, casting stone or impression material. Software allows for image manipulation and super positioning of serial images of sealants. From such devices, surface quality, edges of sealants, volumes, and depths of penetration could be visualised and further analysed.

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CHAPTER 3.5

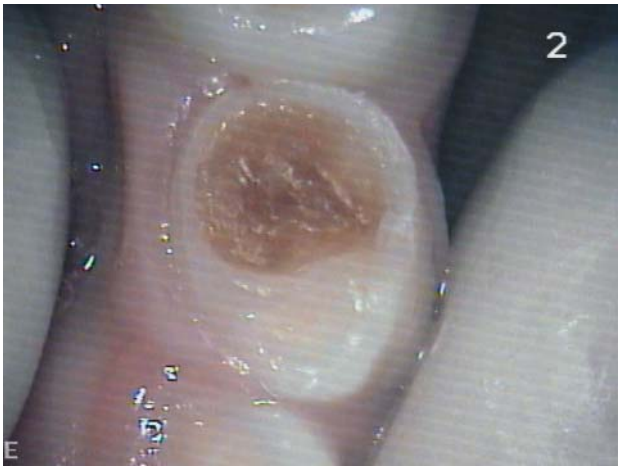
Clinical Management of Deciduous Caries using Ozone

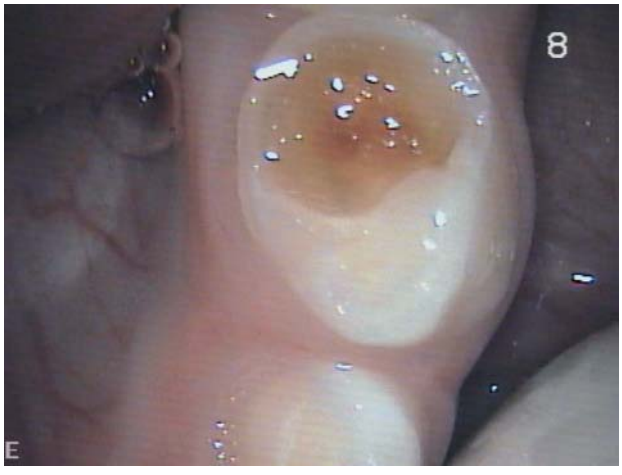
Ola Abu-Salem, Mousa Marashdeh, Julian Holmes & Edward Lynch

The clinical management of caries in the adult or the permanent dentition is described elsewhere in this book. According to Ekstrand et al (2001), dental caries is a dynamic process that takes place in the bio-film on the surface of teeth. Over a time period, a disturbance of the equilibrium between mineral loss from the tooth surface to the saliva (demineralisation) and mineral uptake by this surface (remineralisation), can lead to demineralisation dominating in acidic conditions. This can lead to the formation of a protected niche environment and the formation of a cavity.

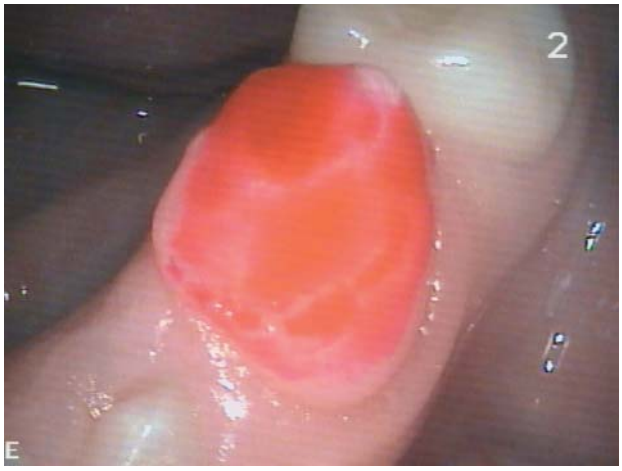
Although the incidence of caries in children (8 to 15 year-olds) has apparently declined, studies have shown that occlusal pit and fissure caries now accounts for the majority of lesions in this age group. A large percentage of children now experience only caries in the pits and fissures in posterior teeth. (Ekstrand et al, 2001).

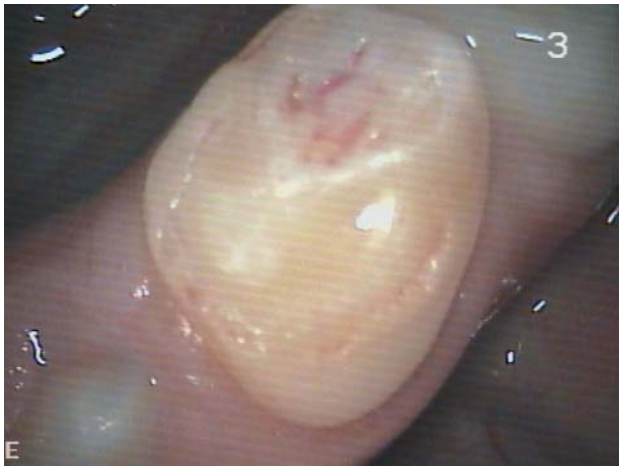
Dental caries is a multifactorial disease but dental plaque is the only causative factor (Fejerskov, 1997). Caries can be regarded as a plaque-related infection. Two bacterial species have been proven to be strongly associated occlusal fissure caries, *S. mutans* and *Lactobacillus* species (de Soet and de Graaff, 1998). Both are acidogenic and aciduric and have the ability to produce intracellular polysaccharides and extra cellular glucans. The clinical signs and appearance of caries starts with a white spot lesion, through to hidden or occult caries. Failure of the enamel surface can lead to frank cavitation (Zero, 1999).



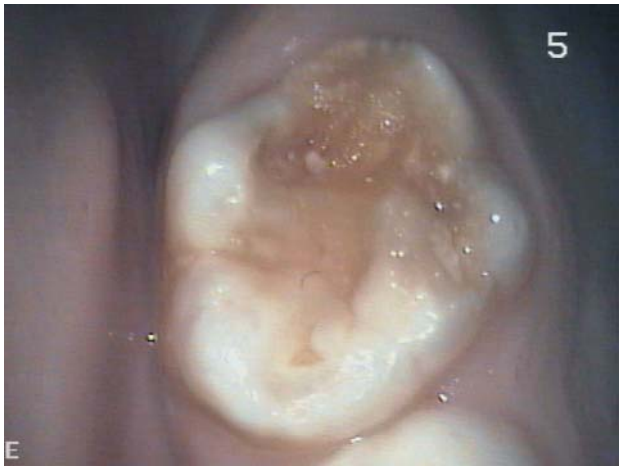


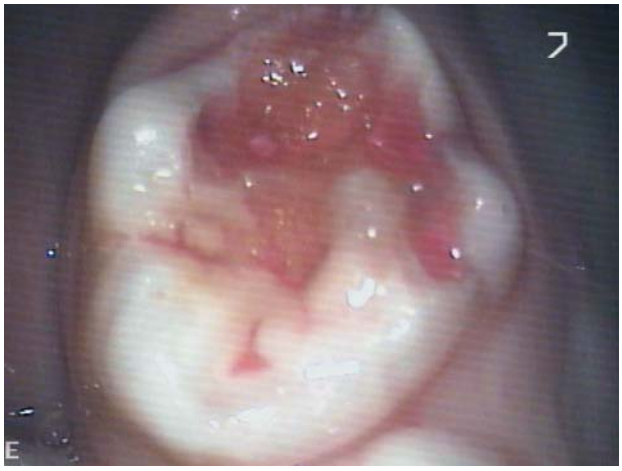
Figures 1 and 2: shows a large carious lesion with all the soft caries removed to a leathery consistency. It was been ozone treated for 60 seconds. And 6 weeks later, this surface is hard and glossy, and totally remineralised (Fig. 2).





Figures 3 and 4: Often there is no need to place any restoration in posterior or anterior teeth. In this example, a previously treated buccal lesion is painted with caries disclosing dye. When the buccal surface is washed, there are no stains visible in the O_3 treated buccal lesion. As the remineralised surface is hard, shiny and easy to maintain, there is no restorative requirement. In most cases, there is no need to drill the tooth with rotary drills. The noise and vibration from traditional 'drill and fill' is avoided, pulpal exposure is avoided, and tooth tissue is preserved.





Figures 5 and 6: In Fig. 5, a deep distal lesion is stained with caries dye in Fig. 6. Pulpal exposure is avoided by combining the ART treatment protocol with ozone.

Progression of caries in primary teeth

There are many features in the structure of primary teeth enamel, which may influence the observed accelerated caries progression (Mortimer, 1970):

- Enamel in primary teeth is about half the thickness of that found on permanent teeth.
- The degree of mineralisation is lower in enamel of primary teeth, which accelerates the rate of caries progression.
- The width of enamel prisms in primary teeth enamel is around 4–7 µm, in comparison with 6– 10 µm in the permanent teeth enamel. Interprismatic spread may lead to faster destruction.

The morphology of fissures in the occlusal surfaces of primary teeth tends to be V-shaped or U-shaped. Marked constrictions are seldom observed, although some of the fissures extend deeply into the enamel. The enamel below the fissure is extremely thin (100–150 µm).

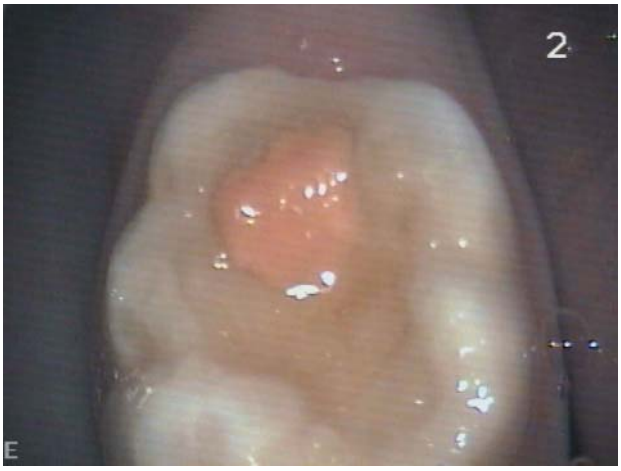


Figure 7: After ozone treatment (40 seconds) the lesion is washed with a remineralising solution, and to prevent debris impaction and potential re-infection, the cavity is restored with FujiVII. FujiIX could have been used for a more aesthetic effect.

- O_3 treatment is less time-consuming than conventional methods. There is no need for the patient to keep their mouth open for long periods which children find difficult; treatment times are around 2 minutes for tooth preparation, 10–40 seconds for O_3 treatment and 2 minutes for restorative placement, if used. In total, about 5 minutes at the most, compared to 20–30 minutes for traditional dental therapy. It is therefore suitable for very young patients.

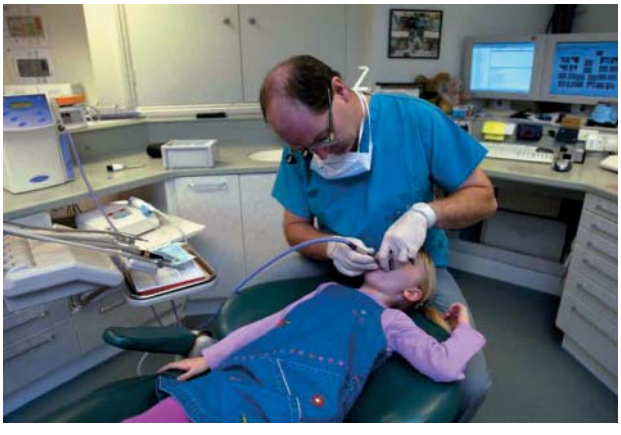
Caries risk assessment

Risk is defined as: the probability of an individual developing a given disease. Thus a risk factor is a factor associated with an increased probability of an individual developing a particular disease (Kidd, 1998). The goal of risk assessment in dentistry is to deliver preventive and restorative care specific to an individual patient.

It is important to identify the caries risk *before* the disease process starts. In young children, previous caries experience is not a particularly useful factor. Low birthweight of a child has been suggested as a caries risk indicator for primary teeth. There is evidence of an association with enamel hypoplasia, or indirectly as a marker for low socio-economic status. Another caries risk assessment is based on the age when the child becomes colonized with cariogenic flora, the baseline caries score, the child's mutans streptococci levels, the presence of visible plaque on the maxillary anterior teeth and the socio-demographic factors such as education.

Systemic and topical fluoride exposure, bottle use, diet and tooth brushing behaviour have been shown to be poor caries risk indicators for primary teeth (Tinanoff and, Douglass, 2001).

Before any dental treatment is started, previous or predisposing factors should be considered. This is used to allocate patients into a high risk or low risk group.



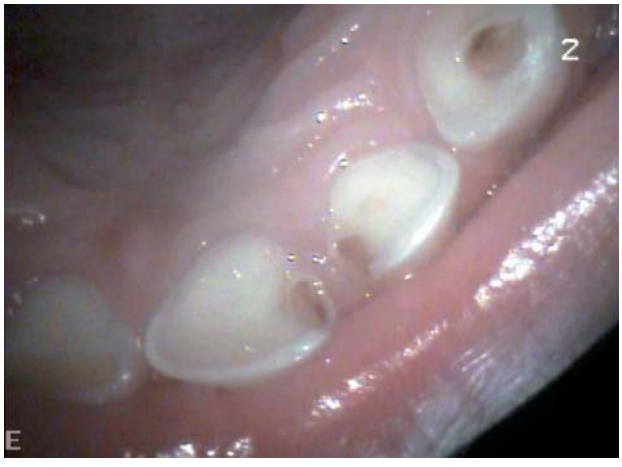


Figure 8 and 9: show young patients being treated. "K" in (Fig. 8) was 6 years old. She is relaxed, and this is her second treatment session. "J" in (Fig. 9) was 4 1/2 years old. He presented with 'bottle-caries', with multiple lesions. He was nervous, and in pain. All lesions were treated by removal of the soft debris to the leathery surface. This was followed by 40 seconds of ozone treatment, and mineral solution wash. The parents were instructed to place a quarter pea sized portion of a remineralising paste (HealOzone toothpaste, KaVo) directly into the treated cavities 2 to 4 times a day. At a 6-week review, all lesions were hard, shiny, and showed no classical signs of decay activity.

This grouping can be used to determine the most appropriate treatment. Monitoring of the caries risk should be routinely recorded to fine tune treatment and support offered. If existing lesions have not progressed and new lesions are not detected, caries activity has decreased. Increased numbers of new lesions or changes in the oral environment (e.g. orthodontic appliances, new fillings, mouth guards) or an increase in mutans streptococci levels may lead to a risk status increase (Tinanoff and Douglass, 2001).

Prevention of occlusal caries

Restorative materials, per se, will not prevent or eliminate disease. Caries is a microbial infection and, until the microflora is controlled, all restorations are at the risk of further tooth structure demineralisation. This leads to cyclical replacement dental therapy. If the cycle is to be broken, the profession must first acknowledge the primacy of prevention (Mount and Ngo, 2000).

Prevention methods:

1. **Dietary counselling:** The process of tailoring recommended changes to the needs of the individual. Dietary analysis (a three-day record of all food/beverage intake) is followed by targeted dietary advice (Harris and Harris, 1998). In general parents, guardians, carers and children can be advised to:

    - Reduce the amount and frequency of refined sugar snacking and consumption particularly between meals and at bedtime (Harris and Harris, 1998). However, if this is difficult to achieve, products sweetened by non-cariogenic sweeteners, such as 'tooth friendly sweets' may be given to children. The use of non-acidogenic sweetener such as xylitol, sorbitol, saccharin and aspartame in a wide variety of products including sweets, candies, chewing gum, oral hygiene products and pharmaceutical products has been shown to reduce the incidence of dental caries (Balakrishnan, Simmonds and Tagg, 2000):
    - Avoid using of bottles and feeder cups for consumption of cariogenic drinks. This would prolong the time tooth surfaces are exposed to sucrose. If a night bottle is given it should contain only water (Harris and Harris, 1998).

2. **Use of fluoride:** The mechanism of action of fluoride includes the enhancement of the remineralisation processes, by shifting the remineralisation/demineralisation rate equation toward remineralisation (Anderson, Bales and Omnell, 1993). Fluoride also leads to an increased resistance of the tooth structure to demineralisation (Levine, 1991) and has an antibacterial action (Balakrishnan, Simmonds and Tagg, 2000).

Fluorides are administrated systemically (e.g. tablets), applied topically at home (toothpastes or mouthwashes) or applied in the dental practice in the form of solutions, gels and varnishes. Fluoride is added to drinking water (Balakrishnan, Simmonds and Tagg, 2000) in certain countries. Water fluoridation has been shown to reduce caries by up to 50% in the primary dentition. This is the same for the permanent dentition (Rugg-Gunn, 1990). Drinking water fluoridation is very effective when considered in terms of cost and frequency of exposure (Kumar and Green, 1998). In order to use fluoride to its greatest potential, it should be available both systemically during tooth development, and topically and daily at low concentration after eruption.

Fluorosis (brown/white discolorations and mottling of teeth due to fluoride excess) can be a common aesthetic problem (Balakrishnan, Simmonds and Tagg, 2000). Young children tend to swallow half of the toothpaste they use and the attractive taste may lead to overdosage. The systemic uptake during tooth development puts them at risk of fluorosis. For this reason it is essential that children under 6 years should use a small pea-sized blob of low-fluoride paste with adult supervision (Harris and Harris, 1998).

3. **Fissure sealant:** Fluoride is very successful in the reduction of approximal, facial and lingual caries. But its effects are less on the rate of occlusal pit and fissure caries. These pits and grooves are impossible to thoroughly debride with a manual toothbrush and during normal routine oral hygiene. The fissure sealant can prevent caries in these locations. A fissure sealant is usually an unfilled resin, placed in the pits and fissures of teeth. This occludes the deep patterns, preventing the development of the acid niche environment and hence caries (Murray and Nunn, 1993). Although primary posterior teeth have not normally been regarded as teeth to be sealed, there are some situations

when this might be required. These situations are applicable, if there is no permanent successor and the primary molar is to be kept in the mouth for some time, or if children fall into a priority groups for fissure sealant selection (Shaw, 2000):

- Children and young people with physical disabilities and limitations of manual dexterity.
- Children with learning disabilities who cannot cooperate for fissure sealants to be applied.
- Difficulty of moisture control.
- Disabled individuals whose general health would be jeopardized by either development of oral disease or the need of treatment.
- Children with significant medical conditions such as cardiac problems, immunosuppression, bleeding disorders, blood dyscrasias, metabolic and endocrine problems. These patients may be at risk if caries develops.

The rational for resin-bonded pit and fissure sealants for primary molars is the same as for sealing permanent teeth. Before sealant placement it should be confirmed, both clinically and radiographically, that the tooth is caries-free (Harris and Harris, 1998). Other preventive methods include antimicrobial agents, caries vaccine and laser treatment. These are outside the scope of this chapter.

Treatment of caries in deciduous teeth

Retention of the primary dentition is important, as their removal may have severe effects on the development of malocclusions, create orthodontic nightmares, and create an anxious and dental phobic that is difficult to treat. This would make his individual more at risk to caries. Prevention of caries and tooth retention is important:

- To avoid pain, sepsis and potential damage to permanent successor (Harris and Harris, 1998).
- Space maintenance for the permanent dentition, and to prevent drifting and tilting of adjacent teeth. This aim can be achieved by early diagnosis of caries, before marginal ridge breakdown and space loss so that the teeth can be restored to their original mesiodistal dimension (Harris and Harris, 1998).
- Mastication (Stoner, 1967).
- To avoid the creation of an anxious, dental phobic (Harris and Harris, 1998).
- Retention of a primary tooth into adulthood, e.g. missing permanent successor (Harris and Harris, 1998).

Restoration of primary teeth is significantly different from that of permanent teeth. Primary teeth have a limited lifespan, children exhibit variable levels of cooperation and there are significant potential difficulties in the isolation of primary molars. Cavities tend to be wider and shallower than in permanent teeth (Fleming et al, 2001). Masticatory and biting forces applied to restorations in primary teeth are lower than forces applied to restorations in the adult dentition. Provided that primary teeth exfoliate, the need for restoration longevity is less in primary dentition than in permanent one (Fleming et al, 2001).

In comparison to permanent teeth, primary teeth:

- Are smaller and more bulbous (Harris and Harris, 1998).
- Have broad flat proximal contact areas (Stoner, 1967; Tinanoff and Douglass, 2001).
- Have relatively larger pulps. The mesio-buccal pulp horn, particularly of the primary lower first molars, is the largest (Stoner, 1967; Harris and Harris, 1998).
- Have thinner enamel and dentine. Usually there is less than 2 mm of tooth structure between the pulp and external surface (Stoner, 1967; Tinanoff and Douglass, 2001).
- Have different fissure morphology (Tinanoff and Douglass, 2001).

Restorative dental materials in paediatric dentistry

It is a challenge when restoring a primary tooth to choose techniques and materials that are likely to avoid the need for later retreatment. Restorative therapy only becomes necessary when there is preventative failure. Restorative therapy in primary teeth is essential where there is a need to restore tooth integrity to prevent space loss or disease progression into dental pulp. However, restorative therapy is a nonreversible procedure that makes a tooth susceptible to cyclical decay and fracture (Tinanoff and Douglass, 2001).

Restorative materials for primary teeth include:

1. **Amalgam:** amalgam is banned in some countries for restorative care for children.
2. **Glass Ionomer Cements (GICs):** GICs are an alternative to amalgam for restorative care of primary molars. GICs are adhesive and tooth-collared restorative materials that leach fluoride (Kilpatrick, 1993). The ability to leach fluoride helps to prevent recurrent caries in the interproximal region it may reduce the risk of caries starting in the opposing surface of the adjacent tooth (Derkson, Richardson and Jinks, 1989).

GICs are recommended for use as a restorative material in primary teeth where the occlusal stresses are reduced (minimal cavities) and over relatively short periods (Harris and Harris, 1998; Kilpatrick, 1993). GICs adhere to both enamel and dentine, and offer the potential to minimise the amount of mechanical retention by tissue destruction incorporated into cavity design (Kilpatrick, 1993).

3. **Composite Resins:** The potential advantage of resin-based composite is their ability to bond to etched enamel. This can reduce tissue destruction as the classic Black-design is not required (Kilpatrick, 1993). Other advantages of composite resin restoration of primary teeth include aesthetics and the excellent physical strength of the fully polymerized material (Croll, 1995). However, the success of these materials is highly technique sensitive and their correct placement requires excellent moisture control that can be difficult to achieve in young children. These factors make composite resins time consuming (Harris and Harris, 1998; Kilpatrick, 1993; Croll, 1995). Composites have little or no caries-preventive properties (Christensen, 2001). Lastly, shrinkage of the resin systems during light curing can lead to marginal microleakage at the resin/tooth interface. Marginal leakage ultimately leads to restorative failure. All the above factors are considered as the main problems with the use of this type of materials (Croll, 1995).
4. **Compomers:** Compomer is becoming one of the most promising and very popular restorative materials for paediatric dentistry. These materials are suitable for restoration of load bearing restorations in primary teeth (Fleming et al, 2001). Compomers are composed of a hydrophilic resin containing glass particles. These dissolve when oral fluids activate the acidic material, releasing fluoride. Advantages of this material are; no mixing required, putty-like consistency, non-sticky, handled easily and fluoride-releasing. However, the enamel and dentine-bonding agent minimises fluoride's ability to penetrate into the tooth. It is a relatively weak material, has poor resistance to wear compared to a resin-bonded composite and can be difficult to light-cure (Christensen, 2001).
5. **Hybrid Ionomer (Resin-Modified Glass Ionomer R-MGI):** R-MGI is a combination of glass ionomer and resin, where the incorporation of the resin significantly improves most physical properties of glass ionomer without diminishing the favourable properties of this material (Croll, 1995; Christensen, 2001). R-MGIs require hand or mechanical mixing. The low viscosity of the mix can make R-MGIs more difficult to handle and to place in a tooth preparation than compomer (Christensen, 2001). R-MGIs have many advantages; for example, it's chemical bond to dentin and enamel, a variety of shades, initial set within 60 seconds of light cure, a coefficient of thermal expansion similar to tooth structure, and a greater fracture strength than traditional glass-ionomer. Most importantly, it releases fluoride ions to the adjacent tooth structure (Croll, 1995).

R-MGIs cures chemically and with standard curing lights. These materials can be used in situations where light-cure is difficult, such as a large restoration or the wrap-around restoration. R-MGIs release higher levels of fluoride than compomers and they do not require a bonding layer, so the fluoride is released directly into the prepared tooth. These factors make it possibly the best restorative material when treating caries-active patients (Christensen, 2001).

6. **Enhanced-Strength Glass Ionomer (E-SGIs):** These products have similar properties to conventional glass ionomers but with enhanced strength. E-SGI materials need to be mixed either by hand or machine and applied to the tooth preparation in the putty stage. They are set by a typical acid-base reaction and do not require light curing. E-SGIs are easy to finish and clinicians reported that these restorations served for several years (Christensen, 2001).

Alternative treatment regimes used with these restorative materials

1. Atraumatic Restorative Treatment (ART)
2. Chemo-Mechanical Caries Removal (C-MCR)
3. Laser
4. Air Abrasion (AA)
5. Ozone (O_3).

Ozone in dentistry

Atraumatic Restorative Treatment (ART) and Air Abrasion (AA) are discussed in other chapters in this book. Other chapters describe the combination therapies with O_3 dental treatment in the permanent dentition. Lasers are outside the scope of this book, although laser and O_3 therapies could be combined into an effective treatment protocol.

Antimicrobial effect of Ozone on dentine caries

Recent published studies have looked at the effects of ozone (O_3) on treating dental caries. An in vitro study conducted by Baysan, Whiley and Lynch (2000) studying the effect of O_3 on micro-organism associated with primary root carious lesions showed that O_3 application for a period of 10 seconds was capable of reducing the numbers of *S. mutans* and *S. sobrinus* on saliva coated glass beads and this treatment was an effective, quick, conservative and simple. The safety of the HealOzone system has been extensively reported. In another study by Abu-Naba'a (2002), the effect of O_3 on fissure caries in permanent teeth showed that O_3 treatment produced significant remineralisation in lesions in the treatment group regardless of lesion type or location. All age groups in this study benefited from the treatment.

Why O_3 is useful in treating the child patient

A study published by Abu-Naba'a et al (2004) examined the effect of O_3 treatment on anxious patients. The study concluded that O_3 treatment resulted in the least anxiety compared to other routine dental treatments. A reduction in anxiety has also been noted in the accompanying carers or relatives. Compared to traditional 'drill and fill' dental treatment, 99% of all patients stated that they preferred O_3 treatment to traditional 'drill and fill' dental care, and all would have chosen O_3 treatment again.

Evidenced based dentistry studies proving the uses of Ozone to treat deciduous teeth caries

Treatment of Non cavitated deciduous occlusal caries

Extracted data for 5 to 7-year old children from a study published as an IADR Abstract in 2003 at Gothenburg (Holmes J, 2003) showed that non canitated occlusal fissure caries can be managed successfully using ozone in this double blind, controlled, randomised clinical trial. This study examined primary occlusal fissure carious lesions over a 12-month period in 76 patients of 2 to 12-year old children, in a general dental practice. O_3 was applied to each test lesion for 10, 20, 30 or 40 seconds depending on the clinical severity index and the DIAGNOdent assessment whilst the control lesions received only air and no Ozone. If reversal had not occurred, ozone treatment was repeated at a 3-monthly review. Patients were recalled at 12 months and clinically re-assessed. The results of this study showed a 98% reversal in the O_3 treated group. The control carious lesions, which had not received any O_3 treatment, did not significantly change in the study period. The conclusion of this study was that O_3 may be considered to be an effective alternative to conventional 'drilling and filling' for non vavitated deciduous carious lesions in general dental practice.

- Ola Abu-Salem in her thesis examined the effect of ozone to remineralise non-cavitated occlusal carious lesions of varying clinical severity scores in primary molars as assessed in a double blind, randomised, controlled clinical trial.

21 child patients with 74 non-cavitated occlusal carious lesions were recruited. At baseline, thorough cleaning with the ProphyFlex (KaVo GmbH, Germany) system was carried out, and baseline details were recorded:

- Date of examination
- Description of lesion site.

- The clinical severity score
- DIAGNOdent readings and scores
- ECM readings and scores
- Size of silicon cup used in ozone initial application.

Teeth with lesions were randomised into two groups:

Group 1: Teeth received O_3 treatment for 10 seconds, and mineral wash.

Group 2: Teeth only received mineral wash onto the lesions (control lesions).

All subjects received preventive advice and were given standard tooth brushes and toothpaste at each recall. Recalls were set at three-month intervals over one year. At assessment, each tooth was cleaned as at baseline, at each of the recall visits. Group 1 teeth were retreated with O_3 for 10 seconds at each of the recall visits at 3, 6 and 9 months.

Final assessment was carried out at 12 months. The ECM values for O_3 treated teeth increased, indicating remineralisation, and decreased in the control group, indicating further demineralisation. The DIAGNOdent vales increased in both groups, and this was greater in the untreated group. Group 1, O_3 treated teeth may have increased due to uptake of stains during remineralisation. When the overall change in ECM scores was analysed, the Group 1 values decreased, whilst those for Group 2, the untreated control lesions, increased. Lower ECM scores reflect remineralisation after ozone treatment.

Time certainly influenced the overall change over the year in the clinical severity scores (Ekstrand et al, 1998). There was an overall reduction in clinical severity scores in the treated groups compared to an increase in the untreated group. Time significantly influenced this change in the severity scores. Analysis of the clinical severity index showed an improvement in the treated group, as shown by lower scores. In comparison, the control group lesions become more severe with higher scores, interpreted as deeper lesions,

The conclusion of these studies were that Ozone treatment produced significant remineralisation in lesions, regardless of lesion type or location, of non-cavitated occlusal caries in primary teeth. Beside this proven efficacy of O_3 treatment, the non-invasive procedure saves costs, in terms of time, skill required, and finance of behaviour management techniques in children.

Treatment of deep deciduous caries with air abrasion, Ozone and sealing

In 2004, Holmes has completed the analysis of a multi centre study assessing the hardness of ozone-treated dentinal deciduous caries 3 months after sealing, and to compare the incidence of post operative complications associated with this technique with a conventional restoration technique. 94 Children from 3 general dental practices, each with two occlusal carious lesions with radiographic radiolucencies extending approximately 2mm into dentine, were recruited into this study. Air abrasion was used to remove the unsupported enamel over the lesion. Soft dentinal caries was removed to leave a leathery surface overlying the pulpal floor. The lesions were O_3 treated for 40 seconds, a mineral wash applied and each lesion was sealed with a mineral-releasing glass ionomer, FujiVII (GC Japan). After 3 months, the glass ionomer was carefully removed, the cavity floor examined, and then restored with a posterior composite. In the second group, conventional 'drill-and-fill preparation was used, and the cavity restored conventionally using a posterior composite. All lesions were exposed to a remineralising regime. At recall, some members of the group treated with traditional dentistry had post-operative complications. No sensitivity was reported associated with any of the O_3 treated teeth ($P<0.05$). At 3 months all O_3-treated dentine caries was hard and required no additional removal. The conclusion in this study was that air abrasion, O_3 and sealing was associated with reversal of deciduous caries, more conservation of tooth structure and less sensitivity pain than conventional drilling and filling. This method is currently extensively used by many UK Dentists to treat all deep carious lesions in children.

Treatment of deep deciduous caries with the Atraumatic Restorative Technique (ART) Ozone and sealing

Holmes completed a study examining the restoration of ART and Ozone treated deciduous carious lesions. This study assessed the placement time required and durability of restorations placed on ART and O_3 treated primary deciduous carious lesions (PDCL) in the most severe category, (judged to have an almost cariously exposed pulp) compared to conventional treatment. Each subject had one PDCL made caries free using conventional local analgesia (LA), drilling and filling using Optibond Solo Plus and Point 4 (KerrHawe) composite

resin. The remaining PDCLs were treated with the ART technique and O_3 treatment for 20 seconds (Heal-Ozone, KaVo) without LA, followed by filling using Optibond Solo Plus and Point 4. Up to 1 mm of softened carious dentine was left overlying the pulpal floor prior to O_3 treatment. The mean (SD) time required for ART, Ozone treatment and placement of a restoration was 6 (2) minutes, whilst the conventional technique, including LA, required 17 (4) minutes ($P<0.05$). After 18 months 30% of the conventionally treated teeth had symptoms suggesting pulpal necrosis compared to only 3% of the ART and Ozone treated teeth ($P<0.01$). The conclusion in this study has to be that ART combined with O_3 treatment saves time and reduces the chance of pulpal necrosis compared to conventional drilling and filling. Many UK Dentists currently use this technique in these deep deciduous caries lesions with similar success.

Treating caries in anxious children with ozone: parents' attitudes after the first session

Treating anxious children remains a challenge in paediatric dentistry. Sometimes the only options are the treatment with sedatives or in general anaesthesia which includes high cost, certain risks and a child that will stay anxious. Dahnhardt et al (2003) reported on treating caries in anxious children with Ozone and assessed the Parents' attitudes after the first session. The goal was to evaluate parents' attitudes towards the treatment of caries with ozone (Heal Ozone Unit, KaVo) in anxious children. After the first treatment of 20 children from age 2–10 years (which included brushing the teeth, hand excavation (Neos Vanadium Excavator No. 591/3, Hawe Neos Dental, Switzerland), DIAGNOdent measurement (KaVo, Germany) and ozone application), the father and/or mother was questioned about their attitudes toward the ozone treatment.

All parents answered the questionnaire but most children were accompanied by their mothers only (90%). Seventy-five per cent of the children were afraid of going to the dentist according to their parents prior to the ozone treatment but this group lost some of their fear and all children were happy to come back to the next appointment. All of them were happy that they started the ozone treatment. After the first session 75% of the parents would recommend the ozone treatment to family or friends and would agree to have an ozone treatment again. Eighty per cent of the parents were willing to pay more for the ozone treatment than for conventional 'drilling and filling'.

After the first session, the treatment with ozone seems to be well accepted by children and by parents and decreases anxiety in children.

Ozone treatment prior to all fissure sealant placements

Extensive research is underway treating all pit and fissure carious lesions with Ozone before fissure sealing, and the results after 2 years show this to be also very useful in our daily practice whilst caring for our patients.

Ozone treatment to all cavity preparations prior to all placement of all restorative materials

This procedure is practised routinely by hundreds of dentists daily in their clinical practice with the objective of reducing any chance of residual or recurrent caries to reduce the life of the restoration placed.

Conclusions

In stark contrast to traditional dental care, these studies into O_3 treatment show pleasant dental care can take place. Predictable caries reversal and re-mineralisation in soft carious lesions is possible, when treatment therapies (e.g., the use of air abrasion with O_3, and ART with O_3.) are combined. These treatment regimes compliment each other, and the data suggest that conventional treatment protocols will lead to greater tissue destruction, and pulpal exposure that necessitates further destructive dental treatment, pain and entry into the spiral of increasing cost and cyclical reparative therapy.

O_3 treatment often requires no local anaesthesia when treating caries in most cases. Deeper and more extensive caries may need a combined approach of traditional and O_3 treatment.

- O_3 treatment is less time-consuming than conventional methods. There is no need for the patient to keep their mouth open for long periods which children find difficult; treatment times are around 2 minutes for tooth preparation, 10– 40 seconds for O_3 treatment and 2 minutes for restorative placement, if used. In total, about 5 minutes at the most, compared much longer times for traditional dental therapy. It is therefore suitable for very young patients.

Ozone treatment with the HealOzone device has been integrated into many dental practices in Europe. These dental practices have reported less anxiety, less trauma, reduced extractions due to caries, and an increase in happy young patients attending for dental care. The treatment dovetails into the preventative approach to disease management, and there are important considerations for the development of a Preventative Management System to record data, and to develop a personalised treatment plan based on sound prophylaxis and preventive research and protocols. A system described in this book by Volker Scholz in his chapter "HealOzone and Total Quality Management in Dental Practice" is called the OHManager software. Treatment plans can be printed and discussed with each patient. This preventative approach integrated with ozone treatment has an exciting potential.

The use of materials such as FujiVII glass ionomer (GC Japan), a high fluoride-releasing pink coloured restorative combined with O_3 treatment seems to point towards a treatment protocol ideally suited to the primary and developing dentition for larger cavitated deciduous carious lesions.

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CHAPTER 3.6

Antimicrobial Effects of Ozone on Caries

Aylin Baysan & Edward Lynch

Introduction

Root caries is a major reason for tooth loss and is a problem, which increases with age. The resulting, tooth loss can diminish function and contribute to loss of self-esteem in elderly populations. This leads to more complex restorative challenges for dentists since the restoration of root caries poses a number of problems. Visibility and isolation from oral fluids (saliva, gingival secretion or haemorrhage) are particular problems, whilst the maintenance of pulpal integrity through the use of biologically acceptable dressings to the pulp-dentine reduces the depth of lesions but do not provide the aesthetic, physical and mechanical qualities required for a restoration. Furthermore, the plastic materials available for restoration of primary root caries lesions (PRCLs) include amalgam, composite, and glass ionomer cement, but many problems have arisen i.e., microleakage and poor marginal adaptations which have necessitated the frequent replacement of filling materials (Lynch et al, 1989). Whilst all these materials have their merits, they also have limitations in the restoration of carious lesions in conservative dentistry.

The clinical success of amalgam is well established when compared to other dental materials for the treatment of root caries. However, amalgam cannot be used in areas where aesthetics are of prime importance (Seichter, 1987). Therefore, there is a movement in dentistry towards aesthetic restorations. Resin-based composites are now being used as either amalgam substitutes or amalgam alternatives. It should be noted that composites have no caries-preventive effects, and postoperative sensitivity, discolouration and recurrent caries are often observed in root caries restored with composite (Seichter, 1987). Incorporation of fluoride into composite resin materials also failed to show any beneficial effect in reducing demineralisation of root carious lesions when compared to glass ionomer cements (Dijkman et al, 1994; Takahashi et al, 1993; Torii et al, 2001; Vermeersch et al, 2001). Indeed, secondary caries has been reported as being the most common reason for replacement of Class V amalgam, composite or glass ionomer restorations (Qvist et al, 1990). Placement of a restoration may result in the tooth being subjected to a repeat restoration cycle where the restoration may ultimately fail, to be replaced by progressively larger restorations (Elderton, 1996). Glass ionomer filling cements are best used for the restoration of erosion and abrasion cavities and PRCLs however, there are still limitations regarding tensile strength and low impact and fracture resistance (brittleness) (Bowen and Marjennhoff, 1992) (Yip et al, 2001) reported the surface roughness of eight aesthetic restorative materials and relationships with weight changes during fluoride release and uptake. Five specimens for each ChemFil Superior, Fuji IX Dyract, Fuji II LC, Vitremer, Photac-Fil, Ketac-Silver, and Z100 (control) were prepared and immersed in 2 ml of artificial saliva at 37°C. The changes in specimen weight and fluoride release were monitored for 12 weeks. This procedure was repeated after recharging the specimens with 1.23% APF gel for another 12 weeks. These authors reported that there was a significant weight loss for all glass ionomer cements following APF gel application, which correlated with fluoride release. Mean roughness measurements and SEM showed that roughness increased from the resin composite to

the conventional glass ionomer cements. It was concluded that the marked erosive effect of APF gel on glass ionomer restorations could increase surface colonization by plaque micro-organisms, and reduce the longevity of the restorations.

Through the use of new technologies, such as air-abrasive devices, Atraumatic Restorative Material Caries (ART), chemomechanical caries removal, lasers, detector dyes and Negative Air Ion (NAI) system can increase the speed, comfort and success of dental restorations. However, the issue of marginal adaptation has still been an ongoing technical problem.

Reversal of PRCLs is associated with remineralisation(Baysan, 2001) and a corresponding reduction in acidogenic and aciduric micro-organisms (Baysan et al, 2001; Beighton et al, 2001; Lynch and Beighton, 1993; Schüpbach et al, 1995; Lynch, 1994 and 1996). Hence, an anti-microbial method to manage PRCLs would be useful (Lynch, 1994). When lesions become inactive, i.e. hard or arrested, they acquire a smooth and hard surface. It should also be noted that arrested lesions remained unchanged during several years of observation (Lynch, 1996).

The best management strategy for root caries still needs to be developed. The possibility of preventing and controlling root caries for all populations worldwide is a strong incentive. However, compared to enamel caries, there has been relatively limited research available into pharmaceutical management of root caries, and many of these studies have also been carried out *in vitro*. As an alternative management strategy for root caries, ozone can be considered. Ozone has strong oxidisation power and has been used as deodorisation, decolourisation and oxidisation. This powerful oxidant is also effective for the inactivation of micro-organisms. The mechanism of microbial inactivation by ozone is thought to occur by general inactivation of the whole cell in micro-organisms.

The use of ozone in medicine

Ozone has been used in several medical applications (Papas et al, 1987; Belianin and Shemelev, 1994; Shiratori et al, 1993; Paulesu et al, 1991; Bocci, 1994; Riva Sanseverino, 1995; Cooke et al, 1997; Özmen et al, 1993; Romero Valdes et al, 1993; Rodriqueuz et al, 1997; Dolphin and Walker, 1979; Gloor and Lipphardt, 1976). Many studies have investigated the use of this therapy in the treatment of ocular diseases as optic neuropathies, glaucoma, central retinal vein obstructions and degenerative retinal diseases. Furthermore, endovenous ozone therapy, in patients with myocardial infarction, has a beneficial effect on blood lipid metabolism, decreasing blood cholesterol and provoking the activation of antioxidant protection system (D'erme et al, 1998).

Ozone, itself is not oxygen radical, but generates oxidants (ROS). This oxidant reacts with many blood components such as lipo-proteins, plasma proteins, lymphocytes, monocytes, granulocytes, platelets and erythrocytes. In a defence reaction to the generation of ROS, the various anti-oxidant systems are activated and go on to produce anti-oxidant enzymes and scavengers (Hernánndez et al, 1995). Since the oxidising effect of ozone is almost linearly related to its concentration in the blood, above a certain threshold it becomes very cytotoxic and produces haemolysis. The half-life of ozone is short and this oxidant rapidly converts into oxygen via endothermic reaction. Ozone treatment can positively affect the microcirculation. Among ozone biological effects (Belianin and Shemelev, 1994; Bocci, 1997 and 1999; Viebahn, 1999), the improvement of oxygen metabolism, increasing cell energy, the immunomodulator property and the enhancement of the antioxidant defence system are some of the beneficial effects of ozone in medical use. The use of ozone therapy on age-related degenerative retinal maculopathy demonstrated a decrease in lipid peroxidation but an increase in superoxide dismutase and an enzyme scavenger of anion superoxide. In this respect, ozone was capable to minimise the damage produced by lipid peroxidation by increasing antioxidant defence system (Barber et al, 1999; Léon et al, 1998; Paralta et al, 1999; Bocci, 1996a and 1996b; Riva Sanseverino et al, 1990) .

Studies on filtrability of blood after ozone treatment showed an increase in membrane fluidity (MF), whilst a reduction in sedimentation rate. In addition, ozonised blood was shown to have a protective effect on ischaemia-reperfusion injury in different organs such as liver, kidney and brain. Shiratori et al,(1993)investigated that ozone treatment had a positive effect on energy charge (EC) and ATP were well maintained in brain hypoxia. These authors also stated that lactate production was inhibited and survival time was significantly increased.

Interestingly, Copello et al (2001) investigated the effect of ozone application in patients (n=68) with retinitis pigmentosa (RP), which is characterised by progressive night blindness in a controlled, randomised, double blind clinical trial. Patients were treated with ozone by rectal administration (dose=10 mg) for 15 sessions. These authors reported a significant improvement in 88.2% of patients treated with ozone when compared to those in control group (23.5%) and stated that it could be useful to apply ozone therapy in the first stage of the disease and at six-month intervals to enhance visual capabilities in RP patients.

Recently, Zamora et al (2001) also reported the use of ozone (200 ug/250 gb.w) for the management of septic shock (inflammatory response). In their study, groups pretreated with ozone and antibiotics in combination showed a significant increase of survival of rats in comparison with the groups treated only with antibiotics. The survival rate for the groups treated only with antibiotics has less than 25%. These results show clearly that ozone was useful in the inflammatory response. Ozone pretreatment in combination to antibiotics was capable of reducing the mortality. The microorganisms have developed resistance to antibiotics, so that in the pharmaceutical field new germicidal products such as cephalosporine and quinolones are being continuously developed. Ozone applied prophylactically was able to increase or support the antibiotics action. These authors suggested that the prophylactic application of ozone may down regulate the inflammatory response and inhibit the IL-1 expression in the liver.

O_3 is also utilised externally in the form of ozonylated olive or sunflower oils. In this respect, medical treatment with ozone appears to be safe, therapeutically beneficial, and cost-effective. Ozonised sunflower oil (Oleozón) has shown antimicrobial effects against virus, bacteria and fungi (Sechi et al, 2001). Oleozón is a substance produced by the reaction of ozone with unsaturated fatty acids present in sunflower oil, this reaction occurs almost exclusively with carbon-carbon double bonds and produces several compounds such as hydrogen peroxide (Sechi et al, 2001).

The use of ozone in dentistry

The preventive and therapeutic effects of ozone in medicine have been well established (Baysan et al, 1999). Unfortunately, there are very few studies on the use of ozone for dental purposes.

Recently, there is growing concern regarding the quality of water that exits in dental unit waterlines (DUWs). The numbers of micro-organisms that have been found in water samples collected from dental units may exceed current limits for water quality and are perceived as a potential health risk to patients and dental personnel (Barbeau et al, 1996; Williams et al, 1993). Fortunately, ozone has successfully been employed for the treatment of dental unit water lines since 1990s. The microbial effect of ozone on dental treatment units lasted longer when compared to the conventional methods such as hydrogen peroxide/sliver ion solutions *in vivo* and *in vitro* (Putnins et al, 2001; Lee et al, 2001; Filippi et al, 1991; Filippi, 1995 and 1997). Filippi, 1997 tested the effect of ozone on *Pseudomonas aeruginosa*, a potentially pathogenic micro-organisms, which is frequently found in dental treatment units. After ozone treatment (10 μg ozone/ml water), there were no micro-organisms detected in water. Furthermore, there was no evidence of air pollution related to the use of ozone in the treatment area and no ozone detected in water taken from DUWs.

Subsequently, ozonated water was considered to be an alternative to a sterile isotonic solution for oral rinse during dental surgery, or following tooth extraction processes. Filippi, 1998 investigated that ozonised water applied on a daily basis can accelerate the healing rate in oral mucosa. The effect was observed on the first two postoperative days. Between the second and 7th postoperative day, there were no further effects observed related to ozone. However, the author stated that the effect observed in the first 48 hours, modified the final wound closure thereby under the influence of ozone, more wounds were closed after seven days and cell proliferation commenced earlier. These authors stated that the use of ozone is completely safe as ozone dissipates very quickly in water. It is concluded that the influence of ozone lead to a higher expression of cytokines that were important for wound healing, especially TGF-α 1, and important substance for regulation and coordination in the initial wound healing phase. TGF-α 1 had a marked influence on cell proliferation, chemotaxis (monocytes and fibroblasts), angiogenesis, synthesis of extracellular matrix and collagen synthesis. However, medically relevant properties of ozonated water in oral surgery still remain to be proved.

A denture cleaner using ozone bubbles (Ozone concentration approximately 10 ppm) was considered as clinically appropriate in view of its strong disinfecting and deodorising power, and high biological safety (Filippi, 1999). The effectiveness of this cleaner against Candida albicans was investigated and levels of this microbe were found to decrease to about 1/10 of their initial value after 30 min., and to $1/10^3$ after a 60 min. period of exposure. Subsequently, Suzuki et al (1999) also examined the influence of ozone on the surface of removable partial denture (RPD) alloys to determine its usefulness as a cleaning method for RPDs. The researchers reported that ozone treatment caused a slight change in the Au-Cu-Ag-Pd alloy in terms of reflectance. However, the changes were significantly less than those caused by acid-electrolyzed water and one of the commercial denture cleaners.

However, there has been no study concerning the clinical evaluation of ozone on its therapeutic benefits that it may offer for the management of root caries. Recently Baysan et al (2000) for the first time reported that ozone application either for 10 or 20 s was effective to kill the great majority of micro-organisms in PRCLs *in vitro* by the employment of a novel ozone delivery system (HealOzone CurOzone, USA) and this application for a period of 10 s was also capable of reducing the numbers of *Streptococcus mutans* and *S. sobrinus in vitro*.

The authors believe that the use of ozone in dentistry is conservative and harmless. This proposed ozone delivery system has been investigated in *in vitro* and *in vivo* studies. Recent clinical studies, which were also conducted by Baysan and Lynch have demonstrated the effect of ozone on the microbial flora and clinical severity of primary root caries by the ozone delivery system (Suzuki et al, 1999). In addition, these authors investigated the safety and efficacy of the use of ozone for the management of root caries in a longitudinal study. The following will be part of the *in vitro* microbiological studies.

In vitro study 1

In this study, a novel ozone delivery system (HealOzone, CurOzone U.S.A) was employed. The ozone delivery system is a portable apparatus for the treatment of caries with an ozone generator and delivers ozone at a concentration of 2,100 ppm±10%. The vacuum pump pulls air at 615 cc/min through the generator to supply ozone to the lesion and purges the system of ozone after ozone treatment. A disposable removable silicone cup (diameters ranging between 5 and 8 mm), attached to the handpiece, is provided for receiving the gas and exposing a selected area of the tooth to the gas. The tightly fitting cup seals the selected area on the tooth to prevent escape of ozone. The ozone is drawn out of the sealing cup through an ozone neutraliser that converts the ozone to oxygen. A suction system then removes any possible remaining ozone whilst the cup is still adapted to the PRCLs (the suction system passed the gas from the delivery system through manganese (II) ions). The system then draws a liquid reductant through the sealing cup to further neutralise residual ozone. The reductant mainly contained deionized water, sodium benzoate, methylparaben, sodium fluoride, xylitol and citric acid.

Study design 1

Forty freshly extracted teeth with soft PRCLs requiring restorations were collected from the Department of Oral and Maxillofacial Surgery at Barts and the London Queen Mary's School of Medicine and Dentistry. These teeth were randomly divided into two groups to test the anti-microbial effect on PRCLs resulting from exposure to ozone for periods of either 10 or 20 s. Plaque was removed using a hand-held standard fine nylon fibre sterile toothbrush with water as a lubricant within 15 min of extraction. Each tooth was dried using dry sterile cotton wool rolls and a dental three-in-one air syringe. Half of a lesion was removed using a sterile excavator. Following sampling, a reductant from the ozone delivery system was only applied to the samples for a period of 10 s in the control group. The excavator blade was used to traverse the lesion in line with the long axis of the tooth across the maximum gingival/occlusal dimension. Subsequently, ozone was delivered into the cup closely adapted to each remaining lesion for a period of either 10 or 20 s at room temperature (23 °C). The suction system then removed any possible remaining ozone whilst the cup was still adapted to the PRCLs (the suction system passed the gas from the delivery system through manganese (II) ions) and reductant filled the cup for a period of 5 s. A further sample was then taken. Each sample was immediately put into a sterile vial and weighed using a Sartorius micro-balance. Instantly, these were

placed in 1 µl of fastidious anaerobe broth (FAB, Lab M Ltd., Bury, Lancs, UK) with sterile glass beads 3.5–4.5 mm in diameter, (BDH, Poole, Dorset, UK) and vortexed for 30 s to facilitate the extraction of any micro-organisms from carious dentine and disperse any aggregates. Dilutions were performed by transferring 1 ml of the resulting suspensions into 9 ml of FAB and this process was repeated with a 10 fold dilution to 10^{-4}. After decimal dilution with FAB, 100 µl aliquots (for both test and control groups) were spread on fastidious anaerobe agar (FAA, Lab M, Bury, Lancs, UK) supplemented with 5% (v/v) horse blood and placed in an anaerobic chamber at 37 °C for a period of 4 days. The total cultivable microflora was assessed by counts of the colony forming units recovered from FAA supplemented with 5% (v/v) horse blood. The number of each colony type was counted and the number of colony forming units (cfus) was then calculated.

In vitro study 2

This study assessed the efficacy of ozone (from the delivery system described above) on *S. mutans* and *S. sobrinus* by the employment of the ozone delivery system. Forty sterile saliva coated glass beads were used.

Test micro-organisms

S. mutans and *S. sobrinus* were maintained by sub-culturing on 5% blood agar (Oxoid, Basingstoke, UK) every 7 days. Cultures were grown anaerobically for 16 hr at 37 °C in 5 ml of Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md., U.S.A).

Preparation of saliva-coated glass beads

5 ml of unstimulated human saliva from one donor was collected into a sterile container for each experiment; 1 ml volumes of saliva were clarified by centrifugation for 2 min. The salivary supernatants were pipetted into a sterile universal bottle and filtered using 0.45 µm and 0.2 µm filters. Three glass beads (3.5–4.5 mm in diameter, BDH, Poole, Dorset, UK) were put into a sterile bijou bottle. Immediately, thereafter, 0.5 ml of filtered saliva was added and left for 5 min.

Test procedure

40 sterile saliva-coated glass beads were randomly divided into two groups (test and control) for *S. mutans* and *S. sobrinus*. Each glass bead was put into a sterile bijou bottle with 3 ml of Todd-Hewitt broth for control and test groups and agitated for 2 s. Each bijou bottle was inoculated with either *S. mutans* or *S. sobrinus* and inoculated anaerobically overnight. The glass beads were then washed with 2 ml of PBS and transferred to a further sterile bijou bottle. Immediately thereafter, ozone was applied for 10 s to each glass bead in the test groups for either *S. mutans* or *S. sobrinus* at room temperature (23 °C), while shaking the bijou bottle to ensure delivery of the ozone gas to all surfaces of the test glass beads, whilst control glass beads for each micro-organism were left in the sterile bijoux and shaken for 10 s. Subsequently, these glass beads (control and test groups) were placed in 3 ml of Todd-Hewitt broth with six more sterile glass beads and vortexed for 30 s. Samples were serially diluted with FAB up to 10^8, and 100 µl aliquots were spread on blood agar plates supplemented with 5% (v/v) horse blood and placed in an anaerobic chamber at 37 °C for 2 days. The number of each colony type was counted and calculated.

Statistical analyses

Microbiological counts from the test and control groups of each study were transformed as $\log_{10}$ (cfus+1) prior to statistical analyses in order to normalise their distributions and ensure variance homogeneity. Statistical analyses of the data were conducted using paired Student t-tests to determine the significance of differences observed between the test and control groups (the threshold of significance was 0.05 for each study). Means and standard errors were also recorded for each experimental condition. All analyses were performed using the SPSS statistical package for MS Windows version 6.1.

Results

Study 1

Results are shown in Table 1. In study 1, there was a significant ($p < 0.001$) difference between the control and test samples for either a 10 or 20 s period of treatment in $\log_{10}$ (cfu+1) as shown in Table 2. Colony forming units for each sample were then analysed by their weights for both time periods. There was a significant difference in $\log_{10}$ (cfu+1) *per* mg between the time periods ($p<0.001$) (Table 1).

Table 1: Mean (±SE) $\log_{10}$ (cfu+1) before and after ozone application for 3, 5 and 8 s

Groups	3 s $\log_{10}$ (cfu+1)	5 s $\log_{10}$ (cfu+1)	8 s $\log_{10}$ (cfu+1)
Control samples	6.31±0.41	5.94±0.18	6.01±0.72
Test samples	5.84±0.29	5.71±0.23	4.98±0.26

Study 2

There was a significant ($p<0.0001$) reduction observed in both micro-organisms (*S. mutans* and *S. sobrinus)* in ozone-treated samples, when compared with the control samples (Table 3).

Table 3: Mean (± SE) $\log_{10}$ (cfu+1) before and after ozone application for *S. mutans* and *S. sobrinus*

Groups	*S. mutans*	*S. sobrinus*
Control samples	3.92±0.07	4.61±0.13
Test Samples	1.01±0.27	1.09±0.36

Discussion

It was demonstrated that exposure of carious dentine to ozone produced by a novel ozone-generating device for periods of either 10 or 20 s substantially reduced the levels of total micro-organisms, to <1% of the control values. Ozone is a powerful oxidant and the rapid inactivation of micro-organisms is one of ozone's outstanding characteristics.

Ozone exposure to PRCLs consistently achieved microbial reduction in the test groups regardless of the application time. The rapid inactivation of micro-organisms is one of ozone's outstanding characteristics. Presumably, ozone dissipates quickly in water and kills micro-organisms via a mechanism involving the rupture of their membranes in the lesions. It was demonstrated that exposure of carious dentine to ozone produced by a novel ozone-generating device for periods of either 10 or 20 s substantially reduced the levels of total micro-organisms, to <1% of the control values.

Saliva-coated glass beads were used to demonstrate the efficacy of ozone was tested specifically on *S. mutans* and *S. sobrinus* with the bacteria adherent to a solid surface as in the oral environment. The use of PBS eliminated the affect of reductants present in culture media.

Results in test groups obtained from saliva-coated glass beads showed a greater reduction compared with the carious dentine samples following ozone application. It can be speculated that in PRCLs, ozone clearly interacted with the abundant organic material in the lesions, which may reduce the anti-microbial effect of ozone. However, there were less organic materials from some salivary proteins on the glass beads than in PRCLs.

Bocci et al (1993) suggested that treating human blood with low ozone concentrations for the management of vascular disorders, chronic viral and autoimmune diseases can actually activate cells of the immune system and this treatment regime would be beneficial. However, it should be noted that ozone concentrations and time of exposure should be considered (Baysan, 2002).In principle, the potential toxicity of ozone should not prevent its use as a therapeutic agent (Hermández et al, 1995). At the correct dose, ozone can be useful as a therapeutic agent. Furthermore, Bocci(1991) reported that human blood treated with the correct dose of ozone can minimise the formation of free radicals and convert oxidants to less toxic species.

Ozone is approximately 10 times more soluble in water than oxygen. Mixed into pyrogen free water, the half-life of ozone is nine to ten hours (at pH 7 and 20 °C); and at 0 °C, this value is doubled. Ozonated water was effective in dental surgery where it was reported to

Table 2: Mean (±SE) $\log_{10}$ (cfu+1) and $\log_{10}$ (cfu+1)/mg before and after ozone application for either 10 or 20 s

Groups	10 s $\log_{10}$ (cfu+1)	10 s $\log_{10}$ (cfu+1)/mg	20 s $\log_{10}$ (cfu+1)	20 s $\log_{10}$ (cfu+1)/mg
Control Samples	5.91±0.15	8.99±0.39	6.18±0.21	9.19±0.36
Test Samples	3.57±0.37	6.79±0.39	3.77±0.42	6.31±0.12

promote haemostasis, enhance local oxygen supply and inhibit bacterial proliferation. Therefore, ozonated water was employed as a mouthrinse during dental surgery, or following tooth extraction procedure (Filippi, 1999).

In conclusion, ozone exposure for either 10 or 20 s under the experimental conditions reduced micro-organisms in the PRCLs. Ozone application for a period of 10 s was also capable of reducing the numbers of *S. mutans* and *S. sobrinus* on saliva-coated glass beads *in vitro*.

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CHAPTER 3.7

Clinical Management of Root Caries Using Ozone

Aylin Baysan & Edward Lynch

Introduction

Most elderly people may encounter significant oral problems that can ultimately have a profound impact on the quality of their lives. Epidemiological studies showed that in general, the oral health of elderly people is poor (Mersel et al, 2000; Ajwani et al, 2001). The most common causes of tooth loss are attributed to dental caries and periodontal diseases, (Chauncey et al, 1989) and this phenomenon can diminish function and contribute to loss of self-esteem in elderly populations.

It is now clear that root caries is a significant problem in older populations. Reversal of root caries is associated with remineralisation and a corresponding reduction in acidogenic and aciduric micro-organisms. (Schüpbach et al, 1996; Beighton et al, 1993). An anti-microbial method to manage root caries would therefore be useful (Lynch and Baysan, 2001; Baysan, 2002). Compared to enamel caries, there has been relatively limited research into the pharmaceutical management of root caries, and many of the studies have been carried out *in vitro*, with limited numbers of clinical trials.

Root caries like other types of dental caries result from the interaction of three factors: dental plaque, dietary carbohydrates, and susceptible teeth. The root carious lesion has some special characteristics. Root caries cannot develop until the root surface is exposed to the oral environment. Once exposed, the cariogenic micro-organisms in the accumulated plaque metabolise dietary carbohydrate to form acids, which set off a chain of events which results in the formation of a carious lesion. Based on numerous microbiological studies performed over the past three decades, it is clear that mutans streptococci (*S. mutans* and *S. sobrinus*) and lactobacilli are high-risk factors. The number of mutans streptococci and lactobacilli in saliva and their isolation frequencies from root surfaces, were correlated with the incidence of root caries (Ravald et al, 1986; Emilson et al, 1988). Accordingly, the total plaque flora is more important in the development of root caries than in enamel caries, and the degree of oral hygiene is more significant. The proportion of acidogenic micro-organisms in plaque is generally higher in older people, mainly due to a decrease in salivary secretion rate and a higher prevalence of removable dentures in the elderly (Fure, 1998). In addition, a gradual loss of manual dexterity in elderly people makes it hard for them to perform adequate oral hygiene.

Management of root caries can be expensive and time-consuming. Many restorative materials such as resin-modified glass ionomer, composites, and amalgams, have been used to restore the root carious lesion. However, many problems have arisen, such as micro leakage and poor marginal adaptations, which have necessitated the frequent replacement of filling materials. Using antimicrobial agents to provide effective levels of continuous fluoride release (such as fluoride releasing restorative materials, especially applied fluoride and fluoride containing toothpastes), may provide some protection for the high caries risk patients. However, clinical trials have been sparse.

As an alternative management strategy, ozone therapy may be considered. Root caries may be successfully treated by increasing the resistance of the lesion to mi-

crobial activity, and by reducing the extent of microbial activity. This may be achieved by applying an effective level of ozone, without the need for restorative materials. Baysan initially tested the anti-microbial effects of ozone on primary root carious lesions (PRCLs), and a significant reduction in total colony forming units (TCFUs) was observed in the ozone-treated groups (Baysan, 2002). Secondly, significant reductions in TCFUs for *Streptococcus mutans* and *Streptococcus sobrinus* was observed in ozone-treated samples. A further *in vivo* study demonstrated a significant reduction in TCFUs. In order to assess the safety of ozone during these treatments, the maximum ozone detectable levels (ppm) adjacent to the point of the application were measured *in vivo* and *in vitro.* These investigations revealed that the mean maximal detectable levels of ozone were all within EU and FDA guidelines. The following *in vivo* longitudinal study assesses the safety and efficacy of ozone in the management of PRCLs.

Study design

Prior to the commencement of this longitudinal *in vivo* study, ethical approval was obtained from the District Ethics Committee of Queen's University Belfast. The author exclusively recruited suitable patients to participate in this investigation. All participants were patients of Queen's University Belfast who were attending its School of Dentistry for routine oral health care. Each subject gave their informed consent for dental examination, and for ozone and sealant treatment to be undertaken. The data was obtained from a total of 140 PRCLs in 44 patients.

Inclusion and exclusion criteria

The following were criteria for the *inclusion* of subjects in this clinical study:

- Male or female ≥18 years of age.
- Leathery root carious lesions (Severity index 2) (Figure 1) on at least one surface, which are accessible for the diagnostic procedure.
- Signed and dated written Informed Consent (IC) from each patient for this protocol, obtained prior to study enrolment.

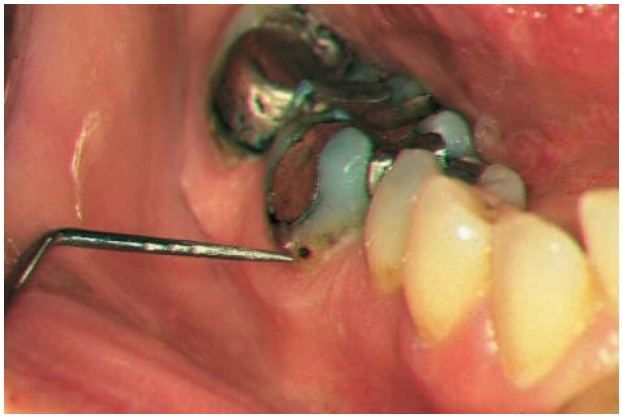

Figure 1: Leathery root carious lesions (Severity index: 2).

The following were criteria for the *exclusion* of subjects from this clinical study:

- No active root carious lesions.
- Presence of advanced periodontal disease in the study tooth (purulent exudate, tooth mobility, and/or extensive bone loss).
- Participation in another dental study during the previous three months.
- Any condition which, in the opinion of the investigator, would preclude participation by the subject (such as cross-infection control risk).
- Pregnancy

The author carefully documented the reasons why patients were excluded from the study.

Equipment used

Ozone delivery system

In this study, a novel ozone delivery system (HealOzone, CurOzone U.S.A) was employed (Figure 2). The ozone delivery system is a portable apparatus with an ozone generator for the treatment of caries and delivers ozone at a concentration of 2,100 ppm±10%. The vacuum pump pulls air through the generator at 615 cc/min to supply ozone to the lesion and purges the system of ozone after ozone treatment. A disposable removable silicone cup (diameters ranging between 5 and 8 mm), attached to the handpiece, is provided for receiving the gas, and exposing a selected area of the tooth to the gas. The

Figure 2: Ozone delivery system.

tightly fitting cup seals the selected area of the tooth to prevent escape of ozone. The ozone is drawn out of the sealing cup through an ozone neutraliser that converts the ozone to oxygen. A suction system then removes any possible remaining ozone whilst the cup is still applied to the PRCLs (the suction system passed the gas from the delivery system through manganese (II) ions). The system then draws a liquid reductant through the sealing cup to further neutralise any residual ozone.

The Electrical Caries Monitor

The ECM III (Lode Diagnostics BV, Groningen, The Netherlands) (Figure 3) was used to measure the electrical resistance of each carious lesion. The ECM measures the electrical resistance of a site on the tooth during controlled drying. By drying the surface, the resistance is determined by the tooth structure, and short-circuiting to the soft tissues caused by the surface liquid (saliva) is avoided. The electrical resistance was measured at 23.3 Hz and <0.3 µA whilst drying the tooth for 5 s at an air flow rate of 5 L min^{-1}.

Figure 3: Electrical Caries Monitor (ECM).

Figure 4: The DIAGNOdent.

The DIAGNOdent

The DIAGNOdent (Kavo, Germany) was used to detect and quantify the severity of PRCLs (Figure 4). This device measures laser fluorescence within tooth structure. As the laser light is propagated into the carious

lesion, the two-way handpiece optics allows the unit to quantify the reflected laser light. A tooth surface that is sound exhibits little or no fluorescence, resulting in very low scale readings on the display. However, carious lesions show some levels of fluorescence, proportional to the severity of the lesions, resulting in elevated scale readings on the display. The DIAGNOdent system records the instant and peak values. The instant reading indicates the real time value that the probe tip is measuring, whilst the peak value refers to the highest level scanned on the tooth. The DIAGNOdent device was turned on by pressing the grey ring on the handpiece. Calibration was performed before each session according to the manufacturer's instructions. The peak value was subjected to statistical analysis.

Study design

This longitudinal clinical study was designed to assess the efficacy and safety of ozone in the treatment of PRCLs *in vivo*. The study involved 44 patients with either two or four PRCLs. The medical and dental history of each patient was recorded, and patients were randomly allocated to one of the following two groups.

Group 1. Ozone Group

At baseline, ECM and DIAGNOdent measurements were performed. Subsequently, clinical criteria used to detect PRCLs were assessed. After cleaning with a sterile toothbrush and water, the tooth surface was isolated and dried with sterile cotton-wool rolls. Ozone was delivered for a period of 10 s into the cup, which was closely applied to each PRCL. Suction was automatically turned on for 10 s. As part of the suction system, captured residual ozone was passed from the delivery system through manganese (II) ions, which acted as an ozone neutraliser (consumer). The reductant filled the cup for a period of 5 s and the suction component of the system removed any possible remaining ozone while the cup was still applied to the PRCLs. As a back up, the dental unit high speed suction system was also used on every occasion. Root caries were not removed after ozone application. After 1 month, the procedures applied at baseline were repeated without ozone application. After 3, 6, 9 and 12 months, the same procedures were performed. In addition, ozone was applied for a period of 10 s.

Group 2. No Treatment (Control Group)

At baseline, the ECM and DIAGNOdent measurements were performed. Subsequently, clinical criteria used to detect PRCLs were assessed as in Group 1. PRCLs received only reductant for a period of 5 s without application of ozone. The same procedures were performed after 1, 3, 6, 9 and 12 months.

If patients presented any form of discomfort, PRCLs for each group were immediately treated with conventional drilling and filling procedures. Patients in both groups received full preventative advice including extensive oral hygiene and dietary advice, together with advice on the use of 1,100 ppm sodium fluoride-containing dentifrices (Advanced whitening toothpaste with soft polish, Natural White, U.S.A) and soft toothbrushes (Natural White, U.S.A).

Examinations

Each lesion was examined at baseline and after 1, 3, 6, 9 and 12 months according to the clinical detection criteria:

Colour: A shade guide was employed with typical descriptions of 'yellow', 'light brown' 'dark brown' and 'black' lesions. This shade guide was used as a reference in classifying the lesions.
Texture: Each lesion was classified as either rough or smooth by passing a dental explorer over its surface.
Hardness: Soft PRCLs permitted a sharp probe to penetrate the surface at 100 g pressure with ease, and there was definite resistance to its withdrawal, whilst leathery root carious lesions permitted a sharp probe to penetrate the surface at 100 g pressure and there was a slight resistance to its withdrawal. Hard PRCLs were comparable to the surrounding sound root dentine.
Cavitation. An estimate of the depth of the PRCLs was made by recording the greatest distance between the existing surface of the lesion and what was judged to have been the original root surface.
Size. A standard periodontal probe marked at 1mm-

intervals was used to determine the dimensions of each lesion. The maximum occluso-gingival and mesio-distal or labio/buccal-lingual/palatal dimensions were assessed.

Severity index. All PRCLs were classified according to the following severity index:

0 All 'Hard' lesions
1 'Leathery' lesions, which were considered to be small, easily cleansable and approaching a 'Hard' texture
2 'Leathery' lesions, which were judged to be shallow, and where the surface of the exposed sound dentine could easily be maintained plaque-free
3 'Leathery' lesions judged to be in surfaces which were difficult to maintain plaque-free, and large, cavitated 'leathery' lesions where pulpal integrity was judged to be at risk
4 All 'Soft' lesions

ECM measurements

During the ECM readings, the measuring probe was applied to a lesion whilst the subject held the reference electrode. Measurements were taken at the centre, mesial, distal, occlusal and gingival points of each PRCL. The monitor recorded the value at the end of the drying period (end value) and the area under the curve during the drying period (integrated value).

DIAGNOdent measurements

Measurements were taken at the centre, mesial, distal, occlusal and gingival points of each root carious lesion. The system recorded the peak and instant values. The peak values were used in the statistical analyses.

Statistical analyses

Clinical data were automatically transferred to a personal computer and data from the ECM and the DIAGNOdent were collected using the dedicated software from LODE Diagnostics. The mean resistance value and DIAGNOdent readings recorded at baseline and after 1, 3, 6, 9 and 12 months were used for data analyses. Subsequently, the ECM readings were transformed using the $\log_{10}$ function to normalise variance for both groups. Means and standard errors for each variable (hardness, cavitation, size, distance from gingival margin, and severity index) were then recorded. For the ozone and control groups, the primary outcome variable was the reversal of severity index.

Results

At baseline, 44 subjects were recruited in this study. Of these, 4 subjects failed to attend at the 12-month examinations. Only subjects attending all examinations were eligible for inclusion in the final data analyses. The mean age (±SD) of the subjects at baseline was 65 (±14) years with a minimum age of 30 and a maximum of 72 years. No adverse effects were observed during this clinical study.

Hardness

At baseline, all lesions were of a leathery consistency. At 1 month recall, 26.5% of PRCLs had become hard in the ozone group, whilst all the lesions remained the same in the control group ($p<0.001$). After 12 months, 47% of PRCLs had become hard in the ozone group and 9% of the lesions got worse in the control group ($p<0.001$) (Figure 5).

Figure 5: Percentages of hardness of PRCLs in ozone only group after 1, 3, 6, 9 and 12 months.

Discussion

The main clinical problem with pharmaceutical approaches to the management of root caries is the difficulty in suppressing or eliminating micro-organisms for extended periods of time. After treatment with selected pharmaceuticals, organisms may proliferate and re-colonise in PRCLs. (Baysan and Lynch, 2001b). In this study, PRCLs for each patient were reviewed in terms of their severity status following the application of ozone. Interestingly, 47% of PRCLs had become hard after 12 months. One can therefore speculate as to why many of the lesions reversed. Ozone is a powerful oxidant and kills the micro-organisms by damaging their cell membranes. An oxidant (sodium hypochlorite) has previously been shown to improve the remineralisation potential of demineralised dentine. Inaba et al, found that the use of an oxidant (10% sodium hypochlorite) on demineralised root dentine lesions improved their potential to remineralise (Inaba et al, 1995). Sodium hypochlorite is a non-specific proteolytic agent and was effective in removing organic components in the lesions Subsequently, Inaba et al, showed that when the root dentine samples were treated with this oxidant for 2 minutes, the permeability of fluoride ions increased (Inaba et al, 1996). Inaba concluded that removal of the organic materials from the dentine lesions was an acceptable approach to enhance remineralisation. This may partly account for the dramatic remineralisation results shown after ozone application in this study. However, ozone is a much more powerful oxidant than sodium hypochlorite (Baysan, 2002). Therefore, the results may also indicate that ozone has the ability to remove proteins in carious lesions, and to enable calcium and phosphate ions to diffuse through the lesions, a phenomenon resulting in remineralisation of some of the PRCLs after only a single application in cross sectional and longitudinal studies.

After the initial suppression of the numbers of total micro-organisms, re-colonisation of the micro-organisms may be retarded by a resistance of the normal commensal oral flora against intruding organisms into lesions. In addition, the ecological niche of these acidogenic and aciduric micro-organisms would be severely disrupted, which in turn could interfere with re-colonisation by these specific microflora. This may result in long-term suppression of acidogenic and aciduric micro-organisms in PRCLs. Emilson also reported that after a short-term intensive treatment of the dentition with 1% chlorhexidine, *S. mutans* was suppressed *in vivo* for a significant length of time (14 weeks) (Emilson, 1981). However, re-colonisation time differed amongst the subjects. In addition, in subjects in whom mutans streptococci had been reduced by 99% after the treatment, micro-organisms returned more slowly than in subjects with less microbial reduction.

It is possible that hypermineralisation is less likely to occur following the application of ozone. Since ozone is a strong oxidant, it will undoubtedly oxidise PRCLs' biomolecules and hence open the dentine channels in the lesions. Furthermore, ozone may have prepared a base for the lesion to allow the diffusion of calcium and phosphate ions through depth of the lesion. Moreover, it should be noted that the patients used a dentifrice containing a standard amount of fluoride. In the literature, it has been reported that low concentrations of fluoride have the capacity to remineralise carious lesions to their full depth. (Martens and Verbeeck, 1998). In this respect, future studies are required to determine the significance of these postulates.

With regard to the safety aspects of the therapeutic application of ozone, it should be noted that during *in vivo* and *in vitro* safety studies, only the tooth received ozone treatment when subjects with PRCLs were treated with this agent for a period of either 10 or 20 s. In these studies, ozone was drawn through the handpiece into the sealing cup by a vacuum. Subsequently, this powerful gas was drawn out of the sealing cup through an ozone destructor, which converted ozone into oxygen. A liquid reductant was then applied to PRCLs through the sealing cup to further neutralise any possible residual ozone. In addition, when this system was operated by suction only, the pathway for ozone was under negative pressure, which meant that ozone could not leak out if an incomplete seal occurred, since the flow sensor in the system shut down the ozone generator. The tightly fitting design of the cup of the delivery system provided a good seal and prevented any leakage of ozone gas. The detectable levels inside subjects' mouths conformed to permissible ozone levels in air as documented by the EU and FDA. Furthermore, the liquid reductant used at the end of each ozone application was essentially equivalent to a standard dental mouth rinse and contained agents currently known and in use in dentifrices and mouth rinses. It can therefore be speculated that xylitol especially acted as a reductant

during ozone application. In clinical studies, the ozone delivery system was used for either 10 or 20 s and no adverse events asoociated with ozone and/or the reductant were observed in these studies. Patients were happy to receive this novel treatment and there were no reported complaints.

Many detailed reviews of studies regarding the health effects of ozone on humans and animals have been published. The main purposes of these studies were to identify the biological molecules that react with ozone as it crosses the air/pulmonary tissues barrier, and the products formed in these reactions. Ozone is very reactive towards unsaturated lipids, certain amino acid residues in proteins, and many antioxidants. However, it cannot penetrate far into the air-tissue boundary before it reacts (Pryor, 1992). The principal targets for the reaction of ozone probably lie in the fluid layer covering the internal surfaces of the lung. The lung lining fluid layer is a patchy and highly dynamic material consisting of lipids, proteins, and antioxidants such as ascorbate, glutathione and uric acid. Studies concerning the reactions of ozone with lipids have identified lipid ozonylation products that can relay the toxic effects of ozone to deeper tissue strata where ozone itself fails to reach. Although reactive itself, ozone can also give rise to the generation of various further reactive oxygen species such as the hydroxyl radical ($OH^{\cdot}$). The oxidants react with many blood components such as lipo-proteins, plasma proteins, lymphocytes, monocytes, granulocytes, platelets and erythrocytes. As a defence to the generation of oxidants, various anti-oxidant systems are activated, including anti-oxidant enzymes and scavengers (Bocci, 1999). Since the oxidising effect of ozone is almost linearly related to its concentration in the blood, above a certain threshold it becomes very cytotoxic and produces haemolysis. The half-life of ozone is short and it rapidly converts into oxygen via an endothermic reaction. Ozone treatment can positively affect the microcirculation. However, it should be acknowledged that the current literature addresses the potential of adverse events in the event of ozone exposure beyond FDA and EU recommended levels. Pulmonary oedema, chronic respiratory disease, decreased lung function, inflammatory reactions, and eye irritation are common findings at higher levels of ozone exposure for medical purposes.

There are animal studies discussing a possible mutagenic effect of ozone in the literature. Dillon et al, reported that induced genetic damage resulting from ozone treatment may not be readily investigated due to its high toxicity, and difficulties in generating and administering controlled concentrations (Dillon et al, 1992). These investigations found that mutagenicity was dependent on the ozone flow rate and total exposure time, with variations in the optimum dose-time regimen leading to toxicity or complete inactivity. Their data showed that ozone was a very weak bacterial mutagen and only when tested under narrowly prescribed sub-toxic dosing conditions. It has been reported that ozone is an air pollutant due to its formation in the atmosphere by photochemical reactions between volatile hydrocarbons and nitrogen oxides (Wilkins et al, 2001). However, in the ozone delivery system used in this reported study, such chemicals were not produced. Aromatic volatile hydrocarbons, which are produced by combustion in cigarette smoke and some nitrogen oxides, such as nitrogen dioxide also produced in cigarette smoke, are carcinogenic, but clearly, it is a question of dose and duration of exposure.

The United States National Institute of Health (NIH) reported an Immediately Dangerous to Life or Health Concentration (IDLH) of 5 ppm for a 30 minute exposure based on acute inhalation toxicity data in humans. King also reported that ozone exposure at 50 ppm for 60 minutes would probably be fatal to humans based on animal studies (King, 1963). It should be noted that some animal and human studies investigating the effect of ozone for medical purposes, have not fully addressed the consequences and adverse events of this therapy on lung tissues in detail. Data on short-term exposure to ozone at various levels is not yet available.

Results obtained from this ozone study were promising. The use of ozone is safe, cost-effective, cost-efficient, and time-efficient. The economic consequences of simpler and less prolonged dental treatment would clearly be beneficial. In the past, cost analysis was based principally on the comparative market price of new treatments compared with conventional therapy. Now, issues such as quality of life, early return to occupation, and subjective symptoms of pain and discomfort caused by a treatment are also critically evaluated. Ozone treatment can easily be applied to the general population. In addition, oral care becomes more difficult for elderly people since compromising somatic and mental conditions affect this growing population. These compromising situations can easily be overcome using preven-

tion and early intervention strategies to reduce the risk of root caries for elderly people. In this respect, the use of ozone can be considered especially appropriate for medically compromised patients, domiciliary care patients and house-bound elderly people. No injection involved in ozone treatment, and the ozone delivery system is portable. Therefore, elderly patients who have limited access to dental services can benefit greatly from this treatment.

In conclusion, the ECM is a sensitive and useful piece of equipment which provides an objective quantifiable method to assist in diagnosing the clinical severity of root caries. The novel treatment regime of the application of ozone is capable of clinically reversing leathery root caries and can be considered a revolutionary alternative to conventional "drilling and filling" for the management of leathery root caries.

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CHAPTER 3.8

Use of Sealants Over Ozone Remineralised Root Caries

Aylin Baysan & Edward Lynch

Introduction

Demineralisation starts from the action of acidogenic micro-organisms on the tooth, subsequent to the ingestion of fermentable carbohydrates (Hoppenbrouwers et al 1987). The various organic acids diffuse within the plaque to the root surface, and give rise to the dissolution of the hydroxyapatite material. Root caries are formed in the same manner as enamel caries, except that demineralisation begins at a higher pH (Atkinson and Wu, 1994). As demineralisation proceeds, more mineral is lost, exposing collagen in root surfaces which is degraded by the plaque-forming micro-organisms (Ship et al, 1991). Active root caries may become inactive by the formation of a hard outer surface through the process of mineralisation. Hence, the killing of micro-organisms in PRCLs can prevent acid formation and collagenase production.

Reversal of PRCLs is associated with remineralisation and a corresponding reduction in acidogenic and aciduric micro-organisms (Schüpbach et al 1996; Lynch 1994; Lynch and Beighton 1994; Beighton and Lynch 1993). An anti-microbial method to manage PRCLs would therefore be useful (Baysan and Lynch 2001). Compared to enamel caries, there has been relatively limited research into the pharmaceutical management of root caries, and many of the studies have been carried out *in vitro*, with limited numbers of clinical trials.

It is now recognised that the most desirable treatment for root caries is remineralisation (Allen et al 1999). Using anti-microbial agents and fluoride-containing dentifrices (the latter to provide effective levels of continuous fluoride release) may provide some protection for high caries risk patients. However, it is difficult to extrapolate the recommendations for fluoride application on enamel to root dentine since, on the basis of demineralisation and remineralisation studies, it has been shown that more fluoride is required for the remineralisation of roots than for enamel (Herkströter et al 1991). Moreover, the delivery of fluoride in various vehicles has been shown to be effective in preventing and arresting root carious lesions. Clinical studies have demonstrated that water fluoridation (Hunt et al, 1989) and fluoride rinsing (Wallace et al, 1993) can arrest root caries. Although fluoride-containing dentifrices have also been shown to have a beneficial effect on root caries (Hicks et al, 2000), such data are sparse. To date, only limited clinical studies have demonstrated the effect of fluoride-containing products on root caries. Consequently, it is important to identify the best delivery system, and the optimum frequency of fluoride delivery for the inhibition of root caries.

Another approach to the management of PRCLs might be the application of a sealant directly over root caries. This technique would have the benefit of being non-invasive, and would reduce the need for local anaesthesia and specialised equipment. Use of a sealant could serve to prevent the re-colonisation of micro-organisms in PRCLs and could act as a barrier between any remaining micro-organisms and their substrate.

A reduction in the number of cultivable micro-organisms from infected dentine has been shown after use of fissure sealants for pit and fissure caries in enamel (Primosch and Barr 2001; Wendt et al 2001).

There has been limited research on the interaction between PRCLs and adhesive materials. The shear bond

strength of four adhesive systems to PRCLs with sound dentine acting as a control was studied by Burke and Lynch in 1998. OptiGuard in combination with Opti-Bond FL Prime and OptiBond Adhesive had the highest bond strength, and this was not influenced by the lesion status.

Recently, Baysan and Lynch conducted a clinical study to assess the efficacy of a root sealant (Seal and Protect, Dentsply, Germany) on the non-carious abrasion cavities to reduce hypersensitivity, and the effect of this sealant on caries-associated micro-organisms in the overlying sealant plaque (Baysan and Lynch, 2003). Clinical assessments, impressions for three-dimensional analysis and modified United States Public Health Service (USPHS) criteria (Burrow et al 2002) (n: 24) were carried out at baseline, 3, 6 and 19 months. Plaque samplings were performed at baseline and after 3 and 19 months. At 19 months, 20 patients completed the clinical trial. There was a significant reduction in the sensitivity scores. The $\log_{10}$ of total colony-forming units (cfus+1) from overlying plaque significantly reduced. The percentage of mutans streptococci reduced after 3 months ($p<0.01$) and yeasts also reduced after 19 months ($p<0.01$). The mean overall wear (±SD) (µm) on the sealant was 78.8±21.3 at 3 months; 95.4±7.3 after 6 months and 136.5±9.8 after 19 months (Figure 1). It was concluded that there was a significant reduction in sensitivity scores compared to baseline after 19 months. The protective sealant was found to be capable of covering the cervical surface to prevent further wear. In addition, there was a significant reduction of some representative caries associated micro-organisms in the overlying plaque. This new root sealant was therefore considered to be useful either with ozone or without ozone in the treatment of PRCLs, and its use in this study is reported.

Study design

Prior to the commencement of this longitudinal *in vivo* study, ethical approval was obtained from the District Ethics Committee of Queen's University Belfast. The author exclusively recruited suitable patients to participate in this investigation. All participants were patients of Queen's University Belfast who were attending its School of Dentistry for routine oral health care. Each subject gave their informed consent for dental examination, and for ozone and sealant treatment to be undertaken. The data was obtained from a total of 80 PRCLs in 35 patients.

Inclusion and exclusion criteria

The following were criteria for the *inclusion* of subjects in this clinical study:

- Male or female ≥18 years of age.
- Leathery root carious lesions (severity index 2) on at least one surface, which are accessible for the diagnostic procedure (Figure 2).

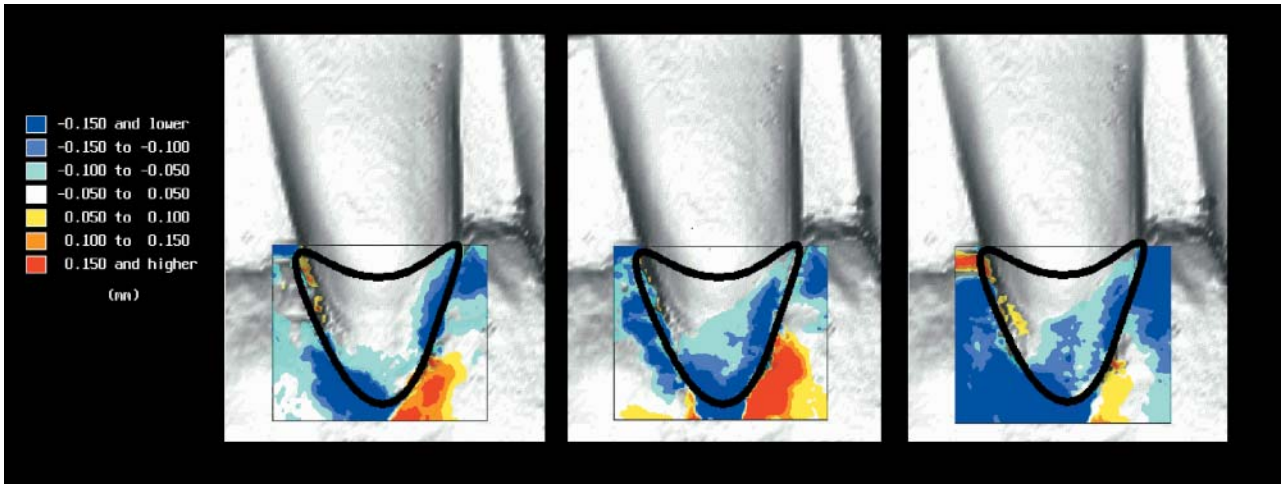

Figure 1: Sealant wear at 3, 6 and 19 months – Subtraction maps.

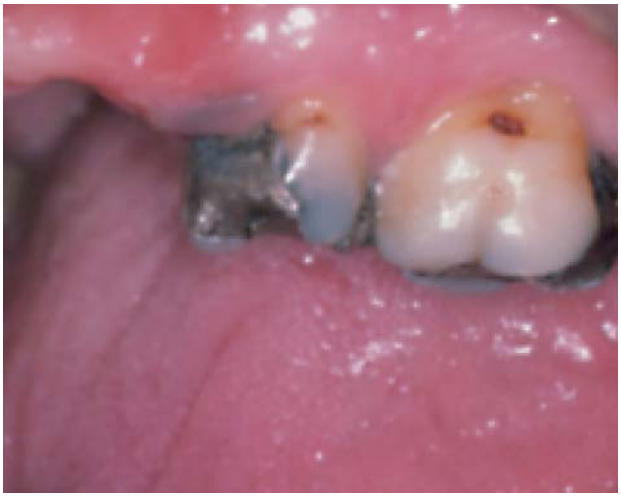

Figure 2: Leathery root carious lesion in severity index 2.

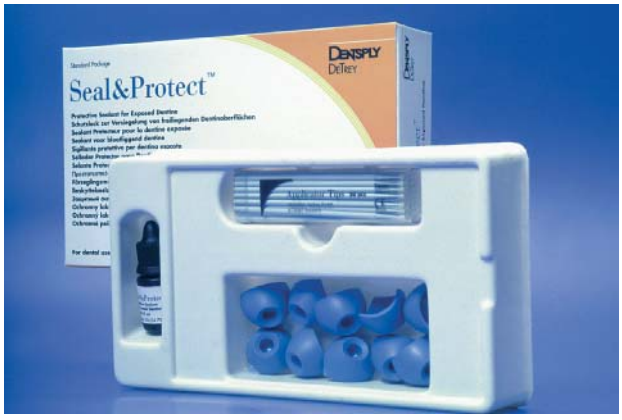

Figure 3: The root sealant.

– Signed and dated written Informed Consent (IC) from each patient for this protocol, obtained prior to study enrolment.

The following were criteria for the *exclusion* of subjects from this clinical study:

– No active root carious lesions.
– The presence of advanced periodontal disease in the study tooth (purulent exudate, tooth mobility, and/or extensive bone loss).
– Participation in another dental study during the previous three months.
– Any condition, which, in the opinion of the investigator would preclude participation by the subject (such as cross-infection control risk).
– Pregnancy

The author carefully documented the reasons why patients were excluded from the study.

Equipment used

Ozone delivery system

In this study, a novel ozone delivery system (Heal-Ozone, CurOzone U.S.A) was employed (Figure 3). The ozone delivery system is a portable apparatus with an ozone generator for the treatment of caries and delivers ozone at a concentration of 2,100 ppm±10%. The vacuum pump pulls air through the generator at 615 cc/min to supply ozone to the lesion and purges the system of ozone after ozone treatment. A disposable removable silicone cup (diameters ranging between 5 and 8 mm), attached to the handpiece, is provided for receiving the gas and exposing a selected area of the tooth to the gas. The tightly fitting cup seals the selected area on the tooth to prevent escape of ozone. The ozone is drawn out of the sealing cup through an ozone neutraliser that converts the ozone to oxygen. A suction system then removes any possible remaining ozone whilst the cup is still applied to the PRCLs (the suction system passed the gas from the delivery system through manganese (II) ions). The system then draws a liquid reductant through the sealing cup to further neutralise any residual ozone.

The Electrical Caries Monitor

The ECM III (Lode Diagnostics BV, Groningen, The Netherlands) was used to measure the electrical resistance of each carious lesion. The ECM measures the electrical resistance of a site on the tooth during controlled drying. By drying the surface, the resistance is determined by the tooth structure, and short-circuiting to the soft tissues caused by the surface liquid (saliva) is avoided. The electrical resistance was measured at 23.3 Hz and <0.3 µA whilst drying the tooth for 5 s at an air flow rate of 5 L min^{-1}.

The DIAGNOdent

The DIAGNOdent (Kavo, Germany) was used to detect and quantify the severity of PRCLs. This device measures laser fluorescence within tooth structure. As the laser light is propagated into the carious lesion, the two-way handpiece optics allows the unit to quantify

the reflected laser light. A tooth surface that is sound exhibits little or no fluorescence, resulting in very low scale readings on the display. However, carious lesions show some levels of fluorescence, proportional to the severity of the lesions, resulting in elevated scale readings on the display. The DIAGNOdent system records the instant and peak values. The instant reading indicates the real time value that the probe tip is measuring, whilst the peak value refers to the highest level scanned on the tooth. The DIAGNOdent device was turned on by pressing the grey ring on the handpiece. Calibration was performed before each session according to the manufacturer's instructions. The peak value was subjected to statistical analysis.

Materials used

A root sealant

The root sealant (Seal & Protect, Dentsply, Germany) is a self-adhesive, light-curing, translucent sealing material and contains a mixture of dimethacrylate resins in a solvent (acetone), with triclosan and fluoride (Figure 3). This sealant showed promising results for the management of cervical sensitivity for 19 months[17].

Study design

This longitudinal clinical study was designed to assess the efficacy and safety of ozone in the treatment of PRCLs *in vivo*. The study involved 35 patients with either 2 or 4 PRCLs. The medical and dental history of each patient was recorded, and patients were randomly allocated to one of the following two groups.

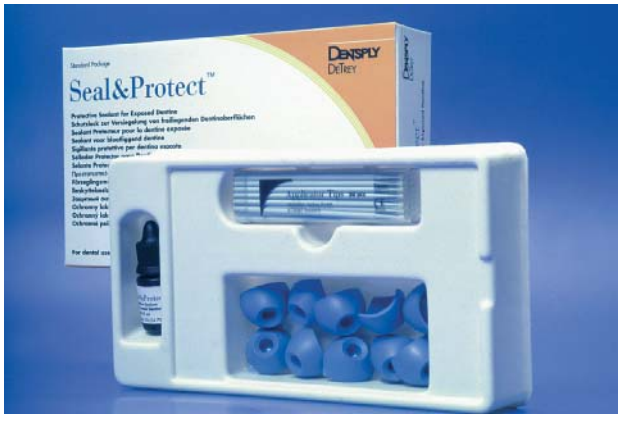

Figure 3: The root sealant.

Group 1. Ozone and Sealant Group

At baseline, the ECM and DIAGNOdent measurements were carried out. Subsequently, clinical criteria used to detect PRCLs were assessed. In this group, a root sealant (Seal and Protect) was placed on top of the PRCLs without removal of the carious dentine after the application of ozone for a period of 10 seconds. As a back up, the dental unit high-speed suction system was also used on every occasion. After 1 month, the procedures applied at baseline were repeated without ozone application. Clinical assessments according to the modified USPHS criteria of the sealants were also undertaken after 1 month. Sealants were applied again only if a partial or complete loss of the sealant was suspected. After 3, 6, 9, and 12 months, the same procedures were performed. In addition, ozone was applied for a period of 10 s. Sealants were also applied only if a partial or complete loss of the sealant was suspected.

Group 2. Sealant Group

At baseline, electrical resistance and DIAGNOdent measurements on each lesion were carried out before and after application of the root sealant. Clinical criteria used to detect PRCLs were applied. In this group, following application of reductant without ozone for a period of 5 s, this root sealant was placed on top of the PRCLs without removal of the carious dentine. Electrical resistance measurements, clinical assessments and the Modified United States Public Health Service (USPHS) criteria were also performed after 1, 3, 6, 9 and 12 months. Application of the root sealant was performed if they were partially or totally lost at follow-up visits.

If patients presented with any form of discomfort, PRCLs for each group were immediately treated with conventional drilling and filling procedures. Patients in both groups received full preventative advice including extensive oral hygiene and dietary advice, together with advice on the use of 1,100 ppm sodium fluoride-containing dentifrices (Advanced whitening toothpaste with soft polish, Natural White, U.S.A) and soft toothbrushes (Natural White, U.S.A).

Examinations

Each lesion was examined at baseline and after 1, 3, 6, 9 and 12 months. In addition, each sealant was scored according to the USPHS criteria rating system for evaluating restorations.

ECM measurements

During the ECM readings, the measuring probe was applied to a lesion whilst the subject held the reference electrode. Measurements were taken at the centre, mesial, distal, occlusal and gingival points of each PRCL. The monitor recorded the value at the end of the drying period (end value) and the area under the curve during the drying period (integrated value).

DIAGNOdent measurements

Measurements were taken at the centre, mesial, distal, occlusal and gingival points of each root carious lesion. The system recorded the peak and instant values. The peak values were used in the statistical analyses.

Clinical criteria used to detect PRCLs

Colour: A shade guide was employed with typical descriptions of 'yellow', 'light brown' 'dark brown' and 'black' lesions. This shade guide was used as a reference in classifying the lesions.
Texture: Each lesion was classified as either rough or smooth by passing a dental explorer over its surface.
Hardness: Soft PRCLs permitted a sharp probe to penetrate the surface at 100 g pressure with ease and there was definite resistance to its withdrawal, whilst leathery root carious lesions permitted a sharp probe to penetrate the surface at 100 g pressure and there was a slight resistance to its withdrawal. Hard PRCLs were comparable to the surrounding sound root dentine.
Cavitation: An estimate of the depth of the PRCLs was made by recording the greatest distance between the existing surface of the lesion and what was judged to have been the original root surface.
Size: A standard periodontal probe marked at 1-mm intervals was used to determine the dimensions of each lesion. The maximum occluso-gingival and mesio-distal or labio/buccal-lingual/palatal dimensions were assessed.
Severity index: All PRCLs were classified according to the following severity index:

0 All 'Hard' lesions
1 'Leathery' lesions, which were considered to be small, easily cleansable and approaching a 'Hard' texture
2 'Leathery' lesions, which were judged to be shallow, and where the surface of the exposed sound dentine could easily be maintained plaque-free
3 'Leathery' lesions judged to be in surfaces, which were difficult to maintain plaque-free and large, cavitated 'leathery' lesions where pulpal integrity was judged to be at risk
4 All 'Soft' lesions

Statistical analyses

Clinical data were automatically transferred to a personal computer and data from the ECM and the DIAGNOdent were collected using the dedicated software from LODE Diagnostics. The mean resistance value and DIAGNOdent readings recorded at baseline and after 1, 3, 6, 9 and 12 months were used for data analyses. Subsequently, the ECM readings were transformed using the $\log_{10}$ function to normalise variance for both groups. Means and standard errors for each variable (cavitation, size, distance from gingival margin, and severity index) were then recorded. For the two treatment groups, the primary outcome variable was the marginal adaptation of the root sealant.

Hardness and severity index
The differences in the number of lesions becoming hard and reversed into a less severe index were tested if the sealants fell out.

Results

Modified USPHS Criteria
All sealants were clinically assessed for colour match, marginal discoloration, anatomic form, marginal adaptation, surface roughness and secondary caries after 1, 3, 6, 9 and 12 months.

1 month follow-up

At the 1 month follow-up in the ozone and sealant group, there were

no discolorations, 21 anatomic form failures, 15 marginal fractures and 5 sealants with surface roughness.

At the 1 month follow-up in the sealant only group, there were 20 anatomic form failures, 18 marginal fractures and 12 sealants with surface roughness.

12 month follow-up

At the 12 month follow-up in the ozone and sealant group, there were no discolorations, 18 anatomic form failures, 12 marginal fractures and 7 sealants with surface roughness.

At the 12 month follow-up in the sealant only group, there were 21 anatomic form failures, 16 marginal fractures and 14 sealants with surface roughness.

The ozone and sealant group also had greater improvements in the ECM and DIAGNOdent values when compared to the sealant only group after 12 months ($p<0.05$). In addition, Modified USPHS criteria revealed that 61% of sealants were intact in the ozone and sealant group, and 26.1% of sealants were intact in the sealant only group ($p<0.05$).

Discussion

Root sealant application in conjunction with ozone application failed to show any significant additional benefits. However, root sealants in the ozone and sealant group performed better when compared to those in the sealant only group. This observation may be related to the oxidative and permeability effects of ozone allowing remineralisation of PRCLs.

In the ozone and sealant group, some of the PRCLs became hard even though the sealants fell out, whilst none of the lesions became hard in the sealant only group. Recently, Burrow et al,[18] in an *in vitro* study reported that the penetration of bonding agents into demineralised dentinal substrate depended upon the properties of the materials. The acidity of the solution is one of the key properties, since it can produce a porous surface and provide a route for materials to impregnate the dentine. In their study, Single Bond was used as a prime/bond adhesive and was applied after etching and water rinsing, and was not required to be acidic. Self-etching/self-priming systems, Prime & Bond 2.1 and Liner Bond 2, had greater acidity compared to Single Bond. However, this variation in the acidity of the solution did not cause any significant differences in the penetration of bonding agents into demineralised dentine. The viscosity of the materials also influenced their ability to penetrate into demineralised dentine. Another possibly relevant factor was the solvent in which the adhesive was carried onto the surface of the dentine, since ethanol- or acetone-based bonding agents had a high affinity for demineralised wet dentine. The ability of sealants to penetrate into carious dentine depends not only upon the chemistry of the material itself, but also the nature of the carious lesion. This root sealant when combined with ozone application appeared to be retained better on some of the PRCLs over a period of 12 months and reversed the severity of some of the PRCLs. The failure of some sealants to be retained intact over a period of 12 months may be attributed to variations in lesion structure. The root sealant was designed for the exposed dentine surfaces. The efficacy of this root sealant on cervical hypersensitivity has previously been investigated (Baysan and Lynch, 2002). In that study, the reduction in the levels of selected micro-organisms observed persisted for 3 months following application of the sealant. This might have been associated with several factors including smoothness of the resin surface, release of cytotoxic constituents from the sealant, the different contact angle and/or surface energy of the sealant when compared to the previous teeth surfaces. Sealing of the rough exposed dentinal surfaces with this smooth sealant may also have been associated with less plaque accumulation. In addition, the operative procedure itself might have had some impact on these results. This root sealant provided a long-term seal of the patent dentine tubules on some of the teeth and three-dimensional analyses showed that some sealant was still present after 19 months. Furthermore, the mean wear was less than the mean sealant thickness at the end of the study. A total of 5 increments of the root sealant were applied to the cervical dentine surfaces at baseline, despite the manufacturer's directions stipulating the application of the sealant in only two increments. The reason for a 5-increment application was to ensure that this novel sealant could be detected by the employment of the three dimensional superposition technique and also clinical assessments. The average thickness of each increment

was approximately 35 µm at baseline and the average wear on the sealant was approximately 28 µm for each month, especially in the early months.

It is widely known that *S. mutans* is the predominant micro-organism involved in the initiation of root caries. Baysan and Lynch[17] also reported that the numbers of isolated mutans streptococci, yeasts and lactobacilli showed significant reductions after 3 and 19 months on the sealed surface and not surprisingly, there was no initiation of carious lesions on these cervical surfaces during the 19-month period.

In the current study, some of the ozone treated PRCLs reversed to a less severe stage, whilst lesions in the sealant only group remained unchanged when the root sealant failed to remain intact. It was also surprising that some of the lesions failed to remineralise following ozone and sealant application when the sealant fell out. It can be speculated that this phenomenon may be caused by various factors including failure of the anti-caries effect of the sealant, cavitation of PRCLs, position of the lesions and the time period of the sealant failure with respect to the follow-up appointments. The root sealant may have fallen out just before the follow-up appointment which would have left insufficient time for the lesions to remineralise. Furthermore, a small amount of resin may have remained on the surface of the PRCLs and this may have prevented remineralisation of the lesions.

In conclusion, after 12 months, modified USPHS criteria revealed a number of debonds, marginal disintegrity and anatomic failures for both the ozone and sealant group and the sealant only group. However, the root sealant can be retained better on ozone treated leathery PRCLs.

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CHAPTER 3.9

Arresting of Non Cavitated Root Caries using Ozone

Julian Holmes & Edward Lynch

The current recommendations for use of the Heal-Ozone (KaVo Gmbh Germany) is for single surface caries. Other cavity types that involve more than one surface are being researched at present, but the potential of routine treatment of these is problematic due to the delivery cup design, and maintaining a seal over these multi-surface cavities. This chapter will look at the treatment of non cavitated leathery root caries, and in particular, a recent published study with 18-month data (Holmes, 2003). In this chapter, the 24-month data is presented, with further comment and discussion.

Elderly populations and root caries

The demographic profile of developed countries has moved from a young to an older population. This ageing is associated with better nutrition, increased standards of living, and advances in medical and pharmacological management of disease.

In a 1996 study, 2,280 subjects who were 60-years-old or over from three different areas of the UK were examined clinically to assess their dental health and needs. Root caries was found to be common and there was an age-related increase in risk of the disease (Steele et al, 1996). In a national survey of adult dental health conducted in the Republic of Ireland in 1989/1990, a total of 1,527 subjects aged 25 and older were examined for root caries. It was suggested that the prevalence of root caries was highest in older age groups, residents of non-fluoridated communities, and those earning low incomes. As tooth loss masked the potential prevalence of root caries and as more people are retaining their natural teeth into middle and old age, the incidence of primary root surface caries (PRCLS) was thought likely to increase (O'Mullane et al, 1992).

Ageing individuals tend to have gum tissue recession, and the associated exposed root surfaces are more susceptible to caries (Hellyer et al, 1990; Galan and Lynch, 1993). A survey conducted in the United Kingdom in 1988 by Downer (1991) found prevalence in the 55–64-year-old group with a mean of 0.7 teeth having root caries, and 1.2 having had restorations placed in their roots. The prevalence of PRCLs was reported to be over 80% in elderly institutionalised people (Banting et al, 1980; Beck, 1993). Hand et al (1988) reported that 1.8 in 100 susceptible exposed root surfaces in adults over age 65 years became carious annually. Saliva contains a number of important minerals such as phosphate, calcium and fluoride that could aid PRCL remineralisation in the right environment, and acts as an important buffer for oral acids (Silwood et al, 1999; Silwood et al, 2002). As salivary flow decreases, for example in the pharmacological management of disease, there may be an increase in the incidence of decay. The micro flora of PRCLs has been shown to contain large numbers of acidogenic and aciduric microorganisms, which correlate with the severity of root caries (Baysan, 2002; Beighton et al, 1993; Brailsford et al, 1998; Lynch and Beighton, 1993, 1994; Lynch, 1996; Ship et al, 1991). Ageing is also associated with a requirement for a plethora of medication that can have a dramatic effect on salivary flow.

The detection of root caries

More accurate detection systems need to be developed for the detection of PRCLs (Baysan, 2002; Lynch, 1986; ten Cate, 1996). Root carious lesions are classified as soft, leathery or hard based on differences in the degree and pattern of mineralisation. Nyvad and Fejerskov (1987) suggested that soft lesions showed extensive demineralisation with no evidence of an intact mineralised surface layer, whilst hard lesions appeared to have a generally uniform distribution of mineral throughout the lesion, and leathery lesions had a broad range of histological appearances. These authors concluded that soft and leathery lesions were active, whilst hard lesions were arrested. Soft lesions are the most severe type of root carious lesions according to a validated clinical severity index and contain more micro-organisms (Beighton et al, 1993; Lynch ,1994). Research suggests that soft and leathery lesions can remineralise and may become hard (Hellyer et al, 1990; Beighton et al, 1993; Lynch, 1994; Hellyer and Lynch, 1991). Remineralised lesions acquire a smooth and hard surface and remain unchanged over many years.

The management of root caries

The term 'root caries' was used by Hazen et al (1973)and the term primary root carious lesion (PRCL) was proposed by Lynch in 1986 (Lynch, 1986; Lynch, 1994). A PRCL is defined as an area on the surface of the tooth, at or apical to the cemento-enamel junction, that has undergone a carious process.

Restorative management of root caries is a challenge in view of the difficulties of visibility, moisture control, access to carious lesions, proximity of the pulp, proximity to the gingival margin, and the high organic content of the dentine. Many restorative materials on roots of teeth are associated with problems such as microleakage (Taylor and Lynch, 1992)and marginal adaptation (Taylor and Lynch, 1993) necessitating frequent restorative replacement (Lynch and Tay, 1989).

Previous studies have shown that root caries prevalence increases with age (Banting et al, 1980; Vehkalahti et al, 1983; Manji et al, 1989) from about one in 9 root surfaces at risk for under-30s, to approximately two in three for the over 60's (Galan and Lynch, 1993). Attachment loss and exposure of root surfaces to the oral environment are accepted to be nearly universal prerequisites to the development of root caries (Stamm et al, 1990; Katz, 1995; Galan and Lynch, 1994).

Papas et al (1992) emphasised the high involvement of posterior teeth with root caries The microbial colonisation of PRCLs has been extensively investigated. Attention has focused on the causative micro-organisms in the aetiology of root caries (Beighton et al, 1993; Lynch, 1994; Beighton and Lynch, 1993; Collier et al, 1993; Beighton et al, 1991). These studies showed the importance of the acid-producing group of bacteria in PRCLs. Further studies linked salivary and plaque conditions (Fure, 1998; Beighton and Lynch, 1995). It is now recognised that the most advantageous treatment for root caries is remineralisation (Allen et al, 1999). The use of pharmaceutical agents (Lynch, 1996; Baysan and Lynch, 2001)and fluoride-containing dentifrices may provide some protection for high caries risk patients. Various pharmaceutical agents have been used, such as fluoride (Lynch et al, 2000; Lynch and Baysan, 2001; Baysan et al, 2001; Duckworth, 1993), and chlorhexidine or chlorhexidine in combination with thymol (Lynch et al, 1995).

Keltjens et al (1993)concluded that high-risk patients with dentures supported by natural teeth with high salivary mutans streptococci, root and enamel caries would benefit both from fluoride and chlorhexidine therapy. Root coverage by removable partial dentures correlates with root caries prevalence (Wright et al, 1992).

Recent publications conclude that ozone should be considered as an alternative pharmaceutical management strategy (Baysan, 2002; Baysan et al, 2000, 2001; Baysan and Lynch, 2004; Holmes,2003; Lynch, 2003) rather than the traditional drill and fill approach. Ozone (a pale blue-coloured gas, chemical formulae O_3) plays an important role as a natural constituent in the higher layer of the Earth's atmosphere. It has been used for many years in medicine, and within recent years in dentistry. A device that has a CE mark, know as the HealOzone (CurOzone, USA and KaVo GmbH, Germany) has been available commercially in Europe for more than 2 years. Ozone is a very powerful antimicrobial agent. Recently, Baysan et al (2000) and Baysan and Lynch (2004) reported that ozone application either for 10 or 20 seconds was effective to kill the great majority of microorganisms in PRCLs (>99% microbial killing after 10 seconds Ozone application).

O_3 is naturally produced by the photo-dissociation of molecular O_2 into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating $HO_3^{\cdot}$, which, in turn, decomposes to hydroxyl radical. Further reactions convert O_3 to an even more powerful oxidant, the hydroxyl radical ($OH^{\cdot}$)

In view of its powerful oxidising properties, O_3 can attack many biomolecules such as the cysteine, methionine and the histidine residues of proteins. The effects of ozone on cell structures, metabolism and micro-organisms is well documented in published papers (Baysan, 2002; Bocci, 1992, 1996, 1999; Bocci et al, 1993) in both dentistry and medicine. Research has shown that ozone disrupts the cell walls of micro-organisms within seconds, leading to immediate functional cessation. This effect within a very short time is of great clinical significance, as the potential for microbial resistance to this treatment modality is insignificant. Baysan (2002) and Baysan et al (2000) have published reductions from Log_{10} 6.0 to Log_{10} 0.46 colony forming units after just 20 seconds of ozone. Studies have shown that just 10 seconds of ozone treatment is sufficient to produce reversal of PRCLs (Baysan, 2002; Baysan et al, 2000, 2001; Baysan and Lynch, 2004; Lynch, 2003).

Aim

The aim of this study was to repeat the study by Baysan (2000) assessing the effect of an ozone delivery system, and to combine ozone treatment with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCLs, in an older population group.

Method

The method is described in a 2003 paper by Holmes (2003). All participants were recruited from consecutive subjects presenting with 2 leathery non cavitated PRCLs at a Dental Practice in Berkshire, UK. Each subject had given their informed consent for both dental examinations and ozone treatment to be undertaken. A total of 89 subjects, all over 60 (age range 60–82, mean±SD, 70.8±6 years) with 2 leathery lesions each (178 PRCLs in total were entered into the study). All lesions entered fulfilled the criteria of the middle severity lesion group in the Perceived Treatment Need Index (Beighton et al, 1993; Lynch, 1994). Lesions were randomly assigned into two groups: Group 1, treated with ozone; or Group 2, no ozone for the control PRCLs.

All subjects were prescribed a course of oral hygiene instruction by a member of the practice hygiene team followed by scaling and polishing non fluoride containing paste at baseline only. Professional instruction was given on brushing, the use of floss and interdental brushes by the practice hygiene team. Each subject was advised not to consume fermentable carbohydrates between meals. They were informed of the relationship between caries incidence, and increased frequency of consumption of fermentable carbohydrates.

Lesions were examined using a visual/tactile method at baseline. Each PRCL was classified subjectively in terms hardness and severity (Beighton et al, 1993; Lynch, 1994). Leathery lesions were selected which were deemed to require drilling and filling. All subjects were offered a pharmacological treatment as an alternative to the traditional drilling and filling method and informed consent obtained. All subjects accepted the pharmaceutical approach to manage their PRCLs rather than the traditional drill and fill method.

The lesions were assigned into two groups by a dentist, using a computer generated random table; Group 1 lesions were treated with 40 seconds of ozone, and Group 2 lesions were left as controls. Following initial oral hygiene instruction, subjects were given ozone or air treatment. The treatment method was explained and demonstrated. Two dentists were involved in this study; the first assessed the PRCLs and the second dentist assigned them to Group 1 or 2 with a computer-generated random table. The first dentist then carried out the treatment for 40 seconds, applied the mineral wash, dispensed the remineralising products, and instructed the subjects. A double blind system was employed, and the ozone treatment was applied by a different operator than the one recording the clinical criteria used to define the severity of the lesions.

The ozone delivery system, HealOzone (CurOzone USA and KaVo, Germany) was employed. The HealOzone is a device that takes in air and produces ozone gas. The ozone is then delivered via a hose into a disposable sterile cup at a concentration of 2,100 ppm±10%. The ozone gas is refreshed in this disposable cup at a rate of 615 cc/minute changing the vol-

ume of gas inside the cup over 300 times every second. The cup forms a seal around the lesion being treated so that ozone cannot leak into the oral cavity.

The HealOzone unit was fitted with a modified control integrated electronic chip. The HealOzone unit's display and sound were exactly the same when delivering ozone or air. In this way the dentist treating the subjects was unaware which tooth had been treated with ozone, and which tooth was used as the control lesion. The second dentist recorded if the PRCL was treated or untreated. If the lesion was assigned to Group 1 and ozone treated, the HealOzone was switched to produce ozone. If assigned as a control, the HealOzone was switched to produce air only. Otherwise, the HealOzone unit functioned as normal so that the first dentist was unaware if the PRCLs were treated or untreated.

After treatment (lasting 40 seconds) a professionally applied remineralising solution containing xylitol, fluoride, calcium, phosphate and zinc (HealOzone remineralising solution) was applied to the lesion.

Instructions were given to each subject to use the remineralising tooth paste twice each day, and the mineral mouth wash on two separate occasions each day, and to use the remineralising spray, sprayed into the mouth 4 times a day after breakfast, lunch, dinner and supper. Standard soft toothbrushes were dispensed, and subjects were advised to use a new toothbrush every month. Subjects were recalled at 3, 6, 12, and 18 months. At each appointment, the lesions were re-treated to the original treatment protocol, again employing two dentists. The subjects were given further supplies of the remineralising products and new toothbrush supplies were dispensed.

The reproducibility of the data was tested at the 12-month recall. 1 week after assessment by the first dentist, 15 subjects (30 PRCLs) were recalled and examined by a third dentist. There was a good agreement in the classifications of hardness and severity of PRCLs (kappa=0.80). Twenty Subjects with 40 PRCLs were also re-assessed 24 hours later, at the final 18-month recall visit, by the usual dentist who had used the same criteria throughout the study period. Two lesions that were marked as soft were re-assigned to leathery on the second visit (kappa=0.95). Data sets were collected at each recall and the codes were only broken at the end of the 18 month period. Statistical analyses using chi square statistics was carried out on these collected data sets.

Results

Eighty-nine subjects started this study at baseline. At 24 months, 87 subjects had completed the study. Two subjects had moved out of the practice area, and were not available for the 12 or 24-month re-assessment visits. There were no observed or reported adverse events at any of the treatment sessions or afterwards (Table 1). After 3 months, in the ozone treated group, 61 PRCLs (69%) had become hard and none had become worse, whilst in the control group, 4 PRCLs (4%) had become worse ($p<0.01$). At the 6 month recall, in the ozone group, 7 PRCLs (8%) remained leathery, the remaining 82 (92%) PRCLs had become hard, whilst in the control group, 10 PRCLs had become worse (11%) and one had become hard ($p<0.01$). At 12 and 18 months, 87 Subjects attended. In the ozone group at 12 months, 2 PRCLs remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCLs had progressed from leathery to soft, i.e. became worse, 65 PRCLs (75%) were still leathery, and one remained hard ($p<0.01$). At 18 months, 87 (100%) of ozone treated PRCLs had reversed, whilst in the control group, 32 lesions (37%) of the PRCLs had worsened from leathery to soft ($p<0.01$), 54 (62%) PRCLs remained leathery and only one of the control PRCLs had reversed ($p<0.01$). At 24 months, 87 (100%) of ozone treated PRCLs had reversed, whilst in the control group, 45 lesions (51%) of the PRCLs had worsened from leathery to soft ($p<0.01$), 42 (47%) PRCLs remained leathery and two of the control PRCLs had reversed ($p<0.01$).

Table 1 shows the data sets (numbers of lesions in the first column and percentages in the second column) at baseline, 3, 6, 12, 18 and 24 months.

Discussion

Statistics show that the proportion of elderly people is rapidly increasing in all developed countries. The conventional approach of drill and fill, or tissue amputation, to treat a carious lesion is problematic in view of the difficulties of visibility, moisture control, access to the carious lesion, proximity of the pulp and proximity to the gingival margin. The high organic content of the dentine leads to potential problems with bonding restorative materials to achieve a long-lasting seal. Many

Table 1

Group 1	Ozone treated		2 patients had dropped out at 12 months									
	Baseline		**3 months**		**6 months**		**12 months**		**18 months**		**24 Months**	
Soft	0	0	0	0	0	0	0	0	0	0	0	0
Leathery	89	100	28	31	7	8	2	2	0	0	0	0
Hard	0	0	61	69	82	92	85	98	87	100	87	100
	89	100	89	100	89	100	87	100	87	100	87	100
Group 2	ontrol Grou		tients had dropped out at 12 months									
	Baseline		**3 months**		**6 months**		**12 months**		**18 months**		**24 Months**	
Soft	0	0	4	4	10	11	21	24	32	37	45	51
Leathery	89	100	85	96	78	88	65	75	54	62	42	47
Hard	0	0	0	0	1	1	1	1	1	1	2	2
	89	100	89	100	89	100	87	100	87	100	87	100

elderly subjects have medical conditions that make dental treatment a challenge, such as sudden muscle spasms and movement. And many elderly patients are on a large number of pharmaceutical products that have a dramatic effect in reducing salivary flow. This study aimed to assess the effect of a novel ozone delivery system, combined with improved oral hygiene and the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCLs, in an older population group, which if successful would be a preferable treatment option to 'drilling and filling'.

The restoration of root caries poses a number of problems, in particular visibility and isolation from saliva, gingival secretion and haemorrhage. Restorative materials (Lynch and Tay 1989)used to restore PRCLs have required frequent replacement. Preventive treatment regimes for PRCLs may be considered to have a better long-term prognosis than restorative treatment options (Arneburg, 1989).

Clinical observations suggest that carious lesions can be arrested at any stage of lesion development i.e., even at the cavitation stage if plaque-free conditions are maintained (Nyvad and Fejerskov, 1986). In this respect, Bradshaw et al (1990) reported that mutans streptococci become increasingly sensitive to fluoride ions as the pH falls. It is possible that the routine topical application of fluoride ions could to some extent inhibit the metabolism of such cariogenic organisms. The remineralisation observed in clinically arrested lesions and the conversion of clinically active to inactive lesions supports the non-restorative management of root carious lesions using dentifrices containing fluoride (Fluoride congruent to 0.1% w/w) for a period of 18 months (Nyvad and Fejerskov, 1986). Papas et al (1999) reported the efficacy of a dentifrice containing 1,150 ppm sodium fluoride with soluble calcium and phosphate salts. Keltjens et al (1993)concluded that high-risk patients with dentures supported by natural teeth with high salivary mutans streptococci; root and enamel caries would benefit both from fluoride and chlorhexidine therapy. However no study has approached a reversal rate of 100% with any of these preventative regimes.

If the results of previous European studies are to be extrapolated to this study, it would be expected that more than 2 lesions in the control group would arrest and reverse, just with improved oral hygiene and care. In the Holmes study (Holmes 2003) each patient had an intensive course of oral hygiene instruction, and treatment, as well as supplies of a professional remineralising system. This study has highlighted that ordinary people, i.e. patients, do not follow strict oral hygiene protocols even in studies, and probably reflect the 'true' state of the general public. If this is accepted, then it is hardly surprising that the 'ordinary' patient does not follow previous study results in lesion arrest and reversal with just oral hygiene measures alone. The key to the success of this study and the 18-month study is not just the ozone which eliminates the acidic niche environment, but the combined use of specialist oral hygiene products that increase the concentration of oral bio-available minerals for the remineralisation process.

This study has shown that dentistry has the ability to reverse lesions with just 40 seconds of ozone treatment. At 18 and 24 months, 100% reversal and remineralisation had been achieved. The studies by Baysan

(2002), Baysan et al (2000, 2001) and Baysan and Lynch (2004) and this study draw together important strands of research and publications on fluoride, oral health and hygiene, and the microbiology of caries. It is possible to eliminate the 'protected' niche environment of aciduric and acidogenic microorganisms, and oxidise the bacterial by-products that are responsible for the perpetuation of the acidic ecological niche. Of the 87 non-ozone treated PRCLs, only two lesions showed spontaneous arrest and reversal, despite improved oral hygiene instruction and care, regular brushing and the use of a remineralising dentifrice, spray and mouthrinse. This study shows that these oral hygiene and care measures alone cannot produce predictable caries reversal. This study has shown that over time, leathery PRCLs can gradually become worse, becoming soft if left untreated, despite good oral hygiene care and the use of professional products.

The requirement for intervention is a necessity, and these lesions should not just be observed and left, especially in this caries risk population, who had presented with 2 active caries lesions and who resided in an area with no water fluoridation. This new technology has the potential to have a dramatic effect on the improvement of the dental health of our ageing populations. There is not a single study to show that once a lesion had remineralised, it is ever involved in the active carious process again. This study used a validated set of clinical detection criteria (Beighton et al, 1993; Lynch, 1994, 1996; Lynch and Beighton, 1994; Baysan 2002; Baysan et al, 2000, 2001; Baysan and Lynch, 2004) for PRCLs and hard root caries lesions are arrested (Beighton et al, 1993; Baysan, 2002; Lynch, 1994; Holmes, 2003).

Ozone has the unique feature of decomposing to a harmless, non-toxic and environmentally safe material (oxygen). Ozone has been used in medicine for many years. The first ozone generator was developed by Werner von Siemens in Germany as early as 1857, and the first report of it being used therapeutically was for the purpose of purifying blood by C. Lender in 1870. In 1885, Dr. Charles J. Kenworthy first published medical applications of ozone. To date, ozone therapy has been a recognised treatment modality in sixteen nations. Research by Baysan showed that the HealOzone system has no potential to leak into the oral cavity (Baysan, 2002) due to the unique ozone delivery system. No side effects have ever been documented in either the dental research centres or the 1000+ dental practices in the UK and Europe that use this technology, and it can be considered to be completely safe. Important features of the HealOzone for use in ozone treatment are that it is entirely self-contained, requiring only a power source; it uses air (no cost); each treatment is of low cost; is very fast (40 seconds, compared to 25 minutes for an average filling); and no injections or tissue destruction is involved.

Recent surveys of adults aged more than 65 years in the UK revealed that all were vulnerable to root caries (Emilson et al, 1993). The proportions of people with restorations on root surfaces ascribable to root caries rose steadily with 35% of 55–64 year olds, and 43% of those aged 65 and above in 1998 (Anusavice, 2000). It is important to remember that in 1968, only 21% of people aged 65–74 years had any teeth compared to 66% in 1998. Such data illustrates the need to provide an increasing dentate elderly population with a simple, effective means of preventing and reversing root caries. The purpose of this study was to build on recent research (Baysan, 2002; Baysan et al, 2000; Baysan and Lynch, 2004; Holmes, 2003), and further investigate this treatment regime for the management of root caries.

Filling materials fail at alarming rates. Costs can be measured in terms of pain, discomfort, and in financial terms such as lost productivity. In England and Wales, restorations carried out in the NHS dentistry cost a total of £1.25 billion in 2001. This does not include private treatment, which is currently estimated to be 50% of dentists' income. The total costs of all dental treatment in England and Wales probably exceeded £ 3.26 billion in 2001 (General Dental Council UK, Annual Statistics, 2001). Most of these fees are ascribable to fillings, root fillings, dentures, crowns and bridges. Published reports suggest 50% of restorative items are replacements for previous restorations, and about half of these restorations are being replaced due to secondary caries. If only 50% of all fillings could be avoided with the use of ozone, enormous sums of money could be saved. The cycle of filling preparation and subsequent replacement eventually may eventually lead to more complex restorative care requirements with increasing cost implications, such as the progression from a simple cavity, to a multi-surface one, to the fracture of the crown requiring root canal treatment, followed by restoration with a crown and core.

In the United States, dental treatment is estimated to cost $52 billion per year, and half of this cost may be associated with restorative treatment and the cost of missed workdays and lost production due to oral disease. Despite advances in clinical and laboratory research, approximately 50% of the U.S. population over the age of 65 shows evidence of root caries (Anusavice, 2000) In all countries, from the advanced to poor and developing countries, there is a huge potential for a cost-effective way to prevent and reverse caries. In the ageing population, and those with reduced manual dexterity, a preventative and early intervention strategy needs to be found. In this respect, the use of ozone should be also considered for medically compromised patients, domiciliary care patients and homebound elderly people. The equipment required is limited and essentially portable compared to that required for conventional drill and fill. Therefore elderly patients who have limited access to the dental services can benefit from this treatment. In many poor, developing and highly populated countries, equipment, dental supplies, and dental services are inadequate due to high costs and lack of dentally trained personnel.

The benefits of ozone treatment can represent one of the major prevention strategies for these high-risk population groups. The processes involved have been shown to be multi-factorial. Traditionally, clinicians have detected root caries by visual-tactile or visual methods, which disclose cavitation, but fail to reflect the dynamic process of carious lesions. Increased understanding of the process of carious lesions and new management strategies in reversing the clinical severity of PRCLs can radically alter traditional drilling and filling of lesions and shift the emphasis to a pharmaceutical approach to the management of root caries using ozone.

Conclusions

The restorative management of PRCLs has become challenging, especially for the high percentage of the elderly population and particularly for those people in special care units that are experiencing reduced financial support from health services. There is a major requirement for an improved management strategy for root caries. Emilson et al (1993)reported that it was possible to convert active root caries to inactive lesions by an intensive prophylactic program. The pharmaceutical approach for the management of root caries in elderly people should therefore be considered.

This study showed that regular ozone application for 40 seconds and the use of remineralising products arrested leathery non cavitated primary root caries in a general dental practice population, without the need for dental tissue removal. The use of ozone may supply the key to predictable caries arrest and reversal. The 18- and 24-month results show that there is a great potential to expand this treatment modality into other areas of dental care, and with alternative delivery cups, ozone treatment can be expanded into multi-surface lesion treatment.

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CHAPTER 3.10

Clinical Management of Caries Using Ozone and a Modified ART Technique

Julian Holmes & Edward Lynch

The ART-technique (Atraumatic Restorative Treatment) is now well-established and is a treatment choice for children and adult patients who require dental restorative care. The ART technique involves caries removal and tooth restoration with adhesive restorative materials using only hand instrumentation. This restorative service is usually performed by auxiliary personnel who have limited experience in dental procedures and often provided under primitive field conditions. While small ART-glass-ionomer cement restorations are effective short-term replacements for lost tooth form, many larger ART-glass-ionomer cement restorations are defective after two years. Conventional glass ionomer cements tend to be more soluble in saliva, and stain quickly. Presently, resin-modified glass-ionomer cements are available which require no special activation equipment, handle easily and are being used with the ART technique as an alternative to conventional glass-ionomer cement.

Fuji IX (GC Corporation, Japan)

Fuji IX is a member of a class of self-cure glass ionomer restorative materials that has been categorized as "densified", "condensable", or "viscous" by various authors. Fuji IX GP was developed by GC International as the restorative material for the World Health Organization's Atraumatic Restorative Treatment technique.

Fuji IX GP has a smaller mean particle size than earlier self-cure glass ionomer restorative materials. This smaller particle size is purported to give improved wear rates and faster setting time than earlier glass ionomer materials. Fuji IX GP powder is composed of 95% by weight alumino-fluoro-silicate glass with 5% polyacrylic acid powder. Fuji IX GP liquid is composed of 50% distilled water, 40% polyacrylic acid, and 10% poly-basic carboxylic acid. Fuji IX GP is prepared with a powder/liquid ratio of 3.6/1.0 and should be mixed in 25 to 30 seconds. Fuji IX GP has a stated two-minute working time with a net setting time of two minutes and twenty seconds. Final finishing and polishing may be initiated six minutes from the start of mixing the material. The material should be protected with either Fuji Varnish or Fuji Coat LC during initial setting and after final finishing to prevent material degradation from corresponding moisture contamination or desiccation. Also, this restorative material is promoted as possessing excellent clinical handling characteristics. Fuji IX GP's purported advantages over current glass ionomer materials are decreased moisture sensitivity, improved wear characteristics, and no requirement for a visible light curing unit (if Fuji Varnish is used). Fuji IX GP is available in both powder/liquid and precapsulated delivery systems.

Pain during invasive treatment of dental caries is a

common phenomenon if no local analgesia has been used before cavity preparation. Atraumatic restorative technique (ART) is a suggested procedure, which is at least less traumatic for the patient. Although the ART approach has been received well by both children and adults who belong to population groups hardly ever exposed to regular oral health care, it has not yet been proven that this particular procedure really causes less pain, compared to more conventional techniques with rotating instruments. In a study (Rahimtoola, et al, 2000), pain was reported in connection with tooth restoration in 19.3% of the cases when the ART technique was used, which is significantly less than with a conventional restorative technique (35.7%). Moreover, a significant operator-related variation was found in the portion of treatments reported to be painful (from 5.9% to 44%). Finally their results showed a clear relationship in the pain reports between the first and the following treatments in both ART and the conventional technique groups.

Atraumatic Restorative Treatment has a number of advantages. First, it reduces anxiety to the patient, and any relatives accompanying them. Second, it reduces damage to the tooth and supporting structures, as well as surrounding tissues and structures. Third, it is less stressful on the dental team who are treating the patient.

ART is centered on minimal preparation, and restoration with simple materials with a predictable outcome. Now, with the advent of the ozone technologies, the systems have become more predictable.

Some papers have seemed to indicate that incomplete caries removal was not a problem, as the high flouride content of these ionomers and their pH, controlled any remaining bacteria, rendering them inactive. The flouride was taken up into the remaining tooth material, and thus the tooth was repaired, and the glass ionomer remained as a filling material.

The problem with this approach would seem to be the residual micro-organisms. A specific ecological niche of acidogenic and aciduric micro-organisms can remain, and so any leakage will result in continued caries progression, and eventually leading to failure of the restoration.

Various products have been developed and launched into the dental market to reduce this failure, such as caries indicator dyes, and systems, such as mechano-chemical caries removal (eg Carisolv) that selectively aid the removal of carious tissue, whilst leaving the un-altered tissue intact. Rotary instrumentation is very inaccurate, and can lead to tissue destruction and removal.

An improved minimal tissue removal system is air abrasion, and there are many forms of this system available from very basic systems which offer little control, to those that allow total control of air flow and pressure, and particle-air mix, such as The PrepStart (Danville Engineering) or CrystalMark (DPS, UK).

KaVo Rondoflex (KaVo GmbH, Germany)

Air abrasion works by the abrasive qualities of a very fine air-borne particle. These are typically about 27um (micrometer) in size, and the control boxes allow a degree of control of the air pressure and air flow. The working tips vary from 0.3mm wide upwards, so very

small cavities can be prepared with a high degree of accuracy. As there are no rotary components, pain and sensitivity does not tend to be a problem with this technology when used to prepare cavities, and local anaesthesia is often not required.

Incorporating ozone care into ART is a natural marriage of these two technologies. Air abrasion is used in the initial preparation of the cavity, without the need for local anaesthesia, in cases where it is deemed that the extent of the carious dentine exceeds that which could be easily penetrated by ozone. This helps to reduce anxiety. Ozone is then used to eliminate the ecological niche of caries promoting micro-organisms. The use of ozone also eliminates the need for tissue amputation that may weaken the tooth and reduce the lifespan of the restoration. The cavity can then be restored with a mineral releasing glass ionomer cement, such as FujiVII.

Holmes has published the results of his study entitled "Restoration of ART and Ozone treated primary root carious lesions" (Holmes 2003).

The aim of this study was to assess the placement time required and durability of restorations placed on ART and ozone treated primary root carious lesions (PRCL) compared to conventional treatment. Sixty subjects, each with 2 soft PRCL in the most were selected. After randomisation, each subject had one PRCL made caries free using conventional local analgesia (LA), drilling and filling using Optibond Solo Plus and Point 4 (KerrHawe) composite resin. Eleven pulp exposures were detected during cavity preparation and RCT was also performed on these teeth. The remaining 60 PRCL in the 60 subjects received only the ART technique and ozone treatment for 20 seconds (HealOzone, KaVo) without LA, followed by filling using Optibond Solo Plus and Point 4 composite resin. Up to 1 mm of softened carious dentine was left overlying the pulpal floor prior to ozone treatment. The mean (SD) time required for ART, ozone treatment and placement of a restoration was 10 (2) minutes, whilst the conventional technique, including LA, required 23 (4) minutes ($P<0.05$). After 18 months, 56 subjects returned for recall. All restorations scored alpha in every recorded USPHS criteria. All restorations were scored aesthetically as excellent in appearance in 100% of cases. However, an additional 4 teeth in the conventional treatment group had required RCT in the intervening 18 months. RCT was required by 25% of the conventionally treated teeth, whilst none of the ART and ozone treated teeth required RCT ($P<0.01$). ART and ozone treatment saves time and reduces the need for RCT compared to conventional drilling and filling techniques for treating soft PRCL.

To conclude, ozone treatment used with the ART technique shows great promise and potential for not only developed, but also developing and poor countries. ART is a particularly useful technique where dental facilities are limited or very basic. By eliminating the remaining micro-organisms and promoting remineralisation of caries affected tooth structure, whilst having no effect on the bond strengths of materials to tooth substance, ozone should provide greater longevity of restorations when combined with the ART technique.

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CHAPTER 3.11

Combining Airbrasion and Ozone; A Method for Treatment of Approximal Dental Caries Using a Tunnel Preparation With Airbrasion

Chris Clifford, Julian Holmes & Edward Lynch

An introduction to Airbrasion & Ozone

The concept of micro-dentistry is relatively recent. The cavity designs proposed by GV Black served the profession and the patient well through most of the 20th Century but, in the later decades, many were questioning the value of these designs. In an age when silver amalgam and gold were the main restorative materials, the principles behind Black's design (extension for prevention and the removal of unsupported enamel etc.) were valid. Black's principles are an interesting but historical phase of the development of modern dentistry. This is the era of micro-dentistry; beneficial for both the patient and the dental team.

It has long been held that patients' greatest fears were those of the drill and the needle. Airbrasion has provided an alternative method of removing hard tooth structure, in many instances, without the need for any anaesthesia. This was good news for the patient; the lack of the sound of the drill was much appreciated; nervous patients chose to see a dentist as the threat of the drill reduced. From the clinician's point of view, minimal access cavities could be cut, which were ideal for adhesive materials (Goldstein & Parkins, 1994 & 1995). However, airbrasion would not remove soft, infected dental caries; this still required some rotary drill or hand excavation, often defeating the benefits for many patients.

The use of ozone to treat caries offered a chance to avoid the mechanical excavation of infected dentine. Thus, a new treatment modality was available that was very acceptable to patients and preserved natural tooth structure. This was fine as long as the caries was easily accessible, such as found in buccal or occlusal decay. Sadly, this could limit the use of ozone therapy in general practice. However, by accessing the caries with airbrasion and then treating the exposed decay with ozone true minimally invasive dentistry is achieved. The tools for effective micro-dentistry are now here, and this chapter explores how airbrasion can extend the use of ozone therapy, particularly into the approximal lesion.

Airbrasion theory

Air abrasion was introduced to dentistry, by Dr RB Black in 1945 (Black 1945) and the first commercial units were produced in 1951 by SS White Airdent, USA. The restorative materials that were available at the time did not lend themselves to the type of cavity cut by airbrasion (the word coined by Black). With the development of adhesive materials the technique enjoyed resurgence in the early 1990s. Interest in minimally invasive dentistry has further fuelled its use (Goldstein & Parkins, 1994, Rosenberg, 1996).

Black's word 'airbrasion' is possibly a misnomer as the effect is not really abrasive. It is the kinetic energy within the particle that is released on impact with a surface, which creates the effect. The harder the substance the more rapidly the energy is released. The result is removal of material at the site of impact. As each particle is very small, nominally 27 µ or 50 µ, the amount of tooth substance removed is small. Hence, there is little pressure and friction applied to the tooth resulting in virtually pain-free cutting. As millions of

particles per second are used the resultant removal of tooth substance is on a par with conventional techniques.

Generally, 27 µ particles provide the most accurate cut and least sensitivity. 50 µ provides good surface modification increasing bond strength of adhesives and cements. Some investigations suggest there is little difference to bond strength whichever size particle is used. A higher practical bond strength is claimed by Clifford's research if the final surface modification is done using the larger particle (97% retention with 50 µ micron powder, as opposed to 83% using 27 µ micron after 5 years). It should be remembered that the quoted particle sizes are nominal and may contain powder of any size. All suppliers of aluminium oxide powder for dental abrasion should state the range of sizes their product may contain. The smaller size particles have a potential danger and no product should have particles of less than 15µ as these can lodge in the lung alveoli producing respiratory disease.

Airbrasion Techniques

It is not the intention of this chapter to provide a treatise on airbrasion but to give a summary of the benefits and disadvantages as applied to ozone therapy. The comments made here may help the inexperienced user and, perhaps, be of some use to those more experienced.

Many units offer a wide range of nozzle sizes; Clifford is not convinced of any benefit from such a range and a fine nozzle of around 0.35 mm internal diameter will suffice for most cases. By varying the working distance of the nozzle, and its angle of approach to the target (carious tissue, enamel, dentine or restorative), the cutting efficiency and the effect upon the surface may be changed. For most purposes a single nozzle size will allow the operator to achieve excellent results.

The ideal working distance of the nozzle for most procedures is around 0.5 mm to 1.00 mm from the surface. This provides the accuracy and speed of cut needed to gain access to the caries with minimum tooth loss, maximum patient comfort and effective use of time. Any surface modification needed to clear any debris (loose caries, food particles and pellicle layers) can be easily achieved by working further away from the surface (say around 2 to 4 mm).

The correct angulation of the nozzle to the target surface will result in a fast rate of cut and minimal stray powder. An angle of particle flow of about 85° to the target surface allows the rebounded particles to clear incoming powder and allows easier aspiration. For surface modification a shallower angle of around 60–70° works well.

In general, a high air pressure will provide a high rate of cut, but that may often mean greater patient discomfort. However, operator technique has a greater influence on cutting efficiency than any other factor. Airbrasion lacks tactile feedback for the operator. All dental students have been trained on rotary instruments where tactility has become the essence of operative technique. As the working tip is not touching the surface, there is no feedback to the operator. As a consequence, there is a considerable learning curve before maximum efficiency is achieved.

Inexperienced users of airbrasion tend to move the hand piece tip in small, often random, pecking movements. It is better to move the hand piece slowly along a predetermined path then stop and repeat the action until sufficient material is removed. When removing enamel on an axial surface of the tooth follow the orientation of the hydroxyapatite crystals as this will speed the rate of tissue removal. In all cases, keep the tip moving. Directing the powder to one spot without moving can result in a very deep fine hole being cut, risking pulpal exposure. Airbrasion, because of the lack of tactile feel and the fact that the surface is very clean as the cut proceeds, can be very deceptive, often cutting faster than an inexperienced user may appreciate. So, keep the tip moving and check with an explorer frequently until one becomes more familiar with the technique.

If a patient reports sensitivity to hot and cold stimuli prior to operation then start with a low pressure (around 40psi) and after a couple of passes over the carious dentinal surface the pressure can usually be increased. Most patients will accept a short twinge of initial sensitivity if it avoids an injection. Rarely does the sensitivity continue but, should this not be the case, local anaesthesia may be required. Good communication with the patient can go a long way to making the treatment very acceptable. Why the sensitivity should decrease is not fully understood. It is thought that aluminium oxide particles and removed tooth substance block the openings to the dentinal tubules thereby reducing sensitivity.

Many units have far too high a powder flow rate

such that the incoming particles collide with the reflected particles (even if the ideal angle of approach is used) thus reducing the efficiency of the system.

Combine a high powder flow with a less than ideal technique and the result is excessive powder contamination of the mouth, risk to the operators and powder dust in the clinic. Good technique, with minimal powder flow and excellent aspiration, is the secret to successful and efficient airbrasion, and avoids one of the major objections many dentists have to the use of airbrasion.

Gaining access for ozone treatment

Treating approximal caries with ozone can present a problem if the lesion is not open to an accessible surface. This is where airbrasion can help. By gaining access to the carious dentine, ozone can be applied. This can be a minimally invasive procedure rarely requiring any anaesthesia and without producing the stress fracturing that can be associated with rotary instrumentation.

In general, there are two methods of approaching an approximal lesion; from the occlusal surface or from the buccal or lingual/palatal surface. Selection of the best site is dependent on a number of factors:

1. Location of the carious lesion
2. Adjacent restorations
3. Ease of access for both airbrasion and ozone

Radiographic and physical examination will help to decide which site is appropriate for gaining access to the lesion. In the younger patient, caries is more likely to be at or around the contact point, making occlusal access a possible choice. Older patients may present with caries nearer to the cervical margin or on the root surface, and so a buccal or lingual approach may be easier. Any adjacent restoration may prove to be the best selection as minimal tooth structure will be lost. Whichever site is chosen it is essential that access can be gained. Trying to position the HealOzone cup over the site will help to confirm that access can be achieved.

Protection of adjacent tissue

The protection of the adjacent tissue is essential during preparation.

For an occlusal approach the use of a conventional matrix band works well however the author's preference is for the use of a dead soft matrix (Den-Mat). A short strip is inserted in the approximal area and wedged, if necessary, to aid adaptation. Whichever method is used it is prudent to move the matrix from time to time to ensure that the airbrasion process has not perforated the material and thereby caused damage to the adjacent tissue. In larger lesions additional protection of the soft tissue may be necessary (the wedge itself may not be enough). Although easy to staunch, airbrasion can cause haemorrhaging of the gingiva making both the application of the ozone and the restoration placement difficult. To overcome the problem a thin strip of rubber dam can be inserted into the interproximal space **prior** to the placement of the matrix and wedge.

The buccal or lingual approach is best done first, protecting the soft tissue by using rubber dam or strips of the rubber as for the occlusal approach, and secondly protecting the soft tissue by applying a metal matrix around the adjacent tooth not the target tooth. Wedging completes the protection. In the case of a carious lesion being close to or below the gingival margin, protection by instruments may allow adequate protection and retract the gingiva at the same time. A carver such as a 1/2 Hollenbach may be successful, although Clifford has customised some old instruments with good results.

Removal of the overlying enamel can then begin. Using slow movements of the nozzle, follow a straight line or gentle arc. For a buccal or lingual approach try, where possible, to direct along the enamel prism lines which tends to make removal of the enamel structure more rapid. For an occlusal approach ensure the angulation of the nozzle is adjusted as it is moved down a cusp or fissure. Produce a large enough access so that the caries is visible and endeavour to keep as much of the proximal wall and marginal ridge intact as possible. Insufficient evidence has been gathered to show the optimum shape of the access cavity. Trials are continuing to determine this. The main premise is that the cavity is kept as small as it can get, allowing full ozone penetration. Classic cavity design (as developed by GV Black) does not need to be followed and unsupported enamel is quite acceptable.

Debride the cavity with a water jet to remove any residual particles and air dry lightly. All soft caries is gently removed with an excavator.

Establish the seal

The occlusal approach
Establishing a seal on the occlusal surface alone is not difficult. But the approximal lesion may need blocking so that a seal can be established. For the smaller lesions the matrix system as outlined above will work but the application of a very small amount petroleum jelly or vegetable oil to the matrix will help to ensure a seal. It is essential that not too much lubricant be applied as surplus could migrate into the cavity and any excess could compromise the bond of the restorative material. Even more simply, the use of gloved fingers to the buccal and lingual aspects will often work and may be worth trying first!

The Buccal or lingual/palatal approach
This tends to be much harder. The use of a gloved finger to assist the seal on the opposite side of the contact area will often work. Paint-on light-cured rubber dam can also help.

Sealing the cavity

Airbrasion provides an ideal surface for dental bonding and tests carried out by CRA and Reality support this. The tooth surface is micro-etched maximising the available bonding area. Clifford has, for many years, used 50µ powder to modify the surface prior to bonding of composite resin materials (Laurell & Fisher, 1994).

Glass ionomers are regarded as the materials of choice for sealing the cavity as fluoride is released from the filling material into the adjacent tooth, and they form a bond to tooth tissue. For any cavity where ideal aesthetics are important, a glass ionomer base with a composite resin veneer gives very good results.

Methods of assessing results

If any leathery caries is left on the pulpal floor of a cavity in order to attempt to avoid the need for RCT, the current method of approach at review is to remove the restoration and assess if the dentine is hard or leathery. This is the most reliable way and allows for treatment if caries is found. However, for many patients, this is an option they may prefer to avoid. Clifford's studies are reported at the end of this chapter.

Choice of unit

There is a wide range of airbrasion units available to the practitioner. Prices vary for simple units such as the RONDOflex (KaVo) to units with built in compressor and full flow controls, but tests have shown that all units work to some extent. The more sophisticated (and more costly) units have far greater control of particle flow and operating pressures. Some units offer two powder chambers extending the treatment options.

To ensure great accuracy of cut and maximum patient comfort operating pressures should be easy to adjust. A low pressure will produce less sensitivity and reduce the risk of damage to adjacent tissue from the rebound of the particles. Some units will cut adequately, albeit slowly, at pressures as low as 40 psi. Pressure settings up to 160 psi are available on one range of units, providing very rapid tooth removal. In general, an airbrasion system working from 60 to 100 psi will cover most operational requirements. Whichever unit is chosen it must be quick and easy to adjust the pressure. Pre-set buttons are the quickest and most consistent way to achieve this though an infinitely variable control can work well.

It has been mentioned that the powder flow is often unnecessarily high on most units. Systems that have a simple control knob are the best as the powder flow can be fine-tuned to suit the operator's preference. Adjust the flow to provide a good rate of cut and allow easy aspiration of spent powder. Too much powder will reduce the cutting rate as the spent powder cannot be cleared away fast enough to allow new powder to work.

To try to control the powder scatter more effectively some units use a water jet around the powder nozzle. Clifford's view is that this is of little benefit, simply creating a slurry of powder around the operating site and, because of the diameter of the co-axial nozzle, visibility is greatly impaired. Airbrasion lacks the tactile feedback that the use of a rotary instrument provides to the user so good visibility is essential.

Many units come with a wide range of nozzles but for most cutting purposes one size will suffice. An internal diameter of between 0.35 mm and 0.46 mm are suitable for most procedures. The nozzles should be checked regularly for wear as cutting performance deteriorates rapidly once the wear reaches a certain point. The use of a #15 endodontic file can help to establish the time to discard the nozzle. Insert the file in a new nozzle and note the binding point. As performance starts to drop check

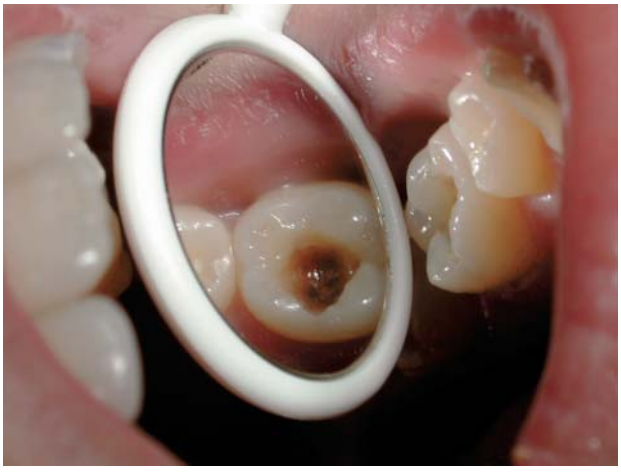
again and note the difference. Nozzles can now be readily checked and changed before their efficiency drops.

Most units use aluminium oxide as the cutting agent. This is chemically inert for all practical purposes and is safe to use as long as the particle size is >15 µ. Most manufacturers supply powder at 27 µ or 50 µ but as mentioned earlier, this is the nominal size and it is the range that is the most important factor as particles under 15 µ can lodge in the lung alveoli with the resultant risk of respiratory disease. Suppliers who quote particle sizes of 26.5 µ or such like are just confusing the issue, as they will not be able to manufacture to that level of precision and it is quite unnecessary to do so.

Reality (2003) has reported that the RONDOflex can be used in virtually every restorative procedure you perform. With its simple connection to your dental unit (assuming you are already using KaVo handpieces and thus have its Multiflex coupler already attached to your air line), it is no more difficult to use than a conventional handpiece. Operationally, it doesn't get any easier either. No dials, no pressure adjustments, no powder regulators, no additional footswitch. Just fill it, attach the tip, snap it on the coupler, and you're ready to perform by simply retrieving it as you would a handpiece, applying pressure to the footswitch, and you are an air abrasion maven.

Reality (2003) also reports that the PrepStart (Danville) is a small, portable machine with surprisingly good performance, probably a cut above the RONDOflex. Being small, it won't take up much counter space. While cutting effectiveness, even with the PowerPlus booster, does not match the units with a built-in compressor, it will still perform adequately.

Case Study



Clifford reported on the successful use of airbrasion in conjunction with ozone treatment (Clifford 2003)

This investigation aimed to establish whether approximal and 'occult' occlusal caries could be treated by using airbrasion, to gain access to the lesion, followed by ozone treatment, and to assess if this conservative method was faster than conventional techniques. Lesions requiring drilling and filling were selected. Thirty-seven patients involving 48 teeth were treated with airbrasion and ozone whilst the time required to restore 48 other teeth conventionally in the same patients was also recorded. Access to the lesion was obtained using airbrasion delivered at 80 psi using 27-micron aluminium oxide as the cutting agent (Abradent DV1). Once access to the caries was established, ozone was applied for 40 seconds using a HealOzone unit. In the treatment of approximal caries, techniques were explored to ensure an effective seal. Glass ionomer restorative [Fuji IX (GC) or Diamond Carve (Kemdent)] restored the cavities. All lesions were successfully exposed and a seal established for the delivery of ozone. Clinically acceptable seals were achieved around all restorations. The airbrasion and ozone technique was significantly faster than conventional drilling and filling ($P<0.05$). Airbrasion combined with ozone treatment, because of its minimal intervention capability, enables carious lesions other than fissure caries to be treated without the need for local anaesthesia and saves time compared to conventional methods.

Clifford reported on the reversal of Caries Using Airbrasion and Ozone-with nine-month results (Clifford 2004)

This study investigated the effects of air abrasion, ozone application and sealing on carious approximal lesions in posterior teeth. Deep approximal dentinal carious lesions that would normally be assessed as 'requiring drilling and filling' were selected. All lesions were visible on radiographs. Thirty-four patients involving 34 teeth were treated with airbrasion and ozone and another 34 control teeth were treated conventionally in the same patients. Treatment times were recorded. Access to the test lesions were obtained (without local analgesia) using airbrasion

(DV1 Crystal Air, Abradent) and after probing, leathery caries, approximately 1 mm in depth, was left covering the entire pulpal floor. Once access to the caries was established, ozone was applied for 40 seconds using a Heal-Ozone unit. A glass ionomer restorative (Fuji VII (GC) restored the cavities. After 3 months the restoration was carefully dissected and the cavity was thoroughly probed again to measure hardness, before a conventional composite resin restoration was placed. All Patients were recalled at 9 months. All ozone treated restorations were symptomless throughout the 9 months. All 34 Fuji VII restorations, removed after 3 months recall, showed hard 'caries' to exploration, proving remineralisation was successful. All lesions were successfully exposed and a seal established for the delivery of ozone at baseline. The airbrasion and ozone technique was significantly faster (lesions were exposed, ozonated and sealed in under 7 minutes) than conventional drilling and filling ($P<0.05$). Approximal lesions were successfully treated using an airbrasion/ozone combination in less time than conventional drilling and filling.

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CHAPTER 3.12

The Effect of Ozone on the Bond Strengths and Surface Hardness of Some Commonly Used Restorative Materials in Dental Practice

D. Campbell, L. Cunningham, D. Hussey, C. Armstrong & E. Lynch

With the increasing use of ozone in the treatment of primary carious lesions, concerns have been raised about the possible effects this gas may have upon adjacent fillings in the same tooth.

Studies were carried out to determine the hardness of commonly used restorative materials, and adhesive bond strength to tooth enamel and dentine, before and after ozone treatment.

Study 1 – Surface hardness

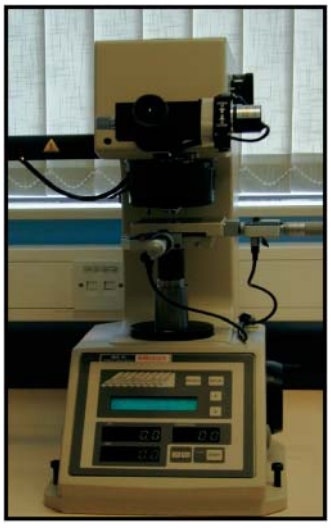
The materials tested were:

1. Amalgam (Disperalloy, Johnston and Johnston Dental Products, East Windsor, NJ, USA).
2. Reinforced glass-ionomer (Fuji II LC, GC Corporation, Tokyo, Japan).
3. Compomer (Dyract AP, Dentsply, Weybridge, UK).
4. Hybrid composite (Spectrum, Dentsply, Weybridge, UK).
5. Flowable composite (Revolution, KerrHawe Bioggio, Switzerland).
6. Composite (Point 4, KerrHawe, Bioggio, Switzerland).
7. Glass-ionomer (Chemflex, Dentsply, Weybridge, UK).

Photograph 1. Testing mould

Each material (n=30) was placed in a preformed plastic hardness testing mould, (photograph 1), and seated on a smooth glass slab to produce a flat surface. The mould was slightly overfilled and the excess material carefully removed. A cellulose strip was placed on the upper surface of those materials needing light-curing, and then cured for the time recommended by the manufacturers.

The hardness testing moulds and the materials which they contained were placed in a pH 7 buffer solution to prevent desiccation and stored for 24 hours.



Photograph 2. Micro Vickers Hardness testing using the Mitutoyo MVK-H1.

The materials were then subjected to Micro Vickers Hardness testing (Mitutoyo MVK-H1), (photograph 2) by applying a load of 1 kg for 10 seconds. The results were recorded.

Ozone was then applied in a constant manner to each specimen for 10 seconds. The surface hardness test was repeated immediately afterwards and the results recorded.

Study 2 – Bond of composite resin to enamel and dentine before and after ozone treatment

Twenty recently extracted non-carious human lower incisors were used for the determination of the shear bond strength of a resin composite restorative material (Esthet X, Dentsply, Weybridge, UK) to tooth enamel. The enamel surfaces were abraded with wet 240 grit silicon carbide paper to produce a flat area of enamel 3.5 mm in diameter.

A further 20 recently extracted non-carious human lower incisors were used to determine the shear bond strength of the composite material to dentine. Again, the enamel surfaces were abraded to expose 3.5 mm of dentine.

Each tooth specimen was randomly allocated to one of two groups:

Group 1 – subjected to a 25 second dosage of ozone from the Healozone unit (Curozone, New York, USA).

Group 2 – no ozone treatment.

The enamel and dentine bonding sites were conditioned with 37% orthophosphoric acid etchant and washed for 15 seconds before the application of a bonding agent (Prime and Bond, Dentsply, Weybridge, UK) followed by 10 seconds light curing.

Using a Teflon mould, cylinders of composite material were applied to the tooth surfaces in increments of 2 mm and light cured (Optilux 501, Kerr, Peterborough, UK) for 40 seconds.

All the specimens were subjected to shear bond testing using a custom made testing device. The tests were carried out on a Lloyds 2000S instrument (Lloyd Instruments Ltd, Fareham, Hants, UK) at a crosshead speed of 1 mm/min.

Results

Hardness Testing

Table 1: The mean surface hardness±standard deviation before and after ozone treatment

Material Tested	Pre-Ozone	Post-Ozone
Amalgam	135.39±22.23	127.21±17.90
Fuji II LC (Reinforced GI)	31.16±8.59	25.75±5.72
Dyract AP (Compomer)	51.23±5.31	46.54±2.72
Spectrum (Composite)	54.68±2.51	51.10±1.96
Revolution (Flowable Composite)	21.06±0.92	19.62±2.42
Point 4 (composite)	52.71±2.03	50.90±2.10
Chemflex (GI)	34.36±7.72	37.99±14.39

The results were analyzed statistically using Student's t-tests.

They revealed that ozone treatment had no significant effect on the surface hardness of any of the materials.

Bond strength

The mean loads (Newton) to debond the composite were as follows:

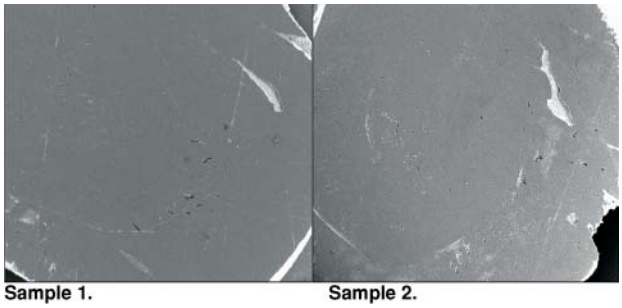
Composite	Mean Load (Newtons)
enamel without ozone	116.4 (SD 50.1)
enamel with ozone	128.6 (SD49.4)
dentine without ozone	54.7 (SD 23.6)
dentine with ozone	51.6 (SD 15.6)

To look in more detail at the surface of a sample before and after treatment with ozone gas, it was decided to carry out a test using a scanning electron microscope.

Two composite samples were prepared by placing them on a glass slab to gain a smooth flat surface. One sample was then treated with ozone for 40 seconds and the other was not. Both samples were then examined under the scanning electron microscope.

Below is a picture of the results. Sample 1 has not had any ozone treatment, sample 2 has been treated with ozone gas for 40 seconds. There appears to be no obvious difference in the surface texture of the two samples.

Scanning Electron Microscope pictures showing two composite samples



Sample 1. Sample 2.

Conclusions

The application of ozone gas for 10 seconds to restorative materials did not significantly affect the surface

hardness of materials tested. Ozone treatment of these materials is not envisaged to have any clinical significant effect of the hardness of these materials.

The 25-second application of ozone had no detrimental effect on the bond strength of the resin composite tested on either dentine or enamel.

CHAPTER 4.1

Ozone and Remineralisation Therapy – Minimally Invasive Treatment of Dental Decay

George Freedman & Fay Goldstep

For the past several centuries, the evolution of dentistry has largely focused on the amputation of diseased dental tissues, at ever-increasing bur rotation speeds, in the preparation of a cavity whose shape was dictated by the limitations of the current restorative material. Unfortunately, the inadvertent indiscriminate removal of both decayed and healthy tooth structures was a corollary development. This inherently incorrect and indefensible approach has been forced upon the dental profession by the limitations of diagnostic and preparation technologies, and the historical absence of suitable tooth-mimicking restorative products.

Natural enamel and dentine are still the best dental materials in existence; hence, minimally invasive procedures that conserve the maximum amount of healthy and remineralised tooth structures are preferable. The ideal of Conservative Dentistry, a treatment process wherein healthy tooth tissue is ***not*** removed during the preparation and restorative processes, is an inherently desirable dental objective. An even loftier goal is the retention of remineralised, previously diseased enamel and dentine and their restoration to normal function and form.

From the patient's perspective, the most significant barriers to seeking dental care include a general fear of pain and a specific fear of intraoral injections. Even in dentally advanced countries, where dentists and dental technologies are widely available, these phobias prevent up to 50% of the population from seeking routine dental care. Fortunately, recent major developments in diagnostic and preparation technologies have partially eliminated many of these objections. The chemotherapeutic restorative materials that have come into vogue over the past quarter of a century have made the restorative interface a stronger and more predictable zone. And finally, recently introduced aero-therapeutic techniques have been shown to successfully eliminate micro-organisms in the remaining tooth structures, permitting remineralisation. When used individually or in conjunction, these advances make dental treatment more accessible and more acceptable, and ultimately will result in a greatly increased utilization of dental services worldwide.

From the dentist's perspective, the most significant areas of concern are the micro and macro environments of the tooth-restorative interface, and the health of the remaining tooth structure. The above-mentioned developments serve to provide the dental practitioner with the tools that are required to access this interface directly and indirectly, and to manage it as conservatively as possible. In particular, the confidence of aero-therapeutically eliminating the damaging biofilm niche of aciduric and acidogenic micro-organisms and chemotherapeutically reversing demineralization offers treatment options that have never been available before.

Differential diagnosis of tooth structures at the interface

In mechanically accessing the decay interface of a tooth, the dentist is likely to encounter three different types of tissues: decayed dentine and enamel (soft), infected dentine in either the demineralizing or remineralising state (leathery), and healthy dentine and enamel (hard) (Anusavice and Kincheloe, 1987; Baysan and Lynch,

2004; Baysan, 2004). Soft lesions demonstrate extensive demineralization and are more difficult to remineralise. Leathery lesions have a uniform distribution of mineral throughout (Baysan and Lynch, 2001).These structures may be undergoing demineralization or remineralisation; the current direction of the process is less important than the potential mineral recharging of these tissues. Current research indicates that given the right environment, these leathery lesions can remineralise and may become hard (Baysan et al, 2000; Baysan et al, 2001; Beighton and Lynch, 1995). Hard dentine and enamel are typically healthy and should be left intact. The first (and ever more difficult) task is to diagnose which tooth materials must be removed and which can be left in place.

Traditional preparation devices, burs made of stainless steel, carbide, and diamond are effective in removing tooth structure; unfortunately, they remove healthy and unhealthy structures indiscriminately. In addition, today's higher-speed X-ray films make radiographic diagnostics less indicative. The primary diagnostic tool for tooth hardness has been the explorer and its tactile differentiation of hard and soft structures. There is extensive research that documents that healthy dentine is "harder" (Beighton et al, 1993; Bocci, 1999). It is not practical, however, to probe the entire surface of the preparation cavity after each pass of the cutting instrument.

The SmartPrep polymer instrument (SSWhite, Lakewood, NJ) is a selective cutting tool that removes softer, decayed portions of the tooth structure, while leaving the harder, healthier portions intact. The SmartPrep is designed to be harder than decay and softer than healthier tissues; thus, in contact with tooth structures, it abrades the softer portions, and upon encountering healthier segments, is in turn abraded itself. It functions as a self-limiting, dual action instrument, providing both *in situ* diagnosis of tooth structure viability, and immediate removal of any tissue that does not pass the hardness test. Since the SmartPrep instrument cannot open healthy dentinal tubules (Bocci, 1996),it is typically used with out any need for local anaesthetic, without patient discomfort (Bocci, 1992, 1996; Bocci et al, 1993; Duckworth, 1993; Ericson et al, 1999). The SmartPrep often leaves areas of discoloured hardened dentine.

The rationale for leaving this discoloured dentine in the tooth is well established (Freedman and Leinfelder, 2002; Fure, 1998; Fusayama, 1993). It is certainly bondable (Fusayama, 1993), and will not compromise the tooth-restoration complex. The only concern with this technique was the issue of any live bacteria that may have invaded the remaining healthy or remineralised tooth structure, a dilemma that might be eliminated to a limited depth of up to only 50 microns by the use of an etching component during the adhesive process. The recent introduction of aero-therapeutic ozone techniques to completely eliminate micro-organisms at and below the restorative surface offers increased predictability to both of these techniques.

Caries Control and Adhesion at the tooth-restoration interface

A successful long-term restoration requires a bondable surface that is free of cariogenic micro-organisms at and below the tooth-restoration interface. The central dogma of tooth preparation has heretofore assumed that once tooth structure is decayed, it can never be made healthy or functional again. This presumption has now been challenged by the observations that clinical caries can be reversed through aero-therapeutic techniques (Fusayama et al, 1966).

Current preparation techniques are designed to eliminate not only the obviously decayed layers of the tooth, but any other structures that *may* harbour micro-organisms. To be on the safe side, dentists are routinely instructed to remove additional dentine beyond the suspected zone of decay. While this approach may effectively eliminate micro-organisms in most situations, it certainly results in the unnecessary removal of healthy tooth structures. In addition, there can be no guarantee that micro-organisms are not lurking just beyond the prepared surface. The etching process is capable of destroying surface micro-organisms and may penetrate enamel and dentine to a depth of 5–50 µm, but it is difficult to ascertain this effect clinically. Over the past 30 years, dental adhesion involving etching as a separate or inherent step has become the most commonly used single procedure in dentistry. Improvements in chemistry and application technique have made bonded dentistry practical and effective, and the latest adhesive agents such as the 7th generation iBond (Heraeus Kulzer, Armonk NY) have made single step bonding very

easy and predictable for the practitioner (Fusayama et al, 1966).

All adhesive research has been conducted with, and assumes, fully mineralized dentine. The prospect of adhering to a less than fully mineralized dentinal surface has neither been proposed nor examined. The high organic content of the demineralized dentine may lead to adhesive problems that prevent the achievement of a long-lasting seal for restorations (Fusayama et al, 1966).Thus, in the context of using currently available restorative procedures, it is ideal to return any demineralized dentine to a fully mineralized state. Fortunately, there is ample evidence that the carious and even cavitation processes affecting dentine can be arrested and these tissues can be remineralised to a bondable state (Hellyer and Lynch, 1991; Hellyer et al, 1990; Holmes, 2003; Hosoya et al, 2000). Furthermore, once these lesions have remineralised, they tend not to be involved in the active caries process again (Anusavice and Kincheloe, 1987; Baysan et al, 2000; Knight, 1993; Kutsch and Everett, 1997; Lynch, 2001, 2003). This feature may be one of the most important long-term benefits of aero-therapeutic treatment.

Once the remaining dentine can be predictably bonded to, the process of placing a definitive restoration, whether direct or indirect, is relatively straightforward. There are numerous composite, compomer, and ceramic materials available to the practitioner.

Predictable Caries Reversal and Remineralisation at the tooth-restoration interface

While the predictability of adhesive dentistry is well established, the process of clinical remineralisation has been a much more difficult task. The major variables are the presence and ongoing activities of aciduric micro-organisms (Lynch, 1996, 2000), and the ability and willingness of patients to control the environment wherein these micro-organisms live on a regular, ongoing basis.

From the profession's past experience with tooth brushing and dental floss utilization, it can be safely assumed that patients are likely to perform easy and pleasant tasks more or less on an ongoing basis, particularly where it concerns their appearance. Those routines that are less immediate in their effect are likely to be omitted and/or forgotten. In fact, it may actually be easier to influence and control the biofilm environment of the mouth through an alternative pharmaceutical management strategy such as ozone therapy (Kutsch and Everett, 1997; Lynch, 1994, 2001, 2003)than it is to modify the daily behaviour of the patient.

Fortunately, human saliva contains several important buffering minerals such as phosphate, calcium, and fluoride that can assist dentinal remineralisation under the proper circumstances (Lynch and Beighton, 1994; Lynch, 1986; Meredith et al, 1996). The other critical factor is that of microbial control. Since research has linked acid-producing bacteria with plaque, and hence carious conditions (Lynch et al, 2000; Lynch, 1996), it can be readily inferred the bacterial control can limit the activity and damage of dental caries. In the absence of aciduric micro-organisms, remineralisation can occur where there is a salivary, oral toothpaste, spray or rinse, or restorative, source of mineral ions (Hellyer and Lynch, 1991; Hellyer et al, 1990; Holmes, 2003; Hosoya et al, 2000; Silva and Thompson, 2003; Nyvad and Fejerskov, 1987).

Ozone has proven to be a very powerful antimicrobial agent. It has been used in numerous medical procedures for many years, and has been commercially available for dental use for more than two years. In fact, an ozone application of 10 seconds has been reported to eliminate more than 99% of the micro-organisms found in the dental caries and associated biofilms (Lynch, 2003; Lynch and Baysan, 2001).

Ozone is produced naturally when there is a photodissociation of molecular oxygen (O_2) into activated ions (O^-) which in turn react with other oxygen molecules to form a transient radical anion (O_3). Ozone eventually decomposes to the hydroxyl radical, a powerful oxidant. Ozone oxidizes biomolecules such as cysteine, methionine, and histidine, disrupting microbial cell structures and metabolism (Silwood et al, 1999, 2002; ten Cate and van Amerongen, 1996; Terashima et al, 1969). Ozone disrupts microbial cell walls in seconds, leading to immediate cell lysis. This extremely rapid bactericidal activity is fundamental for effective dental ozone therapy. Since a 10 to 20 second application of ozone (Anusavice and Kincheloe, 1987; Lynch, 2003)is more than enough to effectively destroy these microbes, a 40 second treatment time covers all eventualities.

Once the aciduric and acidogenic micro-organisms

and the protected biofilm environment that hosted them have been destroyed by the ozone application, remineralisation of tooth structures is not only theoretically possible, but has been achieved (Lynch and Beighton, 1994; Lynch, 1986; Meredith at al, 1996) and clinically proven in numerous clinical trials worldwide. At 18 months, using remineralisation toothpastes, sprays and rinses (HealOzone products, KaVo), 100% caries reversal and remineralisation has been achieved in root caries affected teeth.

The use of remineralising products on a regular basis, for months or years at a time, does require patient compliance and motivation that is not always found. It is also beneficial to consider alternative means of delivering the necessary ions to the tooth surface. Fortunately, materials that accomplish the required remineralisation are available to dentists in the form of glass ionomer cements. These products have been used by dentists for approximately 30 years, and their safety and ion releasing capabilities are well established. The glass ionomer cement is placed in direct contact with the demineralized dentinal surface; the remineralisation occurs directly, without solely relying on patient compliance for rinsing.

Glass ionomer cements are not used in occlusal restorations due to their rapid wear (much faster wear under function than microhybrid composites) and their tendency to stain and pit on the surface. These shortcomings are far more significant in the long-term (years) then in the short term (months).

Microhybrid composites are excellent materials for long-term dental restorations, rivalling and often excelling amalgams. Their use and acceptance has been established over the last quarter century to the point where in many parts of the world, these restorations are the accepted standard. While composites are both functional and aesthetic, they are generally inert; they do not release ions that may recharge the underlying dental surface.

It makes sense to clinically combine these two materials sequentially to take full advantage of their respective properties. The glass ionomer cement is used initially (as a provisional or temporary restoration) to remineralise the dentinal surface after the soft decay has been removed selectively from the preparation. After a period of 12 to 18 months remineralising the carious dentinal surface, the provisional glass ionomer cement is removed mechanically, revealing a hardened, recharged dentine which is now bondable.

The cavity is restored to function and form using currently available adhesive techniques. While the restorative process has now become a two appointment procedure, the conservation and remineralisation of questionable dentine is well worth the extra effort. In addition, all the steps in the technique discussed below are most commonly completed without the need for local anaesthetic, permitting the large segment of the population that avoids dental treatment to access it without fear.

Of even greater significance is the finding that once lesions have remineralised, they are very unlikely to become active in the carious process again (Kutsch and Everett, 1997; Lynch, 2003; Lynch and Baysan, 2001). This, for the first-time, offers the dental profession the possibility of eliminating not only caries, but the cariogenic process itself. These aero-therapeutic dental procedures are already having a dramatic effect on the dental health of patients, and greatly are improving their ability to maintain this situation.

Clinical Technique for aero-therapeutic restorative dentistry

Diagnosis and treatment planning

1. The decayed area is identified visually, tactilely, and/or radiographically (Fig. 1).
2. The patient is advised that the aero-therapeutic technique will be utilized, and that no local anaesthetic is recommended. (There is a standing order throughout the entire procedure that, if at any

Figure 1

Figure 2

time, the patient requests local anaesthetic, it will be administered immediately.)

Minimally invasive preparation

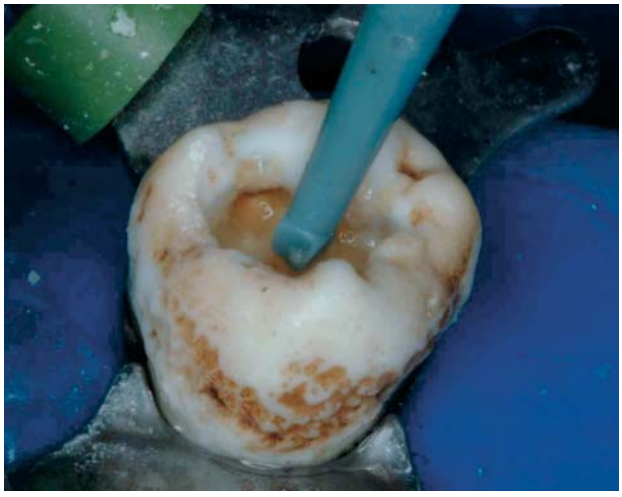
3. Access is gained through the enamel using a Fissurotomy bur (SSWhite Burs Inc., Lakewood NJ) (Fig. 2). Since the length of the Fissurotomy bur assists in limiting its penetration to the depth of the enamel, it is unlikely that this step will cause any patient discomfort. (In some cases no rubber dam is used. While the advantages and benefits of rubber dam isolation are universally recognized and recommended, the prospect of placing a rubber dam clamp on or adjacent to anaesthetized gingiva is less than pleasant. For small to medium size restorations, careful cotton roll isolation and efficient four-handed dentistry may be just as effective as rubber dam isolation (Yamada et al, 1983)).
4. The access opening must be at least large enough to permit the entry of the appropriate SmartPrep instrument (SSWhite Burs Inc., Lakewood NJ). The SmartPrep instrument is used to remove any soft dentinal decay (Fig. 3) (The self-limiting hardness of the SmartPrep instrument can cut neither enamel nor healthy dentine). There is neither extension for retention nor extension for prevention (as with amalgam restorations). The access opening must also provide some visibility to assure that soft decay has not been left in the preparation (Fig. 4).

Healozone procedure

5. The HealOzone (Fig. 5) handpiece tip (KaVo America Corp, Lake Zurich, IL and KaVo Germany), a disposable sterile cup, is used to form a seal around the prepared tooth (Fig. 6). Ozone is then delivered into the lesion via a hose at a concentration of 2,100 ppm. The ozone gas is refreshed in the disposable cup at a rate of 615 cc/minute for 40 seconds (this changes the gas volume inside the cup over 300 times every second). The HealOzone delivery system is a device that takes in normal office air and produces ozone gas.
6. HealOzone remineralising solution (Fig. 7), containing xylitol, fluoride, calcium, phosphate and zinc is applied to the lesion.

Remineralising provisional procedure

7. Multifil GIX (Heraeus Kulzer Inc., Armonk, NY),



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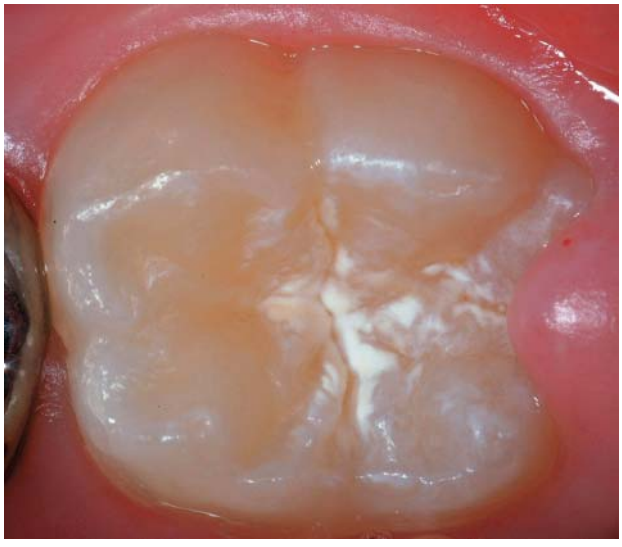

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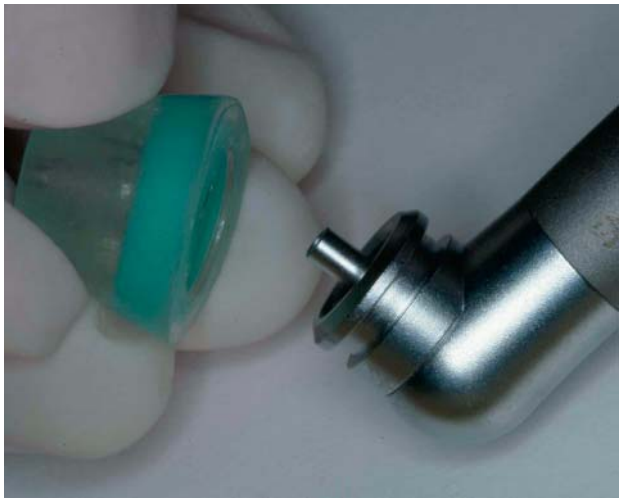

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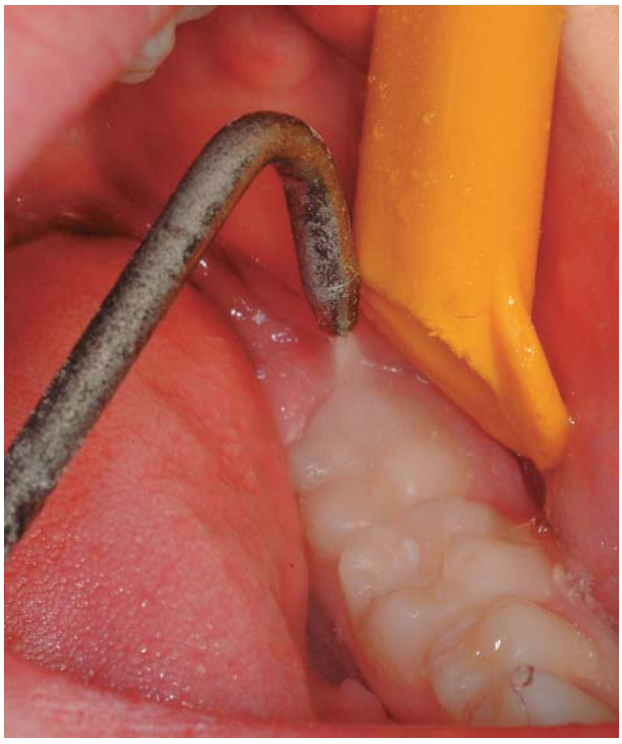

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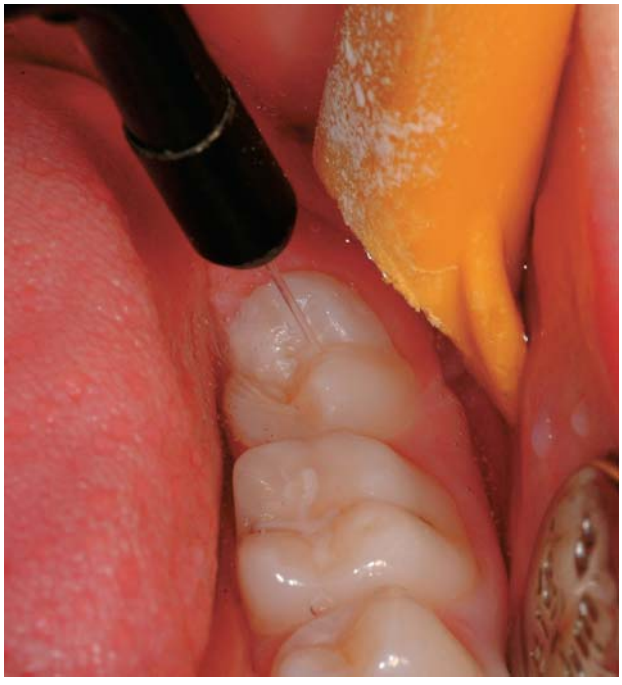

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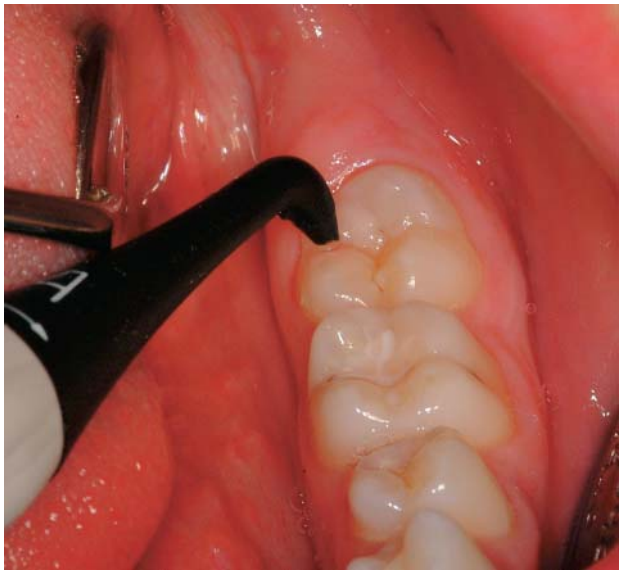


Figure 8

a Glass Ionomer Restorative Cement (Fig. 8) that can assist in remineralising the dental structures is placed on the tooth surfaces as a medium-term provisional restoration. The powder and liquid are dispensed onto the mixing pad.

8. The mixing time must not exceed 1 minute. The

powder is divided into 2 equal portions (Fig. 9); the first half of the powder is mixed with the liquid until homogeneous, whereupon the remaining powder is incorporated and mixed thoroughly.

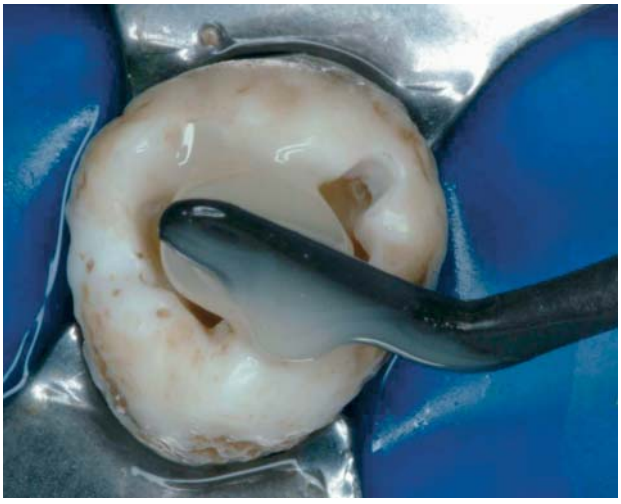
9. The Multifil GIX is transferred to the moistened tooth surface using a suitable placement instrument (Fig. 10). The shaping and contouring must be completed within 2 minutes. The setting time is 3–5 minutes from the start of mixing (Fig. 11).

The Multifil GIX is left in the cavity preparation for the purpose of remineralising the remaining tooth structures for 8–12 months. Most of the research with post-ozone remineralisation has been accomplished through the use of remineralising oral rinses and toothpastes (Fusayama et al, 1966). However, a remineralising provisional restoration does not have the limitation of relying on patient compliance or unpredictable ion access to the area in question. The Glass Ionomer cement is likely to wear, stain, and discolour over its active life. This is of minimal concern because at the second and definitive restorative appointment it will be replaced by a conventional microhybrid composite.

Definitive restoration procedure

Rubber dam isolation may be established

10. The remaining Glass Ionomer Cement is removed with a spoon excavator or a very slow round carbide bur (Fig. 12). This can usually be accomplished without the need for local anaesthetic. If



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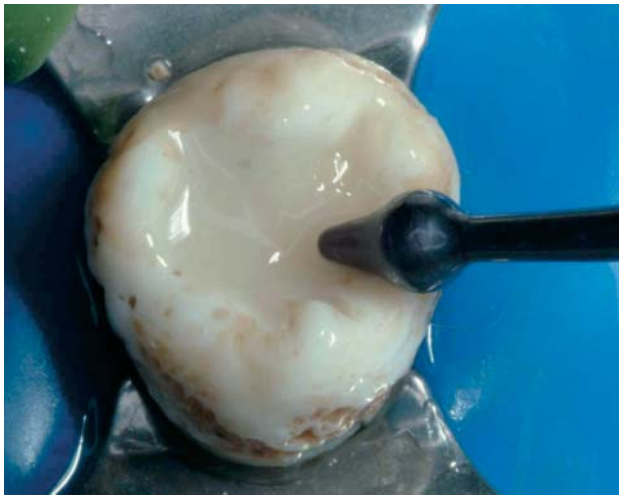

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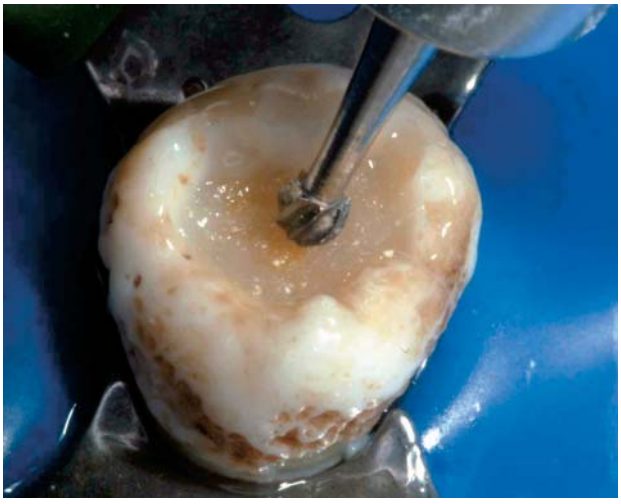

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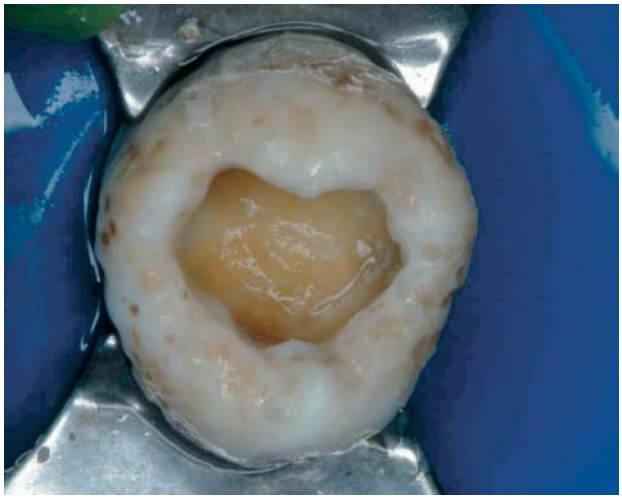

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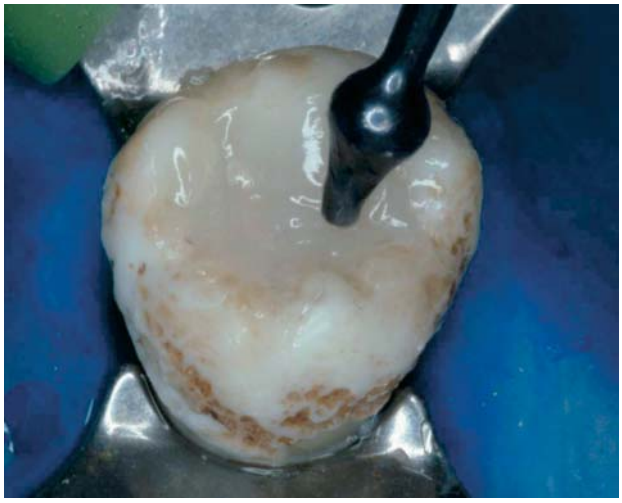

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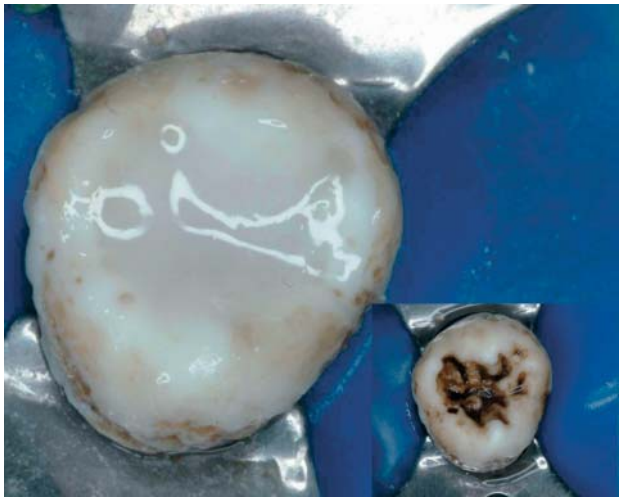

Figure 14

necessary, the preparation is isolated with a disposable matrix (Omnimatrix, Ultradent Products Inc., South Jordan, UT) which in turn is stabilized with a FlexiWedge (Common Sense Dental Products, Nunica, MI).

11. The preparation is then rinsed, and the excess water is removed.
12. The one-bottle, one-step 7th generation adhesive iBond (Heraeus Kulzer Inc., Armonk NY) is applied to the preparation (3 times 10 seconds), agitated on the preparation surface as directed (Fig. 13), and then light cured for 10 seconds. Note that there are no separate etching, conditioning, priming, or desensitizing steps. Seventh generation adhesives incorporate all the necessary chemistry and technique into a single application of one component (iBond is not moisture sensitive; the tooth surface may be dry, moist, or even wet, as long as there is no puddling of the water).
13. A shade-matched composite is applied into the adhesive covered preparation. Venus (Heraeus Kulzer Inc., Armonk NY) is a colour-adaptive microhybrid composite resin that is available in a multitude of shades, and comes with a cookbook-style 2-component shade guide (select the appropriate shade, and the cookbook indicates which colours to use to precisely arrive at the desired result).
14. The surface layers are shaped to correct anatomy with the anatomically designed "duckhead" instrument (Hu-Friedy Inc., Chicago IL) in order to minimize the time and effort required for final polishing after polymerization (Fig. 14). The composite is light-cured at every 2 mm layer.
15. The topmost layer is painted with Seal-n-Shine (Pulpdent Corp., Watertown MA), a composite-sealing liquid that has the unique property of polymerizing hard (without the formation of an oxygen inhibited layer). This leaves a clear, hard, smooth and resilient surface on the restoration.

The completed restoration is functionally designed and aesthetically acceptable. The entire process was accomplished conservatively, without the need for local anaesthetic, and with confidence that all the decay (but only the decay) was removed from within the cavity preparation. Much of the questionable and/or softened dentine has been maintained and remineralised to long-term health and function.

With the advent of aero-therapeutic dentistry, combined with the remineralisation of questionable dentine and truly conservative restorative procedures, dentistry has returned to its original function as a healing art.

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CHAPTER 4.2

The Introduction of Ozone Therapy into a General Dental Practice in Wales, UK – a Personal View

Newton D. Johnson

In the UK, the various Adult and Child Oral Health Surveys have revealed a reduction in the incidence and prevalence of dental caries over the past thirty years. Whilst this is a welcome finding overall, there remain large areas of the country where dental diseases are rampant and continue to pose a major public health problem.

My dental practice is situated in Llanelli, a town of some 100,000 people in south west Wales. The town is documented to have some of the worst dental health in the UK. It also seems to follow that dental phobics are also commonplace in areas of high dental disease. Ignorance of dental health issues and diets high in refined carbohydrates, sugar loaded fizzy drinks and children consuming considerable amounts of sticky confectionary are the norm.

This was one of the primary reasons why my practice was selected to examine the effect of the use of ozone therapy in the treatment of dental caries, coupled with other aspects of the use of ozone in general dental practice.

Every day we come into contact with patients of all ages, with staggering levels of dental disease. Daily events for us include: acute dento-alveolar abscesses, acute necrotising ulcerative gingivitis, pulpitis, broken down teeth and so on. A considerable degree of fear towards dental treatment is also the norm here. Few of us would admit to enjoying having dental treatment, but in our part of the world the level of fear encountered in new patients continues to astonish us.

We launched the practice in 1997 and by the end of 2003 we had some 2,900 registered patients. Over this same period we could have registered double this number of patients, because, despite their fear of the dentist, patients were desperate to 'sign-up' at a practice. Demand is considerable; we are forced to turn away many prospective patients each day. There is a national shortage of qualified dental practitioners in the UK, and recruitment is a major problem. The UK National Health Service (NHS) poses many difficulties to the general dental practitioner, not least financial. In our practice we agree to accept only NHS patients who are disadvantaged, all other patients are seen on a private fee paying basis. The pressure on the practice is always huge, as so many people cannot access dental care. This is uncomfortable for us, as it is for many practices throughout the UK.

The 'Drill and Fill' concept

The generally perceived wisdom has always been that dental caries is an infective process and the only real 'treatment' option was the cutting away of all diseased tissue and its replacement with some form of restorative material.

This invasive process involves needles and drills, which are highly stressful treatments for some patients. This amputation approach has not really changed for over 100 years. What ***has*** improved in recent years is our understanding of the caries disease process. The prevention movement that really got going in the 1970s has attempted to educate people to understand that the real ***treatment*** of the disease is centred on dietary changes, thereby removing many of the fermentable carbohydrates, and improved plaque control. We know

only too well that people are highly resistant to receiving good health education. The typical general dental practitioner does not usually have enough impact in presenting convincing dental health education messages to patients. With the patient sitting in the surgery, surrounded by the tools of drilling and filling, is it little wonder that patients fail to 'hear' what the hard working dentist tells them!

It is a salutary thought that at approximately 9 a.m. each working day, in every typical dental practice across the world, patients are lining up, the dental drills are whistling-up to speed and another day of 'drill and fill' begins. Those UK practitioners who are embedded in the NHS system are super efficient at drilling and filling. I am sure that NHS colleagues will not object to me saying that they may need to treat some forty plus patients per day to survive financially. Taken across the country that represents a massive number of patients entering the 'repeat restoration cycle'. Under the current NHS contract, practitioners are remunerated on the same basis as they were in 1948 when the NHS dental service was launched, i.e. *paid on a piecework system*. Although this system is due to change in 2004, the overwhelming majority of dental work in general practices remains the drilling and filling of teeth.

The 'Real' treatment of dental caries

Modern theory would suggest that the only genuine ***treatment*** of the disease we call dental caries is:

1. **Diet change** – a reduction in the number of fermentable carbohydrate 'hits' each day.
2. **Plaque attack** – high quality oral hygiene measures delivering agents such as fluoride to reduce the cariogenic microbial colonies.
3. **Tooth toughening** – improving the ability of the dental hard tissues to resist dissolution from the acidogenic microorganisms and to improve the potential for remineralization of the tissue.

It is such a struggle to alter the ingrained behaviour activities of individuals. We only have to look at the problems faced by the health promoters and educators who try to persuade people to quit smoking and reduce alcohol consumption, and to cut down on refined carbohydrate and fat intake, and to eat more fruit and vegetables. These valiant people do their best without much success. Thus it is a similar uphill struggle for dental practitioners to successfully alter the behaviour of their patients. It is little wonder that so many practices do not even attempt to run prevention programmes, and allow their practices to become 'drill and fill factories' – particularly in areas with the highest dental disease profiles.

People do not like the dentist!

So many people have experienced a bad time at the dentist. Many are terrified vicariously, as they have been imbued with fear from friends and relatives. We dentists are poor communicators; even excellent dentists often struggle, especially in areas of high disease and fear.

This is hardly a surprise as however delightful, gentle and caring the practitioner and his team may be the patient knows that in the end they are going to face the needle and drill. It is possible for the team to achieve much with our patients by having a gentle and caring approach, soft hands, topical analgesia, high quality needles, and blood-warm local anaesthetic with a slow delivery of the local solution into the tissues. All these elements can result in the elimination of only so much unnecessary pain for the patient. High quality turbines and burs coupled with trained close-support nursing care can contribute as much as possible to a pain-reduced and stress-free treatment experience for the patient. Nevertheless, some dentistry remains old-fashioned and dare I say, barbaric in form.

The ozone alternative

The notion of receiving 'non-invasive' treatment of dental caries is quite special, particularly when it is possible to offer treatment by effectively placing a soft, rubber cup against a tooth for a few seconds only, coupled with some oral hygiene advice and perhaps the use of a special toothpaste, mouth rinse and spray to aid re-mineralization. Compared with a needle and drill, it is nothing short of spectacular.

The scientific basis of ozone therapy in the treatment of carious lesions and its long-term effectiveness is discussed elsewhere in this book. The central notion here is the effect of ozone as delivered in a busy general

dental practice that encounters a tidal wave of disease and a large amount of dental phobia on a daily basis.

In our practice we record, on a formal basis, various aspects of the use of ozone with our patients.

Our analyses include:

1. The natural history of carious lesions, using visual and tactile scoring, bitewing radiography, together with the DIAGNOdent[1] laser probe.
2. The ease, or difficulty, of undertaking ozone therapy and comparing this with conventional drilling and filling treatments.
3. The cost implications: examining the overall financial implications and analysing the time required to provide ozone therapy versus conventional treatment.
4. Recording the attitude of the patient to the whole experience of receiving ozone therapy.
5. Examining the effectiveness of ozone in the treatment of cervical sensitivity.
6. Monitoring the effectiveness of ozone therapy in the reduction of pain in the 'cracked tooth syndrome'.
7. The application of ozone in the conservative treatment of grossly decayed teeth.
8. The use of ozone in treating deciduous teeth exhibiting gross decay, even in cases of toothache.
9. The effect of ozone therapy in the treatment of dental phobic patients.
10. The use of ozone therapy in older individuals, in particular, the medically compromised and those at risk of root caries.
11. Prophylactic use of ozone on occlusal surfaces of erupting teeth in high caries risk children.

Our practice findings

We began using ozone therapy in 2001, and by early 2003 the use of ozone in our dental practice has produced overall results that are nothing short of astonishing. These findings are from a single practitioner dental practice and his team. Much of the results are in preparation for submission to scientific scrutiny for publication in academic journals. However, my personal findings are that patients have responded with great enthusiasm towards the entire concept. There has been 100% acceptance of the treatment utilising ozone.

Taking our ten broad areas of research outlined above, our practice findings have demonstrated significant reversals in primary carious lesions that were compiled in the practice in double-blind, masked, clinical trials, as measured by clinical indices and by the DIAGNOdent instrument.

We have noted that patients tend to respond enthusiastically to the concept of ozone therapy and the DIAGNOdent has proved to be surprisingly popular by patients of all ages. We discovered that patients are keen to become involved in the DIAGNOdent readings. The audible signal produced by the instrument when it encounters a suspect area has proved a revelation in itself. It is remarkable how the patient responds to this signal and child and adult alike remember their DIAGNOdent reading! Children even remember the colour of the ozone cup and often chirp, "You used the blue cup last time, what colour will it be today?"

We have been impressed by the seemingly increased awareness levels of patients after they have been enrolled on the ozone programme. We have noted the subsequent much-improved oral hygiene in the patients when offered the ozone treatment. The patients seem to 'switch on' to the concept, and appear to be much more receptive to oral hygiene and dietary advice, and are keen to participate in the use of mouth rinses.

Our experience in the practice has seen previously nervous individuals, who normally do not like sitting in the dental chair, literally hopping in the chair to have ozone therapy. More smiles, more laughter, more enthusiasm all round.

Our analysis of the cost implications of the use of ozone compared with conventional treatment has proven to be of interest. Initially, the cost of purchasing the ozone machine, hand-pieces and rubber cups does appear to be an expensive investment. However, taken in perspective, we are able to treat several teeth in just five minutes. We do not charge patients for ozone therapy when patients are part of one of our research projects. However, it is interesting to consider the comparison with fee charging at a basic level. If the practice charged a nominal fee, let us say just £10 per tooth, we have found it possible to treat six teeth in five minutes. This represents £60 for five minutes work. Add an additional five minutes for the greeting and saying farewell to the patient from the treatment room, *that*

[1] DIAGNOdent, Kavo, Germany.

equates to £360 per hour in income, with no laboratory fees to pay. The sundry expenses are for cotton wool rolls, some prophylaxis polish and perhaps an aspirator tip and a disposable cup for a mouthwash. This bears little comparison to the hourly income earned from 'drill and fill' treatments!

We asked patients to complete a questionnaire to evaluate their attitudes about accepting ozone therapy. The results of the questionnaire survey predictably revealed that 100% of the respondents said they thought the ozone treatment was better than conventional drilling and filling. A key finding was that an overwhelming majority of our patients questioned said they would be happy to pay more for receiving ozone therapy, even though the treatment typically took just a few minutes to complete.

The patients all scored the highest marks for the procedure being comfortable, and they all confirmed the experience was a positive one. Nervous patients stressed how they appreciated the treatment and that it gave them confidence to visit the dentist. Everyone questioned said they felt motivated to modify their fermentable carbohydrate intake and to improve their oral hygiene as a result of the ozone experience.

This is an interesting practice finding because when questioned further the patients felt rather fatalistic when faced with conventional fillings, even tending towards "why should I bother" in terms of improving their diet and plaque control because "I'll have to have a filling anyway." However, when they received the ozone therapy they reported feeling more optimistic and positive about changing their habits. Certainly, it is our experience that the use of the DIAGNOdent and ozone, coupled with oral hygiene does have a powerful impact on patients.

We have been testing the use of ozone in the treatment of cervical sensitivity and in cases of suspected cracked tooth syndrome with pleasing results. We have recorded success in reducing symptoms in almost all cases with many patients reporting a total cessation in their symptoms.

Allied to this we have been using ozone treatment on deciduous molar teeth with hopeless prognoses as a result of caries. In our part of the UK it is upsetting to find so many children at 3 and 4 years of age with gross decay. For these patients, the usual outcome is a general anaesthetic and extraction at a specialist centre. We have attempted to treat these lesions with ozone and have found that the majority of children are cooperative and actually enjoy the experience. What we have found of great interest is that we have abolished toothache in young children after ozone treatment, with much relief for the parents. We have found the ozone treatment an excellent palliative treatment for these youngsters.

Recently we have been using ozone to treat adults with grossly decayed, but vital, teeth. Initial findings have been encouraging with follow-up assessments showing that soft dentine has appeared almost 'wooden' in texture, with patients reporting no adverse symptoms. In our practice, patients with grossly decayed teeth have a poor attitude to dental health and often attend for dental treatment infrequently. Interestingly when we offer these people the ozone pathway we have discovered a major change in their attitude towards oral health, and they become excellent at keeping their appointments.

As expected, the ozone treatment has proven to be a wonderful help in the treatment of elderly patients exhibiting root decay and in the treatment of people with a disability. So many of our patients are on various medications that result in a reduced salivary flow. These people are at increased risk of developing caries and we have found the application of ozone to be excellent in prevention of lesion development and in the treatment of root carious lesions. The results of the prophylactic use of ozone on occlusal surfaces of erupting teeth in high caries risk children have been excellent.

Added value

Over the past few years the dental profession has enjoyed many technological advances in equipment and materials; we feel proud with our new 'toys' but all too often the patients are not that impressed. In our practice, the introduction of the ozone concept, coupled with the DIAGNOdent has had a major impact on patients, and is a real 'turn-on' for them. It has been quite remarkable.

As mentioned earlier, in areas of the country with high levels of dental disease, dentists often encounter a high level of dental phobia. Our practice has been designed to minimise stress and is highly patient centred and we do everything we can to offer disease control advice and take a preventive approach. Even so it is still difficult to convince some individuals that dentistry can

be delivered relatively comfortably. We have discovered that ozone therapy allows nervous individuals to receive beneficial ozone treatment whilst they develop increasing levels of trust and tolerance. It has helped us convert many phobic patients into cooperative and relaxed participants, who are then happy to accept dental treatment as a whole.

The ozone concept has had a significant impact on the dental team. The dental nurses and front of house staff have been very enthusiastic as they can see the effects of the new treatment on the patients. This adds to the positive atmosphere of the practice towards prevention and disease control. It does much to boost the morale of the team to witness nervous patients in such dental distress evolving into relaxed and appreciative individuals.

Conclusion

The introduction of ozone therapy into our busy dental practice has proven to be exciting, and a huge success. Patients are delighted and it has created a 'buzz' in the town. Any innovation that can help halt dental disease and the fear of the dentist has to be welcomed. Our personal experiences have taught us that the ozone concept enhances our ability to communicate with patients who rapidly warm to the idea. It seems to stimulate their interest and the therapy is a financially highly profitable asset.

From a dental public health point of view, with dental caries being such a problem in large areas of the country and with such a shortage of clinicians here in the UK, the ozone therapy has a major part to play in the prevention and treatment of dental caries. The Healozone[2] machine and the DIAGNOdent are totally portable and it is possible to envisage units being used with great effect in every dental practice and community clinic. Because it takes such little time to treat several teeth it may be possible to cover many more patients compared with conventional treatment. Being so simple to use, dental hygienists and therapists are ideally suited to providing the treatment for all categories of patient.

The research undertaken in centres across the world has delivered the long-term reversal of caries which we had hoped for. Certainly every result published has been excellent. Overall our dental practice has benefited enormously from introducing ozone into our daily routine.

[2] Healozone, Kavo, Germany.

CHAPTER 4.3

Experiences of Using Ozone in 46 General Dental Practices

Edward Lynch

Introduction: ozone treatment in dentistry

In this chapter, the experiences of a number of HealOzone users in various countries have been collated and reviewed. The aim of the chapter is to inform dental health care professionals how some practices have incorporated dental ozone treatment into everyday dental care for the benefit of patients. These practices are located in England, Germany, Hungary, the United Arab Emirates, Ireland, Wales, Scotland, and Italy.

Let us begin with a familiar scenario in a busy general dental practice: an 8-year-old boy presents with occlusal caries in a lower first permanent molar, and the tooth has been occasionally hurting for a few days. The young boy may be difficult to treat, access is poor, and the patient has not had local anaesthesia before.

If the decision is made to restore the tooth, the dentist may to need to subject the patient to an unpleasant inferior dental block injection. The carious area and usually some of the surrounding sound tooth tissue will need to be removed and destroyed, using noisy hand pieces with the associated unpleasant water spray, smells, and vibration. The cavity will need to be restored with a suitable material. If amalgam is used there may be concerns, often from the parents, about mercury toxicity. If composite resin is used, there are the problems of moisture control and the durability of the material. Future dental care may become more difficult due to increased anxiety and fear at subsequent appointments. And in placing a restoration, the dentist has condemned the tooth to a lifetime of filling removal and replacement that may become increasingly more expensive as less tooth is left to support the restorative care. Despite all the modern equipment dental practitioners have, some aspects of dentistry remain old-fashioned and barbaric.

There is another way to treat this patient, which involves no local anaesthesia, no water spray or high volume suction, no noisy hand pieces and is a very quick procedure to perform. The patient and the dental practitioner would undoubtedly prefer this option. Technology is now available to provide such an alternative treatment using ozone gas. The HealOzone unit (KaVo GmbH, Germany) is a portable ozone generator designed especially for treatment of intraoral lesions of the hard and soft tissues and has been in use in dental hospitals and general dental practices for several years.

New technology to aid the treatment of dental patients is always of interest to the forward thinking general dental practitioner (GDP). In recent years, several minor advances have been made to aid the GDP in the provision of dental treatment, many of which are easily integrated into the surgery environment. In making the decision to invest in new advances, the GDP must balance the benefits to patients of new equipment with financial considerations, such as initial expenditure, ongoing running costs, and potential profitability. Ideally, all these factors are in balance, and there may be an additional benefit of enhanced practice image from the use of the technology concerned.

The generally perceived wisdom has always been that dental caries is an infective process and the only real 'treatment' option was the cutting away of all diseased tissue and its replacement with some form of restorative material. This is the teaching that most dental students still receive at dental schools around the world today.

This invasive process involves needles and drills, and it is a highly stressful treatment for everyone concerned. The amputation approach has not really changed for over a 100 years. What has improved in recent years is our understanding of the caries disease process. The prevention movement, established in the 1970s, has

attempted to educate people that the real treatment of the disease is centred on changing the diet and in improving fluoride exposure and plaque control. It is known only too well that people are highly resistant to accepting messages of good health education. The typical GDP does not impact enough in presenting convincing dental health education messages to patients, (sitting in the surgery, surrounded by the tools of drilling and filling is it little wonder that patients fail to 'hear' what the hard working dentist tells them!).

It is a salutary thought that at approximately 9 am each working day, in every typical dental practice across the world, patients are lining up, the dental drills are whistling-up to speed and another day of drill'n'fill begins. Those GDPs in the UK, who are embedded in the country's National Health Service (NHS) system are super efficient at drilling and filling. Some NHS colleagues will not object to it being said that they must treat some 40+ patients per day simply to survive financially. Taken across the country, that is a massive number of patients entering 'the repeat restoration cycle'. Under the current NHS contract, GDPs are remunerated in the same way as in 1948 when the NHS dental service was launched, i.e. paid on a piecework system.

The dental profession has seen a move away from conventional means of restoration of carious tissue to the approach of minimally invasive dentistry, with the associated benefits to both the dentist and the patient. Many techniques, however, still involve the physical removal of tissue before the final restoration is placed. The ideal treatment solution is the simple removal of the disease process with no associated loss of sound tissue and no associated physical discomfort for the patient. This is now available with recent advances in the field of ozone treatment and the HealOzone delivery unit. For the first time, the dental practitioner can break the ongoing circle of restorative dentistry, as it appears that it is no longer necessary to place the initial restoration which will require eventual replacement and subsequent re-treatment.

Over the past 30 years the various UK surveys of oral health of adults and children have revealed a reduction in the incidence and prevalence of dental caries. While this is a welcome finding, there remain large areas of the country where dental diseases are rampant and continue to pose a major public health problem. Parts of Wales and Ireland are reputed to have some of the worst dental health in the Western world. It also seems to follow that in areas of high dental disease activity, dental fear and phobia are also commonplace. Ignorance of dental health issues and diets rich in refined carbohydrates, sugar-loaded fizzy drinks and children consuming considerable amounts of sticky confectionary are the norm.

Every day some dental practitioners come into contact with such patients of all ages, with staggering levels of dental disease. Acute dentoalveolar abscesses, acute necrotizing ulcerative gingivitis, pulpitis, broken down teeth and so on, are daily events for practitioners in these areas, as well as fear and phobia towards dental treatment–people do not like the dentist!

Patient acceptance of HealOzone treatment

In the past number of years continual advances in both materials science and treatment methods have brought outstanding benefits for our patients in terms of simplicity of treatment. Successful dentin bonding systems have obviated the need for the design of a retentive cavity in most cases and hence dramatically reduced the use of the air turbine to design what may be termed as classical cavities. These bonding systems have allowed GDPs to concentrate almost exclusively on the removal of carious tissue while retaining as much sound hard tissue as is possible, a first step towards the minimally invasive approach now advocated. However, this carious tissue has still to be removed whether by use of the hand piece or with hand instruments when used in conjunction with caries-removing liquids and gels (e.g. Cariosolv). HealOzone treatment of dental caries removes the requirement for physical removal of diseased tissue as it promotes remineralization and not amputation of carious dentin.

The benefits to patients are therefore obvious. Most patients' fear and apprehension arise from their perception that the use of the hand piece may be an unpleasant and possibly traumatic experience. This, combined with the requirement for local anaesthesia, the fear of which is very common, has led to the widespread view that the visit to the dental surgery is an unpleasant one. With HealOzone treatment GDPs now have the capability to alleviate those concerns and change the public perception of dental treatment as a whole. Of course, there are still situations where treatment will follow more classical lines. However, these instances are becoming increasingly rare and patient acceptance is

therefore universal. GDPs can offer treatment for a wide variety of carious lesions where there is no need for local anaesthesia and drilling, and treat many lesions in a very short space of time, painlessly and atraumatically. Patients are delighted after treatment and are particularly motivated towards oral hygiene and dietary control when they realize that in improving and concentrating on these areas they can effectively avoid the local anaesthesia/drill approach.

Integration of the HealOzone treatment unit into the surgery environment will totally change the GDP's approach to the treatment of his or her patients. GDPs have to completely reassess the diagnostic criteria when applied to dental caries and potential treatment of the carious lesion. The dental probe is no longer of any significant use in the diagnosis of caries and hence examination is based on the use of a digital intraoral camera combined with selective use of the DIAGNOdent. This has several advantages over the classical approach to the examination appointment. First, it is simple and atraumatic for patients. Second, it involves the patient in their examination (an additional benefit of this is a dramatically increased awareness of oral hygiene), and aids the explanation of any problems which may arise. Third, from the clinician's point of view the advantages of intraoral imaging cannot be stressed highly enough. Images of the dentition can be magnified many times to assist in diagnosis, and the enlarged direct view on the monitor of the region being examined is an essential adjunct to the direct intraoral view. This imaging combined with the use of the DIAGNOdent in areas where caries may be suspected results in an extremely thorough and meticulous examination and gives GDPs a quantitative assessment of any disease process present. Explanation to the patient of the requirement for treatment is very simple when images can be shown on screen and the DIAGNOdent reading explained and only serves to enhance a patient's confidence and educate them in oral hygiene and dietary requirements.

Following the examination, records are made of any positive DIAGNOdent readings and the particular teeth these are related to. Treatment options are explained to the patient and HealOzone treatment recommended where indicated. Images are saved via the intraoral camera of the teeth to be treated and ozone is applied using the protocols as previously described. There is rarely any need to make further appointments for the patient, apart from the ozone review visits. Hence the provision of ozone treatment is extremely time efficient, something which is valuable to clinician and patient alike. The patient's visit to the surgery is completely painless and without any trauma, and they leave well informed and educated on both the reasons for treatment and what is required for a successful outcome. In addition to these factors, GDPs can look at the effects of the employment of HealOzone treatment on themselves as dentists. GDPs naturally subject themselves to a degree of stress as they all desire to provide patients with pain-free treatment as efficiently as possible. Using ozone treatment as the primary approach to the treatment of dental caries completely removes any potential stressors. There is no local anaesthesia to give, no use of the drill and no packing of restorative material. The time spent on providing the actual treatment is also minimal in the extreme. GDPs can therefore provide the most modern and most natural treatment available to their patients without fear that they may cause any physical or mental trauma – all the potential sources of stress for the dental surgeon in restorative treatment of the carious lesion are removed and yet they are providing the very best in dental care. As described elsewhere in this book the use of a flowable composite is recommended to seal in the remineralized dentinal caries four weeks after the HealOzone treatment.

Uses of Ozone in the General Dental Practice

Management of caries

According to 'The Niche Environment Theory', a 'microbial ecological niche' is established within a carious lesion. Progression of the carious lesion occurs when conditions are suitable for acidogenic microorganisms to release acid as a metabolic byproduct. The acid produced may lead to a breakdown of mineralized tooth structure. At times, an equilibrium situation may occur when the rate of remineralization equals the rate of demineralization. Ozone has a microbicidal effect because of its powerful oxidizing properties. This has a severely disruptive effect on the microbial population in the carious lesion and obliterates cariogenic microorganisms, thereby swinging the equilibrium in favour of remineralization. No more acid can be produced within the lesion once the acid-producing microorganisms have been eliminated. The lesion is repopulated with

normal mouth commensal microorganisms, which do not produce sufficient acid, allowing for remineralization to be the dominant process within the carious lesion, after ozone therapy.

Ozone has been show clinically to be effective in the management of root caries lesions, (Baysan et al, 2000; Holmes, 2003). These lesions often present in the elderly who may have associated medical problems, which complicate their dental management. Using ozone therapy, such lesions are easily treated. The portability of the HealOzone unit facilitates its use in the domiciliary setting, and treatment is also simplified because the clinician does not need to carry a range of restorative materials on such visits. Dentists using the HealOzone unit for caries management encourage their patients to regularly use fluoride-containing oral health care products (especially the HealOzone toothpaste, mouthrinse, and spray) that will enhance the efficacy of ozone by promoting remineralization and reduce the frequency of consumption of fermentable carbohydrates.

Treatment of carious lesions in deciduous teeth

Dental treatment in young people can have longlasting effects. If dental care is painful and unpleasant, as these patients grow into adults, they will tend to only attend when if pain. As all dentists know, at this stage, restorative care tends to be more difficult and more extensive. The use of ozone and mineral-releasing glass ionomers can play a significant role in the dental management of these patients. As confidence in the treatment by the patient and parents or guardians is gained, compliance with important oral hygiene messages will increase.

Where caries is present, it is simple to treat and the application of, for example, FujiVII (GC, Japan) will supply long-term fluoride and mineral release, as well as prevent ingress of food debris and re-establishment of the acid-niche environment. Treatment is simple, fast (the average ozone time for practitioners using the HealOzone is 30 seconds) and involves little preparatory work. The loose debris is first cleaned away until a leathery base is reached. This can be done with hand instruments. Ozone is applied and the lesion wetted with the CurOzone remineralizing wash. Then the glass ionomer cement 'sealant' can be applied. This modified atraumatic restorative treatment (ART) technique is described elsewhere in this book.

Allied to this, ozone has been used in the treatment of deciduous molar teeth with poor prognosis as a result of caries. In some parts of the UK, it is upsetting to find many children of 3 and 4 years of age with gross decay. For these patients the usual outcome is a general anaesthetic and extraction. These lesions are treated with ozone and it has been found that the majority of children are co-operative and actually enjoy the experience. Of great interest is that toothache in young children has been reduced and even abolished after ozone treatment, with much relief for the parents. Ozone treatment seems to be an excellent palliative treatment for such youngsters.

Treatment of carious lesions in permanent teeth

Ozone is often used during the eruption of the permanent dentition and as prophylaxis in populations at risk of rampant carious lesions. It is possible that the current fissure sealant technique needs to be re-examined. GDPs are instructed to use a bristle brush and pumice to clean the occlusal surfaces of teeth prior to sealing. However, it is know that food debris and bacteria will remain impacted in the depths of the fissures. Leakage would allow the acidic microbial ecological-niche to resume its activity, and over a period of time, the surface could collapse into a large carious cavity.

Alternative preparation systems, such as the KaVo Prophyflex or similar, or air abrasion could be used. Bristles in toothbrushes are often larger than the fissures being cleaned. However, air abrasion powders will flush out the debris, prepare the fissure for acid etching, and produce a more reliable sealing along the fissure edges.

Treatment of primary pits and fissure carious lesions (PFCLs)

Early diagnosis of primary pits and fissure caries is of great importance in children and adults because of the difficulty to diagnose these lesions with traditional methods such as oral radiographs and probe. Low sensitivity to visual, probing and bitewing examination leads

to a significant number of teeth with dentinal caries not being detected. Lesions have a natural history of deepening into dentin leaving a macroscopically undamaged enamel surface. Minimal mineral loss prevents radiographic evidence of decay, and no macroscopic cavitation means there is no probe stickiness. Systems using indirect light fluorescence have been demonstrated to be effective in the clinical detection of decay in the permanent and deciduous dentitions.

In superficial root caries or early pit and fissure carious lesions, ozone alone may be sufficient to treat these lesions (Reaney, 2002; Abu Naba'a L, 2003). However, in situations where severe breakdown of tooth structure has occurred, ozone may be used initially to promote remineralization and when this has occurred the cavity may be restored with a suitable restorative material.

Practitioners who use ozone place a restorative material, such as FujiVII or a composite to prevent food packing and food trapping. Some cosmetic concerns, such as stained remineralized caries may require placement of a restorative purely for aesthetic reasons.

Following on from the core philosophy of ozone treatment and of minimally invasive dentistry, the group of practitioners who have integrated ozone into their clinical practice will place a restorative material. The material of choice for these practitioners is a composite resin. Restoration using a 15-second etch (enamel) and a maximum of 10-second etch (dentin) followed with a dentin-bonding agent and finally a composite resin is again a totally atraumatic and simple procedure for the patient and dentist alike. Any regions of remineralization that have darkened during the course of treatment can be simply and effectively masked. GDPs have found in many cases that previously active root caries lesions are easily masked using one of the flowable composite materials. These are extremely simple and rewarding materials to use and, if placed correctly require no polishing, simplifying the treatment process even more.

Treatment of the larger carious lesion

The larger lesions need special care. It must be stressed that larger lesions are not those to be treated with ozone alone; most will require a combined approach of traditional therapy, as well as ozone. As before, the aim is to allow natural remineralization to take place on a predictable basis, without the wholesale destruction of tooth tissue. Where the lesion extends deep into the dentin (and is therefore clearly visible on radiograph), the ozone will take longer to act, or may require several treatment periods over time. The basic protocol is the same: the soft debris is removed along with any grossly unsupported enamel. If possible, carious dentin is removed to the leathery layer and ozone is applied for 40 seconds or longer. Then the HealOzone remineralizing wash is applied.

HealOzone practitioners are using two options at this stage of treatment:

- The lesion is left as self cleansing, and the patient is given modified oral hygiene instructions. As well as routine brushing and rinsing, they are asked to place a small amount of the paste directly into the cavity three times each day and especially the last thing at night. They are also advised to spray the HealOzone remineralizing solution directly into their mouths at least three times each day after eating. This increased exposure to HealOzone oral health care products has led to reports from these practitioners of complete hardening and reversal of the carious lesions within six weeks.
- The lesion is restored using a mineral-releasing glass ionomer, such as FujiVII. This will allow remineralization to occur, without the possibility of ingress of food debris and recolonization of the cavity. Where this has been carried out, for example in Class II type lesions extending into the approximal contact areas, practitioners are reporting complete remineralization at three to four months on average. Radiographs show remineralization, which will occur both from the material and the pulpal tissues.

It must be stressed that all these practitioners commented that it is vital to control both their own and their patients' expectations. If the pulpal tissue is already necrotic, no amount of ozone will bring it back to life. Root canal therapy and tooth removal are the only two viable options at this stage. However the good news at this point is that ozone can be used in root treatment cases and to manage potential pain post-removal by eliminating opportunistic infections in the socket and exposed soft tissue before healing has taken place. Ozone may also hasten the healing potential and reduce the time taken for healing.

Tooth whitening

Teeth are whitened using ozone, due its strong oxidizing properties. The situation often encountered is the discolored incisor that has been previously root filled. This condition is readily treated with hydrogen peroxide and sodium perborate mixture in the 'walking bleach' technique. However the application of ozone applied into the access cavity into the mixture will greatly enhance the whitening effect. The chosen whitening agent can be applied to the access cavity in the usual way and ozone applied from the HealOzone unit. The cavity is sealed with an acid-etched composite to retain the mix inside the cavity and the tooth left for one week for the whitening to occur.

Root canal therapy

The aim of conventional root canal therapy is to provide a cleaned, shaped, root canal that facilitates the placement of an adequate root filling. There may be multiple canals, frequently linked by a 'web' of accessory canals. There is the so-called 'apical delta' and the common lateral canals. Until recently, we relied on irrigants reaching these areas to disinfect and dissolve organic debris where it was impossible to instrument mechanically.

In this situation, current procedures can again be modified – as with whitening – to greatly improve the quality of treatment for patients. When irrigating with the usual irrigant solution, for example sodium hypochlorite, ozone can be applied to the hypochlorite solution in the root canals. This technique allows the root canal system to be thoroughly disinfected and possibly even sterilized. In cases where previous root canal treatment has failed, *Enterococcus faecalis* seems particularly prominent and especially difficult to eradicate. Ozone can eliminate this bacterial type (Chang et al, 2003).

Treatment of fractured cusp syndrome

The symptoms of sensitivity and pain on release of pressure related to the fracture of a cusp can again be successfully treated with HealOzone. The exact location of the fracture is helpful in the approach to ozone treatment and can be ascertained through careful examination and the use of intraoral imaging. Once the fracture area has been diagnosed a seal is obtained covering the cusp and fracture line in question and application of 4060 seconds of ozone followed by a remineralizing solution wash leads to elimination of symptoms. This obviously cannot cure the underlying problem of tooth fracture, however, the alleviation of acute symptoms by such a simple means is most beneficial for clinician and patient alike.

Fractured teeth

Posterior teeth with fractures along the pulpal floor often present with symptoms associated with reversible pulpitis. Such lesions have been traditionally difficult to treat, but if the restoration is removed, the fracture site determined and ozone applied, resolution of the pulpitis may be achieved. The base of the cavity may then be sealed with a dentin-bonding agent or glass ionomer cement prior to restoration with a suitable restorative material.

Where patients attend with fractured anterior teeth and exposed pulpal tissue, several practitioners from the pathfinder group have treated such cases up to 48 hours after injury. Local anaesthetics should be used prior to treatment. The exposed nerve tissue can be trimmed if required, and once bleeding has been controlled, ozone applied. Once the exposed pulpal tissue and surrounding tooth structure has been sealed the tooth can be reconstructed. Several pathfinder dentists have maintained vital teeth at 24 months for their patients treated in this way.

Dentin hypersensitivity

Exposure of the dentinal tubules with related symptoms of sensitivity is an extremely common problem presenting to the general dental practitioner. All treatment methods are directed at sealing of these tubules and vary from the application of fluoride varnishes to the placement of next-generation bonding systems on the root surface. The 'hydrodynamic theory' proposed to explain dentin hypersensitivity has been around for some time. As well as fluid movements within the dentinal tubules, bacteria may be associated with the tubules. This problem can be simply and immediately eliminated with the use of HealOzone treatment. Ozone penetrates the exposed tubules, eliminates bacterial contamination, and effectively allows mineral ingress and subsequent sealing. It is vital that the seal obtained allows ozone delivery to the area being treated, and in these cases, the liquid rubber dam is a great help in achieving this seal around the marginal gingivae. Once a seal is obtained, an ozone delivery of 40 seconds is followed by painting the treated area with the sup-

plied CurOzone remineralizing solutions. This protocol is usually sufficient to completely eliminate any symptoms arising from the area undergoing treatment. A final application of fluoride varnish may be done, and the patient is given oral hygiene instructions before leaving to ensure correct brushing technique so that future problems of this nature can be prevented. Over the last 28 months, several of the pathfinder group have only had to re-treat fewer than 5% of cases. It seems that ozone not only allows deep dentinal tubular remineralization, but may also stimulate the pulpal tissue to switch off the pain signals.

Postoperative pain

This is commonly reported, following cavity or crown preparations. Traditionally it has been explained as being due to thermal trauma to the pulpal tissues. Current thinking suggests that, associated with the pulpal trauma, there may also be bacterial ingress into the dentinal tubules. This bacterial contamination of dentin may lead to an acute inflammatory reaction within the pulp. The patient with the resulting pulpitis will complain of hypersensitivity to thermal changes and often spontaneous pain. The pulpitis may become irreversible and this may necessitate endodontic procedures to relieve the symptoms.

If ozone is applied to cavity and crown preparations when completed and prior to restoration placement, the degree to which the dentin becomes infected with bacteria is reduced. This decrease in bacterial count reduces the symptoms of post-operative pain and the need for endodontic procedures in such situations.

Soft tissue lesions

There is anecdotal evidence to support the use of ozone therapy for soft tissue- lesions, such as aphthous ulcers, 'cold sores', and dry sockets. The mode of action is thought to be a reduction in the bacterial population associated with such lesions due to the bactericidal effects of ozone. This use of ozone is also supported by many studies in general medicine where ozone has been used in 'bagging' techniques.

Treatment of dry sockets

Postoperative infection following extraction is unfortunately a common complication. Again, as ozone is bactericidal, in theory GDPs should be able to treat such problems very simply. All the practitioners have experienced great success using ozone for this application. Once a seal is obtained around the infected area, a delivery of 60-second ozone has led to, in all the cases treated, a complete resolution of symptoms within 24 hours. Ozone seems totally effective in the management of dry sockets and reduced the requirement for systemic antibiotic treatment.

Treatment of aphthous ulceration

The symptoms of major aphthous ulceration can be severe and extremely distressing for dental patients. Current modalities of treatment are primarily aimed at symptomatic relief as generally the aetiology of aphthous ulceration is idiopathic. It is simple to form a seal over the ulcer using a large cup and deliver 40 seconds of ozone to the lesion. In all cases, the symptoms have dramatically decreased within 24 hours and in some cases completely resolved within 48 hours. Again, this is a very relevant application as aphthous ulceration can be a very severe problem for some patients. Ozone treatment is an extremely useful aid in the resolution of our patients' symptoms.

Sterilization

Sterilization of all instruments and hand pieces between patients is an essential procedure in general dental practice. Standard autoclave cycles can take up to 6 minutes to complete. Ozone is being researched for this purpose. There is build-up of heat in the instruments and they can be removed totally dry and ready for use with the assurance that they are completely sterile. This is potentially a huge application for ozone use, not only in dentistry but also in any operative environment.

Dental unit water lines

These have been shown to be heavily contaminated with biofilm and high bacterial counts have been recorded in the water from dental units. This does not seem to have any serious effects in the general dental practice setting but may be more worrying where immunocompromised patients are concerned. Biofilm contamination plays havoc with dental units, often causing annoying blockages in couplings, hand pieces, and 3:1 syringes.

Initial research on the use of ozone, applied to water lines via the dental unit water supply, has shown greatly reduced numbers of bacteria present and also a significant reduction in the biofilm present (Al Shorman et

al, 2001). It is interesting to note that the HealOzone unit may be adapted to allow ozone to be applied to the water lines via the 'clean water system' water bottle. Significant savings may be made by the resulting reduction in blockages of hand pieces, couplings, etc.

Prevention of demineralization surrounding orthodontic brackets

Demineralization surrounding orthodontic brackets is a well-recognized problem following fixed appliance treatment in orthodontic cases. Accumulation of cariogenic microorganisms around brackets can lead to demineralization of the areas surrounding the brackets in caries-risk patients. If demineralization is allowed to take place, the optical properties of the tooth enamel will change and white or coloured carious lesions will appear. By regularly eliminating the microorganisms, and supporting this with regular oral hygiene instruction, these lesions may be avoided. Unfortunately, once the lesions have developed, remineralization will not return the optical properties of the affected enamel to its original state.

Ozone treatment has the capability of preventing this presence of cariogenic microorganisms and, when repeated at 8–14 week intervals GDPs and orthodontists have eliminated any demineralization occurring along with the subsequent problems related to cosmetic appearance and susceptibility to decay. When used in a preventive role such as this, ozone is applied to the treatment area for 10 seconds. Achieving a seal around many orthodontic brackets can be troublesome and again in this case the use of liquid rubber dam can prove invaluable. Applied in a circular fashion around the bracket it makes it relatively simple for the operator to seal and deliver the required ozone dose.

Patient compliance

The majority of HealOzone practitioners noted that patients tend to respond enthusiastically to the concept of ozone therapy and the DIAGNOdent has surprisingly proved to be extremely popular among patients of all ages. Patients are also keen to become involved in the DIAGNOdent readings. The audible signal produced by the instrument when it encounters a suspect area has proved a revelation in itself. It is remarkable how the patient responds to this signal and child and adult alike remember their DIAGNOdent reading. Children even remember the colour of the soft rubber cup that was used and often chirp, 'You used the blue one last time!'.

Improved oral hygiene is common after ozone treatment. The patients seem to 'switch on' to the concept and appear to be much more receptive to oral hygiene advice and are keen to participate in the use of the HealOzone oral health care products. The general experience in these practices is that previously nervous individuals who normally did not like sitting in the dental chair, literally hop in the chair to have ozone therapy. More smiles, more laughter, more enthusiasm all round.

In some pathfinder practices, other related research has shown that the patients all scored the highest marks for the procedure being comfortable and they all confirmed the experience was a positive one. Nervous patients stressed how they appreciated the treatment and that it gave them confidence about visiting the dentist. Everyone questioned said they felt motivated to modify their sugar intake and to improve their oral hygiene as a result of the ozone experience.

Another interesting finding is that when questioned further, the patients felt a degree of inevitability when faced with conventional fillings, even tending towards 'Why should I bother' in terms of improving their diet and plaque control because 'I'll have to have a filling anyway'. Yet when they received the ozone therapy they reported feeling more optimistic and positive about changing their habits and felt 'involved'. Certainly, it is the general experience that the use of the DIAGNOdent and the ozone, coupled with the HealOzone products does have a powerful impact on the patients caring for their own oral health.

Interestingly when this group of patients are offered the ozone pathway, the HealOzone practitioners have discovered a major change in their attitude toward oral health and these patients become excellent at keeping their appointments.

Added value

Over the past few years the dental profession has enjoyed many technological advances in equipment and materials. While the dental practitioner and his/her team may feel proud of their new 'toys', all too often the patients are not that impressed. In all these practices, the introduction of the ozone concept, coupled with

the DIAGNOdent, has had a major impact on patients and it has been quite remarkable.

The ozone concept has also had a significant impact on the dental team. The dental nurses and reception staff have been very enthusiastic as they can see the effect the new treatment has on the patients. This adds to the positive atmosphere of the practice towards prevention and disease control. It does much to boost the morale of the team to witness nervous patients in much dental distress evolving into relaxed and appreciative individuals.

Costs of traditional restorative care

Filling materials fail at alarming rates. Costs can be measured in terms of pain, discomfort, and in financial terms such as lost productivity. In England and Wales, restorations carried out in the NHS dentistry cost a total of £1.25 billion in 2001. This does not include private treatment, which is currently estimated to be 50% of dentists' income. The total costs of all dental treatment in England and Wales probably exceeded £ 3.26 billion in 2001 (General Dental Council, 2001).

Most of these fees are ascribable to fillings, root fillings, dentures, and crowns and bridges. Published reports suggest 50% of restorative items are replacements for previous restorations and about half of these restorations are being replaced due to secondary caries. If only 50% of all fillings could be avoided with the use of ozone, enormous sums of money could be saved. The cycle of drilling and filling may eventually lead to more complex restorative care requirements with increasing cost implications, such as the progression from a simple cavity to a multi-surface one, to the fracture of the crown requiring root canal treatment, followed by restoration with a crown and core.

In the USA, dental treatment is estimated to cost $ 52 billion per year, and half of this cost may be associated with restorative treatment and the cost of missed workdays and lost production due to oral disease. Despite advances in clinical and laboratory research, approximately 50% of the US population over the age of 65 years shows evidence of root caries. In all countries, from the advanced to poor and developing countries, there is a huge potential for a cost-effective way to prevent and reverse caries. A preventive and early intervention strategy is needed for the aging population and those with reduced manual dexterity. In this respect, the use of ozone should be also considered for medically compromised patients, domiciliary care patients and homebound elderly people. The equipment required is limited and essentially portable compared to that required for conventional drill'n'fill dentistry. Therefore elderly patients who have limited access to the dental services can benefit from this treatment. In many poor, developing, and highly populated countries, equipment, dental supplies, and dental services are inadequate due to high costs and lack of trained dental personnel.

Even if ozone therapies could save just 50% of all the fillings placed, the cost savings are huge. Clinical trial research using the HealOzone shows that over 90% of fillings can be avoided. In the modern world, where centralized welfare is being reduced to contain finances, the implications are enormous.

The tables below shows the potential effects of ozone treatment and the potential reduction of the number of fillings required in the UK. They also show the cost for dental restorative care in terms of fillings. The costs of provision of crowns, root canal treatments, extractions, and dentures have been omitted. The eventual cost saving are possibly even greater than illustrated here.

Conclusion

In conclusion, ozone therapy provides an excellent treatment modality with enormous benefits for dental patients of all ages. It is applicable to a wide range of conditions of the intraoral hard and soft tissues. The

Table 1

Year	Total Number	50%	60%	80%	90%
1998/99	16,67	8,33	10	13,33	15
1999/00	16,48	8,24	9,88	13,18	14,83
2000/01	15,98	7,99	9,58	12,78	14,38
Reduction in number of fillings over 3 yrs	9,13	24,56	29,46	39,29	44,21

Table 2

Year	Cost (£ millions)	50%	60%	80%	90%
1998/99	126	63	75,6	100,8	113,4
1999/00	127	63,5	76,2	101,6	114,3
2000/01	125	62,5	75	100	112,5
Saving over 3 yrs	189	226,8	302,4	340,2	

Table 3

Year	Cost (£ millions)	50%	60%	80%	90%
1998/99	9,3	4,65	5,76	7,68	8,37
1999/00	9,7	4,85	5,82	7,76	8,73
2000/01	10,1	5,05	6,06	8,08	9,09
Saving over 3 yrs		14,55	17,64	23,52	26,19

treatment of carious lesions is effective and is exceptionally popular with patients. This makes ozone treatment especially relevant to the younger patient, who may find conventional treatment unacceptable, and also for the elderly, who may have medical problems, which may complicate conventional dental treatment. The treatment is simple, completely safe to provide and often renders the need to introduce potentially toxic restorative materials unnecessary. It is not available under the NHS and is available as a private option.

Patients are delighted and it has created a 'buzz' in each area. Any innovation that can help halt dental disease and the fear of the dentist has to be welcomed. Our personal experiences have taught us that the ozone concept enhances our ability to communicate with patients who rapidly warm to the idea. It seems to stimulate their interest and the therapy is also a financial asset.

From a dental public health point of view, dental caries is a problem in large areas of the world and with shortage of clinicians in some parts of the UK and Europe, the ozone therapy has potentially a major part to play in the prevention and treatment of dental caries. The HealOzone machine and the DIAGNOdent are portable and it is possible to envisage units being used with great effect in every dental practice and community clinic. It takes such little time to treat several teeth that it may be possible to help many more patients compared with conventional treatment. Since it is simple to use, dental hygienists and therapists are ideally suited to providing the treatment for all categories of patient.

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CHAPTER 4.4

HealOzone – a Revolution in Dentistry

Carsten Stockleben

Experiences and thoughts of a practitioner who thought it was boring just to keep on drilling.

Background

Just before the first HealOzone Congress in Frankfurt on 11 May, 2003, I traveled through Sardinia on my motorbike. My presentation for this important event was already finished and saved on the computer. Still, while I was enjoying the winding roads and the wonderful landscapes in the brilliant sunshine, I often thought of the congress. I asked myself how my colleagues would receive this new, revolutionary technique. What questions would they have and what would they find difficult to understand? It was clear to me that the therapy with HealOzone represented a quantum leap in caries therapy and that getting used to this new technique would be a difficult process for the human psyche, which always tends to hold onto traditions and needs certainties. Would the other participants of the congress share my enthusiasm?

At that time, I had been familiar with the HealOzone project for two years. In March 2001, Prof. Edward Lynch approached me with his new project of caries therapy with ozone and asked me whether I was interested in joining him. The idea of a painless and substance-preserving caries therapy fascinated and enthused me immediately, as minimally invasive therapy in dentistry had been at the center of my work for a long time. So, a little later, I made the acquaintance of the leading figures in this project and also saw a prototype of the HealOzone device. I immediately imagined how easy it would be to heal caries by simply attaching the silicone cup on to the lesion and applying ozone.

Apply ozone – and everything will be fine! I imagined a bright future for dental medicine.

However, it took another year after that first encounter before the CE standards were fulfilled, and it was not until May 2002, that and a prototype finally arrived at our surgery. In the following months I learned that the application of HealOzone was completely different from what I had expected. Ozone therapy turned out to be far from as simple as I had imagined.

The therapy guidelines were still very vague at that time. There was no documentation, and only limited clinical experience with the new technique. At the congress, I wanted to share our surgery's experience with HealOzone with my colleagues, and with its market introduction in Spring 2003. I was aware that we already knew a lot more by then about therapy with ozone, although the development is still at an early stage. We are just beginning to discover the full spectrum of possible clinical applications.

While I was riding my motorbike I asked myself what the less innovatively inclined dentists would think of this new treatment method. Would they say:

> "Caries can only be treated with the drill!
> We have always done it like that.
> These newfangled things never work anyway.
> It takes much too long and is much too expensive!
> It is too dangerous, untested and unproven!
> I haven't got the right patients for this!"

There are a thousand reasons for resisting change.

Sometimes life itself provides the right answers and, while I was pondering those thoughts on a quiet country lane, an old farmer came towards me, riding his little donkey. There was the answer!

When Karl Benz and Gottlieb Daimler constructed the first horseless carriages about 100 years ago, nobody seriously believed that this idea of transport, so noisy, smelly and technically challenging, would ever prevail. Humankind had been travelling on foot or on horseback for dozens of generations. So, why change this good and proven way of getting from one place to another?. Yet, the new automobile technology triumphed above all initial skepticism and resistance and went on to conquer the world, and now, when I was approaching that farmer, I was riding a motorcycle with fuel injection and catalytic converter.

This was exactly the experience I predict for dentistry. Generations of dentists have treated caries with forceps, drills and fillings. But progress cannot be held back by reference to tradition, for the better is the enemy of the good.

Nothing is more continuous than change

For some years, we have been experiencing a paradigm change in dentistry, away from dental treatments strongly characterized by repair work, towards minimally invasive therapies combined with causal therapy. This transformation of values and attitudes requires an extreme change of outlook, in some instances, on the part of the dentists, dental associations, insurance companies and politicians. The central, almost philosophical question concerning this change is: "What value do we put on the integrity and long-term health of the human body?"' Or more specifically for dentistry: "What is the value of substance preservation, and substance care, with regard to the quality of life and the comfort of our patients as individuals, and the population as a whole?". What are the effects of our present therapeutic methods on the future cost-development in our health care systems? These questions may appear simple, yet the answers carry most important implications for the way we see, experience and practice dentistry. Considering the present development, we can only come to one conclusion. Future dentistry will be radically different from what we have practiced so far. Caries therapy with HealOzone is, therefore, a therapy of the future rather than one of the past. It is different from what we have been doing, not only in terms of its technical application. We also have to grasp it mentally if we want to understand its true benefits and potential.

Presently, we are at the beginning of a new development, a real breakthrough. We still cannot define the full spectrum of possible therapies enabled by this development. And of course, there still are many unanswered questions.

How our thinking was formed

To judge the psychological barriers in the understanding and realisation of new therapy options, it is helpful to have a closer look at the roots of our thinking as dentists. Historically, humans have tried treating diseased teeth and replacing lost ones since the times of the ancient Egyptians and Etruscans. Extraction was usually the only therapy.

As early as 2900 BC, there were Egyptian specialists in the treatment of teeth. Around 1300 BC, the Greek doctor Aesculapius discussed the removal of decayed teeth. At about 400 BC, Hippocrates was the first to describe a forceps for tooth extraction. This makes the forceps the oldest dental instrument, which continues to influence our view of dentistry. Hippocrates also recommended cleaning teeth with honey-soaked wool and subsequent mouth rinsing with a mixture of dill, anise, myrrh and white wine.

In the Roman period, wealthy Romans already attached great importance to oral hygiene. Tooth picks were used as a matter of course. Back in Greece, around 200 AD, Galen classified the dental anatomy and described the pulp as the sensitive part of the tooth. The Arabian physician Albucasis described instruments he had developed for removing tartar in the 11th century. The 17th century saw the first toothbrush, an expensive new instrument invented in England.

Until the late 19th century, dentistry in Europe was regarded as a sideline for blacksmiths, barbers and traveling showmen. The first recommendations to practice daily oral hygiene with dental floss and toothbrush were published around 1840. About the same time, the first molar extraction under anesthesia was performed, representing a major improvement and blessing for patients, who, up to then, had to suffer considerable pain.

The first hand-driven drills also appeared at that time, enabling the treatment of decayed lesions. The development of amalgam as a filling material, which is still with us, followed before the end of the 19th century. Further technological developments, most notably in the second half of the 20th century, have hastened the rate of innovation.

So, nearly 5,000 years of dental treatment characterized by extraction, repair and replacement had to leave their mark on our view of dentistry. The whole emphasis on dentist's education and training is on the search for holes in teeth as a symptom for caries at an advanced stage. He or she has learned to eliminate such damage aggressively. The standard procedure is to extract severely affected teeth and to replace them by elaborate bridges and other prosthetic products. In this way, the patient receives a replacement (prosthesis) that allows him to simulate the chewing function more or less adequately. Yet, the replacement never equals the quality of the original: the patient's own, intact teeth.

Therefore, the conclusion must be that classic, reparative dentistry amounts to the amputation of diseased tissue. The patient suffering from caries or periodontal disease partially or completely loses the function of his mouth. He becomes an invalid, a "mouth cripple". This experience, and the history of dentistry explain the fear of dental treatment still suffered among today's population. In spite of considerable improvements and progress in the area of preservative dentistry, a significant number of our patients are still afraid of visiting a dentist.

"For the modern human being, the visit to the dentist is what a visit of a sabre-toothed tiger was for prehistoric man in his cave."

From extraction to prophylaxis

In the second half of the 20th century, the dental profession began to slowly turn away from extraction. Replacement and conservation of tooth substance became more and more important. Since World War II, and especially since the 1960s, we made important progress in understanding the etiology of caries and periodontitis. In the 1990s, new findings in biofilm research and a further refinement of dental treatments (dentin adhesives, composites, laser) led to a new way of thinking. Gentle, minimally invasive therapy accompanied by tackling the causes through a risk-oriented prophylaxis should be common practice now.

The long-term effects of prophylaxis are not limited to the clear benefits in terms of health improvement and quality of life due to reduction of caries and periodontitis among the patients in our care. They also manifest themselves in a changed appearance of caries. Instead of finding large, open lesions with massive destruction, as was the case in the past, we see a larger share of cases of hidden caries. Such caries is usually strongly localized and more difficult to diagnose, because in most cases the enamel surface is still intact. Furthermore, significantly more teeth are preserved into old age. On the one hand, this situation is welcomed by the patients concerned. On the other, however, it resulted in a development that used to be encountered much less frequently in dental practice: root caries in older patients.

Foreseeable changes

The paradigm change from classic reparative dentistry to minimally invasive dental medicine oriented towards prevention has wider effects for health care systems. Patients are growing older with their own dentition, which means there will be a higher demand for dental treatment, notwithstanding prophylaxis and further improvements of dental therapies. This in turn leads to a further rise of costs for the national health care systems. At the same time, patients' demands concerning the quality of treatment and aesthetic aspects are rising. Patients also increasingly demand more comfort with the treatment options chosen, and as painless a treatment as possible.

The consequences for dental treatment are foreseeable. Prevention and minimally invasive therapy are becoming more important. An accelerated technological development and more differentiated treatment options are accommodating this trend. Our growing knowledge of the etiology of caries and periodontal disease and their physiological implications are transforming the understanding of new treatment methods, new treatment demands and innovative techniques. For the practicing dentist this means, first of all, confusion and the loss of certainties. What has been for a long time the prevailing view, and therefore good and appropriate, for instance "extension for prevention", is suddenly a pro-

fessional blunder. The new maxim is now "prevention of extension".

This creates an understandable insecurity among dentists. To meet the challenges of the increasing rate of innovation and change, dentists must not only put more and more effort into updating their knowledge, but are also faced with rising investment requirements for medical technology. Despite the enormous progress in science and research and the resulting innovative treatment options, there is an unmistakable trend:

Dental treatment only seems to become easier.

In fact, it becomes more and more complex and intricate.

"You only see what you know" (Goethe)

Because of the tradition outlined above, dental treatment is still characterized by reparative, symptom-oriented and surgical treatment systems. Yesterday's rules still determine today's actions. Consequently, dental treatment is being started too late and valuable time is lost. This attitude characterizes the scales of fees in most western countries, and also the training of young dentists. The emphasis remains on classic, restorative therapies, which are often accompanied by considerable destruction of healthy tissue. In caries diagnostics, the predominant symptom, i.e. the massive destruction of hard tooth tissue found in the latest stages of the disease, is still referred to, incorrectly, as "caries". This view and way of treatment completely ignores the etiology and pathogenesis of caries. For instance, this therapeutic approach does not include any accompanying causal therapy, which would most likely prevent a later relapse. Consequently, the patient treated in this way will develop recurrent caries.

Thus, caries diagnostics appears to be medieval. The dentist looks for open caries lesions, visually and with his sharp dental explorer, and takes radiographic images. Within this system, the patient visits the dental surgery once a year for check up by the dentist (looking for holes). Dental hygiene at home is regarded as causal therapy. Obviously, early stages of caries are not diagnosed in this way, and relapses are not prevented either. For the tooth, the diagnosis thus marks the beginning of a fateful journey, via the following stations:

- Secondary caries
- larger fillings
- caries profunda
- crown
- root filling
- core and post/crown
- root esection
- extraction
- drilling the adjacent teeth for a bridge, further destruction of hard tooth substance

These procedures are expensive, accompanied by a lot of effort and pain, and have their price to pay in terms of the patient's quality of life. To break this vicious circle, and to understand the advantages of HealOzone and minimally invasive dentistry, we need to "see" caries differently.

Seeing caries differently

For a better caries management in the future, a brief look into cariology will help us to find the answers to two crucial questions:

1. What is caries?
2. How do we define initial caries and secondary caries?

Caries is often described as an infection caused by bacteria adherent to teeth, often with a multifactorial genesis. The multifactorial model of explanation is very complex, but it can usually be reduced to three factors:

- Host
- Microflora
- Nutrition

When treating a patient, neither the infection model nor the multifactorial model help the dentist to determine the causes of the existing caries, or prevent recurrent caries. Simple pragmatic thinking reveals that to prevent further tooth decay, removing the host, (extracting the tooth), might be useful. But, obviously, this misses the point. Equally unrealistic would be to remove the bacteria, since they are not intruders, but part of the natural mouth flora, which cannot be removed without wider consequences.

Finally, we could remove the nutrition factor and stop eating, which however is the basis for our survival. Doing without food often has the welcome side effect of losing weight, but it would not prevent the growth of the mouth flora, since the latter mainly feeds on constituents of the saliva.

Thus we have to conclude that tooth surfaces, bacteria, saliva, nutrition and all the other things that are usually found in the mouth cannot be regarded as causing caries, since they form part of the basis for life as such. Still, they play their role in the occurrence of the illness called caries. Caries is a local disease starting from local bacterial activity. The bacteria involved are not aliens in the mouth; they are also found in other regions of the body, e.g. in the intestines.

How do natural bacteria of the mouth become cariogenic?

To answer this question, we have to think of the following. The mouth flora live in a continuous fight for survival in a very hostile environment. The mouth is regularly exposed to extreme conditions such as temperature and changing pH, changes in the flow properties and viscosity of the saliva, and the varying chemical composition of food taken up. The strongest attacks come from oral, mechanical forces. For instance during eating or brushing the teeth, when large numbers of microorganisms are ripped from on the surfaces of the oral structures and removed from the mouth by swallowing. The main attachment surface of saliva bacteria is the oral soft tissue, which releases mucosa cells continuously. From the perspective of the bacteria, the hard, stable surface of the teeth therefore is very attractive for helping them to survive.

When looked at more closely, bacteria do not grow with the same intensity on all tooth surfaces. The tooth cusps and incisal-edges, for instance, are colonized little or not at all, while the enamel along the gingival-margin can hold a large bacterial population. The reason is that the areas without a visible bacterial population are subject to regular mechanical wear during chewing. Therefore, bacteria settle and grow better in areas where they are protected from intraoral mechanical disturbance. These are also the areas where, in general, caries occurs. Consequently, mechanical disturbance or strain is an important intraoral factor for bacteria accumulation and growth with a cariogenic potential. Variations in the intraoral mechanical forces, i.e. protection from, or stronger exposure to mechanical abrasion, not only determine the microbial colonisation of the tooth surface. They also affect the environment and development of microbial ecosystems with a cariogenic potential.

When the dental biofilm can develop free of mechanical interference, the conditions for the micro-organisms living there change slowly. The shortage of oxygen gradually favors anaerobic organisms over aerobic ones, whose survival depends on adequate supply of oxygen. In the absence of oxygen, glucose is broken up into the end products of anaerobic glycolysis: lactic acid and alcohol. The energy produced by the anaerobic organisms dominating the bacterial biofilm goes into the formation of acid, which dissolves the enamel under the biofilm. As a first sign, the cariogenic activity of the bacterial plaque causes demineralisation of the underlying enamel. While extraoral glucose from food provides additional supplies for the biofilm, saliva is the main source of energy for the bacteria. This mechanism is supported by the varied flow velocity of the saliva. It is lower in these naturally protected areas and, thus, is slower to remove the acids produced here than in places with a high flow rate. If no measures are taken to remove this cariogenic biofilm, this cycle goes on automatically and slowly leads to visible changes or destruction, which is only noticed years after onset.

What is initial caries?

The question already hints that we consciously notice the symptoms of the disease without paying attention

to the disease as such. When trying to decide if a patient has caries, we actually think of the presence of visible signs of caries, a lesion or a cavity, without considering if there is a cariogenic biofilm or otherwise. The detection of the cariogenic biofilm is a central element of the diagnosis. It is important for the health of each individual patient. Yet, training and research focus almost exclusively on the identification of the signs and symptoms of the disease, with increasing emphasis on the differentiation of the advanced degrees of destruction and on a refined therapy. Therapy means all the professional measures to control or delay the disease.

As dentists, we are fully familiar with the health effects of an advanced destruction of hard tooth substance by caries. Of course, we also know the appropriate treatment methods which we call surgical dentistry. At the same time our clinical experience teaches us that surgical procedures are neither the beginning nor the end of caries therapy. This experience coincides with the biological nature of caries, which is why we should define such measures that arrest or heal the caries without any recourse to surgical or restorative procedures as non-surgical treatment. Thus, non-surgical dentistry is the logical extension of restorative therapy, in order to prevent recurrent caries.

Therefore, early diagnosis of caries is much more important than the search for cavities. The aim is to apply non-surgical methods of treatment as soon as cariogenic or potentially cariogenic biofilms are detected in areas where the protection from oral mechanical forces has allowed the development of anaerobic bacteria.

First signs of caries

The first, invisible sign of acid producing and acid-tolerant bacteria is the micro-dissolution of the enamel surface under the cover of the biofilm. The first enamel dissolution leads to a better physical attachment between the cariogenic biofilm and the underlying enamel surface. In this way, a potential problem spot begins to develop and requires professional intervention. However, if this cariogenic problem spot is allowed to develop undisturbed, this leads to the progressive dissolution of the enamel under the biofilm, to bacterial invasion and the formation of a subsurface lesion, a so-called white spot. The damage is located between the enamel dissolution of the surface and the first, localized micro-activities. Because of the surface dissolution, the damaged area appears white, since it reflects light diffusely while porosities under the surface cause a change in the enamel translucency.

Arrest of the initial lesion

The development of the initial lesion is connected with a local reduction in mechanical forces. Consequently, renewed mechanical abrasion in this area removes the cariogenic biofilm. The enamel demineralisation is thus arrested and any further progress of the caries is prevented. The combination of professional tooth cleaning and regular home-care lead to the ablation of the partly dissolved enamel surface and keep it polished. This reduces the white spot effect, and the surface shine and hardness are restored. This process is often confused with remineralisation. The arrest of the enamel lesion only occurs *in vivo* if the acid forming biofilm has been removed, and it is only maintained if the settling and development of a new cariogenic biofilm is prevented in the long term.

What is secondary caries?

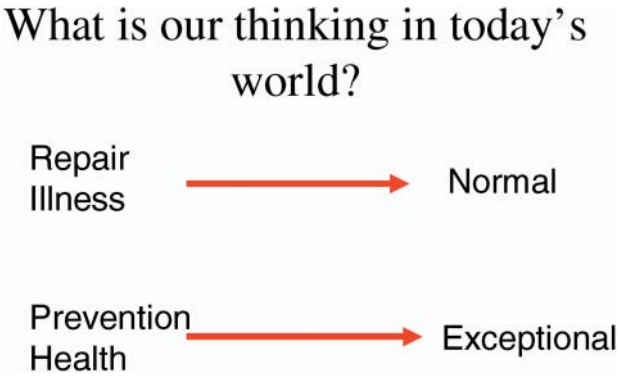
Research has shown that visible open margins, margin-deficiencies and microleakage are not directly responsible for secondary caries. Secondary caries is rather a local phenomenon caused by conditions contributing to the development of a cariogenic biofilm. This means specifically that a filling was made because the patient previously did not succeed to control his cariogenic biofilm. This led to the development of caries at that location. If the patient does not achieve biofilm control there in the future, secondary caries will develop. Hence, this is not a general attack along the entire margin but rather a new lesion that has its origin at the tooth surface next to the margin of the restoration.

Recurrent caries	1	IMG 0119	Recurrent cervical and root caries on a right lower canine of a 55.year old patient. This picture nicely demonstrates the patient's inability to mechanically remove the dental biofilm in this spot. The first approach in conservative, but symptomatic treatment, a composite filling, does not solve the problem and is failing because the biofilm is still around. So is demineralisation and consequently secondary caries, starting close to the margins of the filling. With progressive periodontitis and time, gingival recession opens the door for root caries. Note the layer of biofilm on the surface. A perfect situation for HealOzone treatment in conjunction with professional preventive care to stabilize the situation in the long run.

Prophylaxis is also prevention of recurrent caries

Now it is clear that new, ever larger restorations are not the be-all and end-all of caries therapy. Even the insertion of a crown does not prevent recurrent caries. The exclusive focus on repair in dentistry while ignoring the etiology and pathogenesis of caries does not result in the intended stability of the patients' health, but leads to more repair work again. In the long run it is irrelevant what form of restorative therapy is chosen. There is no long-term stability without professional and continuous biofilm management, which the dental team carries out together with the patient. Therefore, the integration of the patient in a professional prophylaxis program including a recall system is imperative. Recurrent caries can only be prevented in the long run if the patient gets to know his problem spots and also if he is enabled to apply "mechanical forces" regularly to those areas too. Only in this way his health can be restored and maintained. After having achieved this level of care, this is the basic justification for applying minimally invasive treatment methods.

"Dental treatment without prophylaxis is malpractice" (Prof. Per Axelsson)



Minimally invasive dentistry

The term "minimally invasive" has a positive connotation in today's medicine. However, in dentistry it must not be reduced to the preparation of tiny cavities. The underlying motivation behind this comprehensive medical concept is to minimize any injury to the patient and his tissue as far as possible. Dental treatments should be performed in a way appropriate for the indication, the existing damage and the risks involved. The spectrum ranges from preventive measures to reduce the risk of caries, through minimally invasive forms of preparation in cases of a manifest caries, to the struggle for minor gaps with adhesive prosthetics. The aim of minimally invasive treatments is to prevent further damage to the hard tooth substance, the pulp, the periodontal tissue and tooth function. On the other hand, it meets the aesthetic expectations of the patient and minimizes the scale of the treatment and thus the strain on the patient. Compared to conventional, restorative dental treatment, minimally invasive dentistry demands very high competence. Applying minimally invasive treatment techniques requires rethinking, challenging and changing our own therapeutic emphasis. Quite often, such treatments involve demanding and technology-sensitive methods, which also entail the use of sophisticated technical equipment. The emphasis is on more comprehensive and well documented diagnostics for the early detection of damage to the hard tissue and the pulp. Instead of waiting for the development of large defects, as it still is common practice, the dentist deals with problem spots locally and at an early stage. Minimally invasive dentistry involves both surgical and non-surgical techniques. Parallel to restora-

tive treatment, the cause for the disease is treated in a preventive manner, in order to avoid future relapses. We differentiate between the following therapeutic approaches:

non-invasive
- Fluorides
- Plaque-inhibiting agents (e.g. CHX, Triclosan)
- Ozone (antibacterial therapy)
- Prophylactic fissure sealing

selectively invasive
- Extended fissure sealing
- Chemical removal (Carisolv)
- Selective "drilling" (fissurotomy burs, SmartPrep (SS White Burs), laser, air abrasion)

minimally invasive
- Micro-fillings (e.g. initial lesion)
- Repairs (crack formation, fractures etc.)

The advances in the area of restorative, minimally invasive treatment methods are based on the use of adhesive techniques. Contrary to the conventional opinion that large defects are more difficult to treat, the technical demands and difficulties actually increase when the defects to be restored become smaller. The following guidelines should be obeyed:

- Sufficient access for the safe removal of infected entin (this guideline is less strict for the use of HealOzone).
- Sufficient access for the instruments and filling materials to be used (the adhesive must cover all surfaces, and the composite must be applied without air enclosures).
- Reduction of damage to neighbouring teeth (the smaller the cavity, the higher the risk).
- Use of oscillating preparation systems.
- Sufficient access for a technically adequate provision.
- Use of the adhesive technique.

The goals of minimally invasive dentistry can be summarized as follows:

- Maximum tissue preservation.
- Minimum traumatisation of tissues and structures.
- Improvement of the prognosis for tooth and reconstruction.
- Controlling the caries risk.
- Remineralisation of initial lesions.
- Active observation and accompanying of the patient (monitoring).
- Integration of the patient in an active care and treatment concept.
- Reduction of fear.
- Avoiding or delaying of later, major secondary damage and costs.
- Repair instead of replacement.

Minimally invasive versus aggressive

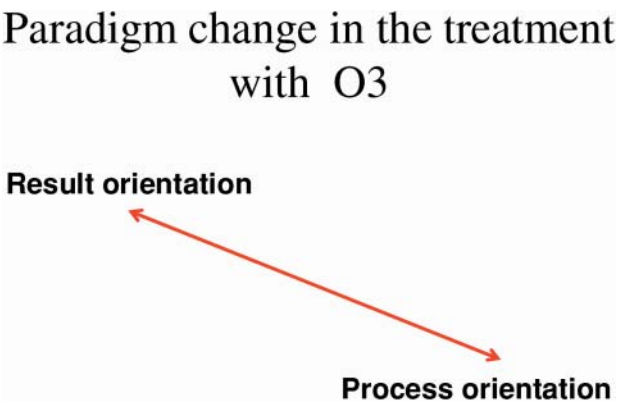
Classic-restorative and prosthetic dentistry comes into play only when the disease and destruction of the tissue is at a very advanced stage. Ultimately, it is a sign of failure or omission of minimally invasive and preventive dentistry. Through their active conduct, both patient and dentist are responsible for any success. The latter offers and carries out minimally invasive dentistry and a professional maintenance program; the former is supposed to support the treatment with his compliance (in many countries, this is a legal matter embedded in the treatment-contract!).

Under these aspects, providing a tooth with a single crown is contraindicated in many cases and can be regarded as inflicting bodily harm. As an expression of aggressive, restorative dentistry, it should be seen as a phase-out model, and soon be a thing of the past. In many cases, even a severely damaged tooth can be treated in a preservative, defect-oriented way.

Also, the annual superficial clinical check-up by the dentist and the unsystematic tooth care at home, should be supplanted by professional diagnostics and integration of the patient in a risk-oriented prophylaxis program. Further information on this forward-looking subject is provided by the European Academy for Minimally-Invasive Dentistry, at *www.acamid.org*

Looking at modern dentistry from this perspective, the application of HealOzone firstly is an important new asset for minimally invasive dentistry. Secondly, it is also of interest for the classic-restorative therapeutic approach. HealOzone is just the beginning of an interesting and exciting development. The full range of applications are not yet defined. To fully exploit the potential of HealOzone, the we should part, at least mentally, with the concept of classic-restorative and

aggressive dentistry and follow the path of a preservative, minimally invasive and preventive concept of dentistry.



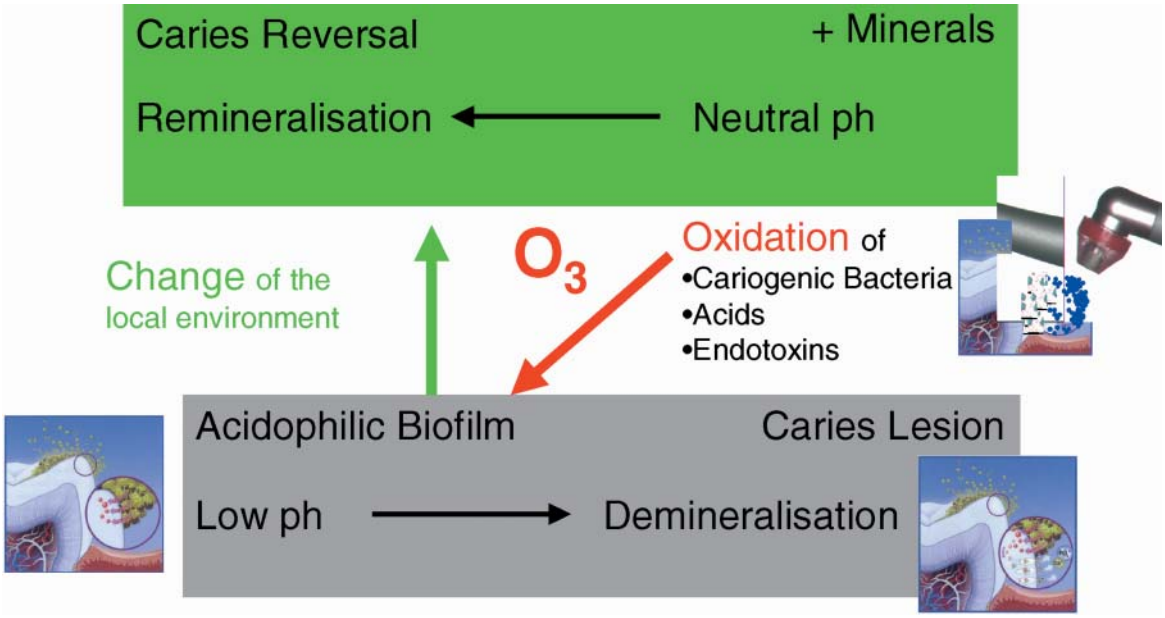
Practical application of HealOzone in the dental surgery

I would like to report on our application of HealOzone and on the experiences we gathered as early pilot-users of this technique in Germany.

First of all, HealOzone is totally different from what we have been doing in dentistry for the last 150 years. It involves the understanding of caries etiology and especially its physical and chemical basis. It also does not show immediate results, as we are used to from drilling and filling which is result-oriented. Using HealOzone is process-oriented dentistry, you have to wait for physics and chemistry to do their work, but then it really works! But just these "simple" things are so difficult to understand for the dental community, that is my impression from years of international lecturing on HealOzone and minimally invasive dentistry. Actually, it is very simple: Diagnose correctly, treat right, wait and see dramatic changes.

The following chart shows how remineralisation works after sterilisation of a lesion with ozone:

Ozone & Remineralisation



The 4 Phases of Therapy

1. Differentiated diagnosis, risk assessment and documentation.
2. Sterilization of the caries with HealOzone.
3. Remineralization.
4. Final sealing or filling of the enamel defect, if necessary.

Hidden caries

Using HealOzone for refining minimally invasive treatment techniques appears to be an obvious idea. When we started working with HealOzone, it was obviously tempting for us and our patients to realise a caries treatment that was painless and did not involve drilling. Thus, within only a few months, we treated about 500 teeth in 180 patients diagnosed with hidden caries. The treatment was carried out in the following sequence:

1. Cleaning with Prophyflex.
2. DIAGNOdent (KaVo) measurement.
3. Documentation.
4. Photographic documentation.
5. Ozone application: DIAGNOdent (KaVo) level between 20–40: 40 secondsDIAGNOdent (KaVo) level above 40: 40–60 seconds.
6. Application of ph-balancer (Reductant), Cervitec (Ivoclar/Vivadent), Fluor Protector(Ivoclar/Vivadent).
7. Patient instruction and handing out the patient kit.
8. Recall after 8 to 12 weeks in the course of routine prophylaxis.

At the recall of these patients we found ca. 50% of them showed an improvement of DIAGNOdent (KaVo) levels, 20% showed unchanged levels and about 30% were measured had worse DIAGNOdent (KaVo) levels. This, of course, dampened our euphoria about a new, revolutionary treatment approach. Consequently, we discussed why our results were in such stark contrast to those of other pilot-users especially in England, where success rates of between 80 and nearly 100 percent had been reported. We had to examine the individual components of our treatment system.

Patient compliance

One precondition for the ozone treatment was the integration of patients in the prophylaxis program. The patients taking part in such programs are usually highly motivated and exercise good mouth hygiene at home. Hence, this could not be the reason.

Standardisation of DIAGNOdent (KaVo) measurements

Each DIAGNOdent (KaVo) user goes through a learning curve in order to obtain secure and reliable data. Although we had this diagnostic system in our surgery for a considerable time, only the introduction of the HealOzone technique led to its regular, systematic application. The equipment was regularly calibrated according to the manufacturers' instructions. Since we are dealing with very delicate and, hence, vulnerable devices, they should be treated with utmost care. The diagnostics tip and its fixing in particular, are very sensitive. Nevertheless, a stronger focus on how we handled the DIAGNOdent (KaVo) equipment did not lead to the desired improvement, either.

After thorough discussions in the German pilot user group and with KaVo we finally came to the conclusion that DIAGNOdent (KaVo) should not be used as first choice for clinical re-evaluation because the instrument does not seem to suited for measuring remineralised dentin. This is being explained by DIAGNOdent (KaVo)s physics and the fact that during remineralisation, coloured particles get embedded in the dentin leading to false negative readings. This is very important to remember when you are using DIAGNOdent (KaVo)!

Application of the ozone

Could it be possible that, in the treatment of hidden caries, the ozone did not penetrate deeply enough through the enamel that covers the caries? Or, if the ozone was reactive enough to sterilize the caries completely, can calcium and phosphate ions penetrate through the enamel-cover to the caries in sufficient amounts to ensure remineralisation? With a heavy heart, we parted with the idea that we would be able to treat hidden caries without drilling, without opening the enamel surface.

On our search for a truly painless and minimally invasive way of opening the fissures of hidden caries, we came across the Fissurotomy burrs from SS White Burs. These steel burs, which were developed especially for diagnosing fissure caries, are characterized by their minute size and well-defined penetration depth. Subsequently, 140 lesions in 45 patients were treated accord-

ing to the modified treatment concept described below. This is how pilot-users sometimes have to learn the hard way. However, when recalling these patients, we were rewarded with good results. Even after a short recall interval, in some cases only 4–6 weeks, we found a significant improvement of the DIAGNOdent (KaVo) levels. The success rate, clinically measured, rose to more than 90%, firstly in terms of the hardness of the surface and its shine in dry condition (the surface very much looks like sclerotic dentin), and secondly – only as an auxiliary measurement – the DIAGNOdent (KaVo) levels measured subsequently. As soon as the clinical results are stabilized, the enamel lesion is permanently closed with an adhesive microfilling. In terms of the results, this is a safe and predictable approach for the treatment of hidden caries. However, it also leads to the conclusion that the preservation of our natural hard tooth substance requires a markedly more intricate procedure. A dentist who wants to follow a very gentle, non-invasive approach of treatment should first try applying HealOzone without having opened the fissures. Then he can decide, at the first recall, whether the lesion under treatment responds well, or otherwise. If the diagnostic results improve, this path is followed further; if a stagnating or even worsening situation is found, fissures can be opened with fissurotomy or, alternatively, air abrasion, and the treatment can be continued in this way.

Healing in progress	2	IMG 6648	Tooth 48 prior to HealOzone treatment. Caries: CSI 5, DIAGNOdent (KaVo) 99. Treatment: 60 Sec. Ozone, Patient-Kit, OHI, PCP.
	3	IMG 8300	After 4 month. DIAGNOdent (KaVo) 32, hard and shiny surface. Treatment: HealOzone 20 Sec. for sterilisation followed by an adhesive micro-filling.

Clinical study

After the above experiences, we finally started a clinical study in February, 2003. The aim was the clinical evaluation of the degree of caries reversal under controlled conditions.

Duration: 5 months
- Patient pool: 178
- Hidden caries: 157
- Root caries: 21

Patient selection: All patients are integrated in a preventive care progamme.
Number of teeth treated: 554
Recall and evaluation after: 8–12 weeks

Cases of hidden caries: 508
Treatment protocol:

- DIAGNOdent (KaVo) readings 15–40 were treated with 40 sec. of HealOzone.
- DIAGNOdent (KaVo) readings >40 were treated with 60 sec. of HealOzone.
- Hidden caries with DIAGNOdent (KaVo) readings >30 was opened with Fissurotomy and stayed open for the treatment period and finished with an adhesive micro-filling after 20 sec. of HealOzone for sterilisation.

Evaluation

Clinical evaluation after 8–12 weeks of the cleaned and dried surface optically with loops and physically with an explorer. To be evaluated "reversed", the surface had to be hard and shiny. DIAGNOdent (KaVo) was not used for clinical re-evaluation because the instrument does not seem to be suited for measuring remineralised dentin.

Cases of root caries: 46
Treatment protocol:
- DIAGNOdent (KaVo) readings 15–40 were treated with 40 sec. of HealOzone.
- DIAGNOdent (KaVo) readings >40 were treated with 60 sec. of HealOzone.
- Soft caries with destroyed collagen-fibres was removed until the consistency became leathery.
- Root caries with a surface defect stayed open for the treatment period and finished with an adhesive-filling after 20 sec. of HealOzone for sterilisation.

Evaluation

Clinical evaluation after 8–12 weeks of the cleaned and dried surface optically with loops and physically with an explorer. To be evaluated "reversed", the surface had to be hard and shiny. DIAGNOdent (KaVo) was not used for clinical re-evaluation because the instrument does not seem to be suited for measuring remineralised dentin.

	Total	Reversal	%	No Reversal	%
Hidden caries	508	475	93.5%	33	6.5%
Root caries	46	40	87%	6	13%

Conclusion

HealOzone seems to produce predictable results in reversing dentin caries in the dental practice. The cases that did not reverse well were correlated to deep and large lesions, where excavation of the soft caries did not seem to be fully performed, and also to poor compliance of the patient during the clinical trial period.

Root caries

The populations of western industrial nations are growing ever older and, as a result of improved dental care, they grow old with their own natural dentition. At the same time, the age pyramid is turned upside down by the present demographic development. In 2030, more than 50% of the population in these countries will be of retirement age. Being able to grow old with your own teeth represents an enormous advance, also with regard to quality of life, yet there is a dark side to it too. With increasing age, more and more root surfaces become exposed to the oral environment. The main reasons for this are periodontitis, lacking professional care in prophylaxis programs and tooth-brushing traumata. Due to their surface structure and because they are often insufficiently cared for, these exposed root surfaces are particularly susceptible to caries. Root caries is one of the main reasons for tooth loss among adults. Root caries develops in the same way as enamel caries, although the demineralisation already starts at a lower acidity (higher pH!). With increasing demineralisation, the collagen in the root surface becomes exposed and broken up by plaque-forming micro-organisms. An active root caries can be inactivated by remineralisation, which leads to the growth of a new hard surface. Killing off micro-organisms in caries lesions results in the prevention of acid and collagenase production.

Conventional filling therapy is only of limited use in the treatment of root caries. And the adhesive technique is not ideal, either, because of:

- poor access
- direct proximity to periodontal structures
- moderate to low shear-strength values because of missing enamel
- the small number of dentin tubuli per square millimetre

The application of HealOzone offers many advantages in the treatment of root caries. Due to the preservative nature of the procedure and the remineralisation of the caries lesion, the long-term prognosis for the tooth is markedly better than for an excavation-and-filling therapy. After a successful remineralisation of the root caries, the surface is very hard and full of minerals and collagen, and it does not show any superficial dentin tubuli. This means that the surface will show less of a tendency to hypersensitivity and is more resistant to recurrent caries in the future. Due to the remaining discoloration, the optical impression of the treated carious lesion is not changed by the therapy but the surface is hard. This is always a reliable sign of a stalled or healing root caries.

In most cases, the access to a root caries from the palatinal or vestibular is easy, making the application of HealOzone an uncomplicated procedure. However, if a root caries extends further towards the approximal, getting a vacuum by means of a silicone cup can present difficulties. A quick and easy remedy for this problem is the attachment of silicone sealing on the opposite side of the approximal cavity and, if necessary, on the occlusal surface. This "silicone-stent" can be retained for possible successive treatments. The reapplication will then be very easy. For such cases, it is recommended that the size or color of silicone cup, is recorded in the patient's file.

Primary root caries lesion on tooth 27	4	Figure 5 PRCL 27	57-year old female patient with a PRCL on the palatinal surface of tooth 27. DIAGNOdent-reading 56, soft to leathery surface. HealOzone application for 60 sec. The patient was very anxious and did not participate in the prophylaxis-program.
	5	Figure 6 PRCL 27 after 2 years	Next check up 2 years later! Even without professional care the surface was hard and well remineralised. DIAGNOdent-reading 58, most likely due to embedded chromophores.

Pushing the limits

Obviously, apart from the classic HealOzone application for remineralizing the caries which is covered by the CE license, users would also like to benefit from the bactericidal, virucidal and fungicidal effects of ozone in other therapy areas.

Poor margins and root caries	6	IMG 7066	Root caries below a poorly-fitting, but old crown, which the patient desperately wanted to keep. Situation 8 weeks after HealOzone treatment of 60 Sec. The surface is hard and shiny, the caries remineralised.
	7	IMG 7071	Adhesive closure of the open margin and root surface for easier cleaning.

Caries profunda

In our experience, HealOzone can also be used as a very beneficial addition in the classic filling therapy. Following the conventional excavation of the caries with a steel-burr, the application of HealOzone results in the complete sterilisation of the dentin surface. Residual caries, which cannot be diagnosed visually although it is detected in up to 80% of conventional lesions after excavation, is safely eliminated by ozone. The bacterial toxins and acids are broken up at the same time which leads to an improved prognosis for keeping the pulp healthy in the long run. Compared to applying the caries detector for exposing and removing residual caries, the application of HealOzone carries significantly lower risks. It is also gentler and allows a better prognosis. In regions close to the pulp, the application of the caries detector can produce false-positive results, increasing the risk of an iatrogenic pulp exposure.

The teeth that we treated with HealOzone very rarely showed a tendency towards postoperative hypersensitivity or pulpal complaints. Even teeth that had shown signs of mild complaint, e.g. hot/cold-sensitivity or a typical lingering pain prior to the treatment, became spontaneously and permanently pain-free after the additional application of HealOzone. In our opinion, the comfort benefits for the patient and the additional certainty for the dentist justify the additional effort.

In cases of typical pulpal complaints, the treatment was not successful in the long term. After a period of relief, lasting for some days or, in rare cases weeks, these teeth were treated endodontically.

Treating deep carious lesions with HealOzone and using the benefits of remineralisation at the same time has great advantages:

- Preservance of tooth substance.
- Avoidance of pain and hypersensitivity.
- Patient comfort.
- Good chances for long term pulpal health.
- Avoiding traumatic and expensive treatment.
- Lowering the long term risk for RCT, core & posts, crowns or even extractions.

There are 2 treatment options in deep caries:

Open remineralisation

- Open carious lesion for access and visibility.
- Remove soft caries with destroyed collagen until the consistency becomes leathery.
- Apply HealOzone for 40–60 sec., or longer in large and deep lesions.
- Apply Reductant, in large lesion cotton pellet soaked with Reductant.
- Recall and re-apply Reductant.
- Apply filling when the surface is hard and shiny.

This is a great treatment especially in those cases, where the caries is close to the pulp and further excavation

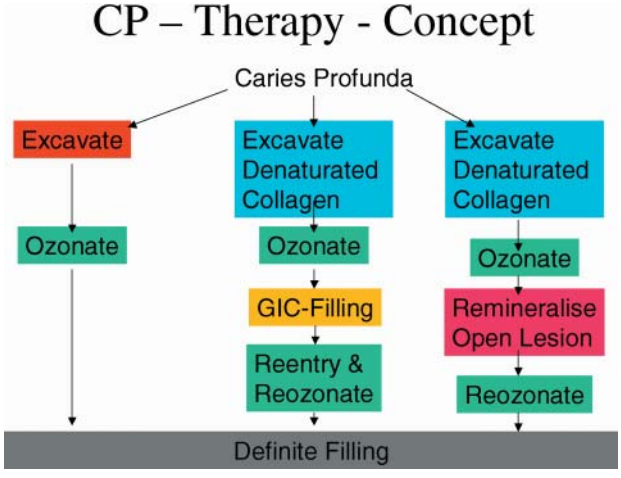
would probably risk pulp exposure and RCT. The benefit is a thicker "security-layer" of dentin above the pulp with a good chance of long term pulpal health.

These teeth do not show senitivity while being used during remineralisation.

Closed remineralisation

- Open carious lesion for access and visibility.
- Remove soft caries with destroyed collagen until the consistency becomes leathery.
- Apply HealOzone for 40–60 sec., or longer in large lesions.
- Apply pH-balancer (Reductant).
- Apply GIC-Filling (remineralisation will take approx. 12 weeks).
- Apply proper filling.

This also is a great treatment especially in those cases, where the caries is close to the pulp and further excavation would probably risk pulp exposure and RCT. The benefit again is a thicker "security-layer" of dentin above the pulp with a good chance of long term pulpal health. It is a good option if you are afraid of sending your patient home with an open tooth (remember: it was open when he came! – and don't worry, it won't be sensitive!), or if you do not see your patient again for a long period of time, or if just want to save chair-time and a second appointment.



Approximal caries

Treating approximal caries lesions is a bigger challenge since that type of caries is much more difficult to detect in its early stages and also more difficult to access and to control. Bite-wing x-rays are imperative but destruction by caries must be over 25% of the hard tissue before the human eye is able to see the lesion. DIAGNOdent is not well suited for detection because it will only measure up to 2mm in depth and interpreting the reading is difficult in those special cases.

Nevertheless, HealOzone is an interesting treatment option which can be performed in 2 ways:

1. Silicone-mould technique: Form a reusable silicon mould around the tooth to get a reliable vacuum for HealOzone application. This works well with an open approximal surface.
2. Trepanation on the occlusal surface: This is appropriate if the x-ray shows an intact enamel surface on the approximal. The opening should be big enough for tactile and visual control. After remineralisation the opening is closed adhesively.

Treatment of approximal caries	8	IMG 7668	Mesial caries on a right lower molar. The old filling on the adjacent tooth was removed for better and minimally-invasive access.
	9	IMG 7683	The mesial wall was opened gently for better access of HealOzone and minerals.
	10	IMG 7688	Silicone mould secures the easy application of HealOzone and a quick vacuum.
	11	IMG 7693	The mould is reusable for following treatments of this lesion.

Endodontics

Being able to sterilise the root canal system completely would significantly improve the prognosis for all teeth to be treated endodontically. The option of a reliable sterilisation would also have an effect on the future of root canal treatments. Presently, conventional endodontic methods cannot achieve this objective. In spite of

the considerable efforts involved in such methods, partial germ removal is the best that can be achieved by them.

The application of HealOzone makes the sterilisation of the root canal systems a real possibility in the near future. Given the present technical possibilities offered by the HealOzone equipment and the existing cups, a tooth that is to be treated endodontically needs to be built up so that the clinical crown is restored. The ozone is applied from occlusal, through the trepanation opening, making the technical realisation quite a simple process.

Since there are only few reports on this experimental application available to date, it is advisable to extend the application time to ca. 120 seconds per application. This will certainly be enough to sterilise the cavum and disinfect the canal to a degree that is comparable to concentrated sodium hypochlorite.

The question remains how deep the ozone will be able to penetrate into the root canal, and what the effect of any proceeding preparation for certain instrument sizes will be. To ensure the safe application of ozone in the apical region too, a modified handpiece with a small cannula to take the ozone to the apex region is under development. However, to explore this interesting application further scientific studies are required.

The application of HealOzone with conventional cups during RCT as a powerfull desinfectant prior to filling the root canal is showing encouraging results.

Another promising application in RTC is the sterilisation of the decay in an inflamed tooth prior to opening the pulp chamber, therefore avoiding bacterial contamination of the chamber and successively the root canals. After sterilisation of the decay the remains should be excavated with a second, sterile, burr and the pulp chamber opened and extirpated. Afterwards HealOzone should be reapplied for decontamination of the root-canal system.

Treatment of viral infections

Because of the high reactivity of ozone, the treatment of infections such as herpes, or aphthous ulcers appears to be another obvious treatment option.

Herpes

Herpes labialis can be treated with HealOzone in an easy and uncomplicated way. The 8-mm cup is attached on the affected site and HealOzone is applied for 40 to 60 seconds. Our clinical experience was:

The earlier after its onset the herpes infection was treated with HealOzone, the shorter was the clinical course of the illness. The uncomfortable tingling and lingering pain at the initial stage of the illness ceases spontaneously after only one application of HealOzone. The infection heals very quickly, without fully forming the typical small blisters and subsequent scabbing. If HealOzone is applied at an early stage and on a daily basis, the herpes infection disappears after three days.

Herpes	12	IMG 7517	Herpes labialis infection day 1
	13	IMG 7516	HealOzone application day 1
	14	IMG 7546	Herpes labialis infection day 2
	15	IMG 7590	Herpes labialis infection day 3 Instant relief and quick healing

Aphthous ulcers

Painful aphthous ulcers in the mucous membrane of the mouth can be treated with HealOzone in a quick and simple manner. For this purpose, a suitable cup is attached on the affected mucous area and exposed to ozone for 40 seconds. Clinical experience shows a single HealOzone application leads to an 80% reduction in the original pain intensity. After about one day, the patients are pain-free. After that, the aphtha heals quickly and without any complications. A single application of 20 sec. is usually sufficient.

Further clinical studies should show if ozone application has an effect on the incidence of relapse.

Aphthosis	16	IMG 6921	Aphthosis after 20 sec, of HealOzone application. Instant reduction of pain and accelerated healing.

Preventing postoperative sensitivity

Postoperative sensitivity sometimes occurs following the definitive integration of crowns, partial crowns or inlays and after the preparation of direct composite fillings. This usually manifests itself in sensitivity to pressure and hot and cold temperatures. The possible reasons are manifold but often micro-organisms and their toxins in the dentin tubuli trigger the irritation of the alpha receptors of the odontoblasts. Leakages in the provisional filling can be the original reason for this problem.

In our experience, the application of ozone for 20 seconds immediately prior to the definitive integration of a restoration leads to a drastic reduction in the incidence of complications referred to above. In practical application however, it is not always easy to achieve a vacuum with the cups that are available so far. Still, with some effort and sometimes imagination, most teeth can be treated this way.

This kind of application of HealOzone is a true bonus, since today's patients expect to be free of any complications immediately after a dental treatment. In this context, HealOzone offers the dentist increased clinical certainty, and the patient enjoys the benefits of immediate chewing comfort. Micro-organisms, their toxins and acids, are securely eliminated in the area of the preparation, which increases the quality of the dental work and the long-term prognosis for the tooth.

Fractured tooth	17	IMG 8020	An extraordinary situation with an unusual solution: Upper left first premolar in a 63.year old, male patient with a history of heavy bruxism. The tooth is vertically fractured with an open and vital pulp. No filling or caries. The deep fracture usually means extraction. Since there was not much to lose, the patient agreed to try HealOzone.
	18	IMG 8022	Mobility of the palatinal cusp.
	19	IMG 8026	Opening of the fracture with a piece of a wooden wedge to give access for O_3.
	20	IMG 8031	Silicone mould around the tooth supports the vacuum.
	21	IMG 8040	The situation after sterilisation of the fracture with Ozone for pulp stability and pain reduction. The fractured parts were put together by adhesive technique and secured against bite-forces by a surrounding connect-ribbon (Kerr-Sybron).

Use of HealOzone in pediatric dentistry

Obviously, clinical applications of HealOzone discussed above also apply to the dental treatment of children. However, the limiting factors here are the lack of compliance from younger patients during treatment and insufficient care at home during remineralisation. Restricted access to the mouth and strong salivation can present additional difficulties. All these factors can either complicate or make impossible the ozone application and the generation of a vacuum.

On the other hand, if a child is very fearful or unwilling to be treated, HealOzone can actually save the treatment session. A typical situation is described here:

A young patient in pain arrives at the surgery as an emergency case accompanied by both parents. The presence of both mother and father already indicates that the patient may present problems during the treatment. The rule of thumb for such cases is:

- Accompanied by the mother – usually no problem.
- Accompanied by the father – difficulties in treatment.
- Accompanied by mother and father – problematic patient.

If the young patient in pain rejects the dental treatment, the dentist is under pressure to succeed. The accompanying parents naturally expect help for their child and thus a solution to their problem. Massive interventions by parents, especially by mothers, are not an

uncommon occurrence while you are trying to treat the patient. Quite frequently, the situation develops into a power struggle between the parents and the child. With any kind of treatment it is psychologically important for the therapy-acceptance of the child that the dentist prevails against the rejection. This does not have to be the pain treatment initially planned. It is rather more important not to support, and thereby consolidate, the rejection posture of the child as his or her pattern of behavior.

In such cases, in our surgery we rely on the following proven measures to defuse the situation.

We invite the parents to have a cup of coffee in the waiting room and engage in a trustful, but unambiguous heart-to-heart talk to the child. In this conversation, the necessity of the treatment is explained, while we signal an understanding for the anxiety of the child. In this context, we clearly state that only as a team can the dentist and child can solve the problem, and that we want to become long-term friends. In the course of the conversation we repeatedly ask for confirmation and agreement of the child. Eventually we agree, between friends, that we will help each other, and that the dentist will explain everything that will happen during the treatment. At this point, HealOzone is introduced to the child as a painless and comfortable treatment method. Our explanations are supported by a demonstration of the technique on a fingertip. After that, we usually get a positive treatment results and leave the treatment room with a smile.

In our experience, pulpal complaints in decayed deciduous teeth require HealOzone application for 60 seconds in order to have a pain-free patient for at least some days to some weeks . The positive experience of treatment described above helps to build trust between the dentist and the patient, and reduces the fear of further treatments. This done, the children are admitted to causal therapy in the prophylaxis program which further reduces their anxiety. Usually, these children will then easily accept other, conventional dental treatment.

Another possible application for HealOzone in the treatment of children is the temporary arrest of caries progression in deciduous teeth or permanent teeth. This is interesting if the compliance or the age of a child has not reached a level yet that would make the child suitable for an adequate filling therapy. The child is cared for in the prophylaxis program and simultaneously the caries lesions are treated with HealOzone at intervals of 3 to 6 months, for 40 to 60 seconds. If the further psychological development of the child allows it, the carious lesions can then be treated with normal fillings. The advantage is the optimal preservation of substance by remineralisation of the caries-related defects, which occurs in most cases. This improves the long-term prognosis for the individual tooth and decreases the traumatisation caused by the progression of the caries and the excavation by the dentist.

Implant surgery and periimplantitis

Creating a sterile surgical environment in the oral cavity would be a desirable prerequisite for implant surgery. To get some way towards this goal, the patient rinses with CHX and the surface-desinfectants are on the skin surrounding the mouth. In order to achieve a sterile surface directly where we perform implant surgery, we apply HealOzone prior to and immediately after surgery. This will also promote healing and stimulate the immune system, an approach that has been used in general medicine for a long time. Our experience is that post-operative patient comfort is increased.

A sterile implant surface is a necessary precondition for the successful treatment of periimplantitis. Once the supraconstruction is removed, ozone can easily be applied with the available silicone cups. Where long supragingival abutments are found, two silicone cups can be attached on top of each other to generate a vacuum. The intraoperative sterilisation of the implant surface with ozone, and the successive use of augmentative procedures with autologous bone and membrane coverage, appears to be an interesting approach to solving the periimplantitis problem. A similar procedure involving the successful sterilisation of the implant surface by means of a CO_2 laser was reported in recent literature. Further scientific studies will be necessary to develop future use in this field.

Periimplantitis Case 1	22	IMG 6702	Old implant suffering from infection and excessive tissue growth. Situation after 3 conventional surgical interventions over the last 3 years.

	23	IMG 6980	Situation after initial professional cleaning and anti-inflammatory treatment
	24	IMG 7083	One week after laser-surgery and HealOzone application for 120 Sec.
	25	IMG 7092	HealOzone treatment of the infected implant with "double-capping" technique. Pus and BOP stopped completely.
Periim-plantitis Case 2	26	G0358805	Murphy's Law: Failing Branemark MK III Implant 3 month after surgical intervention. Unfortunately pre-op picture was not taken. The implant was infected by it's neighbouring tooth 35 after insertion. There were no initial signs of inflammation. Tooth 35 was resistant to different endodontic approaches and is doomed to be extracted. Initially, more than 50% of the bone around the implant was lost, creating a significant bone-crater. The site was opened, granulation-tissue was taken out and the implant surface was cleaned with an AIRFLOW-Handy and CleanPro-Powder. The bony defect was filled with a mixture of autologous bone and Cerasorb and covered with a membrane. HealOzone application was not possible during surgery due to heavy bleeding. The membrane was lost early and we decided to replace the cover screw with a healing abutment, creating access to the implant. HealOzone was applied after removing the healing abutment for 60 sec. on a weekly basis for the following month.
	27	G0358806	6 month post-op
	28	G0358807	9 month post-op
	29	G0358808	12 month post-op Complete bone regeneration up to the first thread.
Treatment of the surgical site	30	Figure 3	Implant site after closure of the flap
	31	Figure 4	Sterilisation of the surgical site immediately after suturing.

Prophylaxis

As described at the beginning of this chapter, the professional and continuous biofilm-management is of crucial importance for stability and health. Apart from professional tooth cleaning in order to remove the biofilm, controlling the reinfection of the cleaned surfaces, qualitatively and quantitatively, plays a crucial role in this regard. The fissures of molars and premolars, which often cannot be cleaned completely for anatomic reasons present an ideal niche for the development of acidophilic, cariogenic plaque. They also are the origin of the reinfection with cariogenic micro-organisms in areas already cleaned. Prophylactic sterilisation of the fissures at 6-months intervals will eliminate the cariogenic plaque, the lactic acid and the bacterial toxins hiding in the fissures.

After sterilisation the main recolonisers are basophilic micro-organisms. After about 6 months, the newly developed biofilm is transformed into a cariogenic, acidophilic flora. The prophylactic fissure sterilisation described above will protect the fissures from caries and prevent the reinfection of other ecological niches. The combination of professional prophylaxis and daily tongue cleaning performed by the patient should give better results than could be achieved by conventional means in individual prophylaxis.

Orthodontics

Fixed orthodontic devices are particular retention niches for a cariogenic biofilm, adding to the problem spots that already exist anatomically. Therefore, patients undergoing an active orthodontic treatment

must be regarded as caries risk patients. This increased risk necessitates intensified care through a professional prophylaxis system. To eliminate the problem zones around the brackets, the same facts apply that we already mentioned in connection with fissure sterilisation. The easiest approach is to apply HealOzone around the brackets with the bows removed, i.e. when the bows are replaced. For practical reasons, this procedure should be performed in the orthodontic surgery.

With some dexterity, HealOzone can also be applied to patients with alloy bows in the dental surgery. To this end, a suitable silicone cup is carefully incised with a scalpel, in two places so that the bow can cross the cup with sufficient sealing maintained to achieve a vacuum.

Sterilisation of brackets 32 Figure 1 HealOzone in orthodontics

Long-term complications

A lesion successfully treated with HealOzone will still show the brown or white discoloration which is typical for caries. The patient may find this irritating or interpret it as a sign of treatment-failure. However, this problem can be controlled by informing the patient beforehand or during treatment. This becomes more difficult in aesthetically sensitive areas. In this situation it may be necessary to remove the brown discoloration carefully with a rotating instrument and to provide the affected site with an aesthetic filling.

Furthermore, severe problems, including a strong disruption of the dentist-patient relationship, can arise if a patient treated with HealOzone visits another surgery, be it as an emergency case or as a new patient. To the untrained eye – or to the eye of a dentist who might have neglected to update his expertise – the lesions treated with HealOzone may be diagnosed as being affected by an active caries. Further unqualified statements concerning "neglect by your previous dentist" will quickly induce strong insecurity in the patient. In this way, a well-intended, innovative treatment method can be brought into disrepute and, even worse, the patient who had been treated successfully and gently, may suffer injury from aggressive treatment methods. To prevent such unpleasant experiences, the following approach should be chosen:

1. Inform the patient of the mechanism just discussed, and advice the patient to inform any dentist he might visit in the future that he underwent ozone treatment.
2. Issue a HealOzone treatment card documenting the areas and lesions that underwent treatment.

Halitosis

Bad breath is a widespread problem which is feared by many people. This is why supermarkets and service stations sell shiploads of chewing gums, mints and sprays to freshen the breath or cover the smell. Contrary to a common misconception, in more than 90% of all cases the origins of bad breath are found in the mouth and not in the stomach area. Therefore, the dentist is the person to address such problems, not the gastroenterologist. Quite often the bad breath of the patient is accompanied by periodontal disease. Most cases of halitosis can be cured with little effort, just by integrating the patient in a prophylaxis program. Bad breath is usually caused by sulfur-producing micro-organisms. Part of the management of a patient is to instruct him in daily tongue and mucous-membrane cleaning. As an additional measure, in the course of a three-part introduction program for prophylaxis, the tongue surface, where the micro-organisms causing the problem often reside, can be sterilized with an 8-mm silicone cup. This is a simple way to make the patient feel better about himself and give him more confidence in his social relations.

Integrating the new treatment method into daily practice

Introducing new treatment methods into existing procedures within a dental surgery is not an easy task. While the clinical application of HealOzone is relatively simple and easy to learn, coming to terms with completely new thinking and a different understanding of caries is the real obstacle on the way to a successful integration. The psychological aspects have already been discussed at the beginning of this chapter. HealOzone

needs to be understood and lived not only by the dentist who applies it, but also from all other members of the dental team in the surgery. Once the professionals in a surgery have understood the advantages of this painless and gentle treatment method, they will be able to communicate their enthusiasm to the patient too. Understanding and enthusiasm are the basic conditions for acceptance on the part of patients requiring treatment. Still, the dentist does not have to be the only one acting as the communicator. Assistants and members of the prophylaxis-team can play their role in introducing patients to the new treatment method. This personal approach can be supported by various accompanying measures and materials:

- Patient information leaflets in waiting rooms or treatment rooms.
- Including brief information about the new treatment system in recall letters.
- Multimedia presentation in waiting-rooms.
- Additional multimedia presentation for direct communication between the dental team and the patient.
- Description of the new treatment system on the homepage of the dental surgery.

The advantages enjoyed by HealOzone patients are manifold and easy to communicate:

- painless
- not inducing fear
- often no drilling involved
- quiet
- comfortable
- Non-invasive to minimally invasive
- Substance-preserving
- No complications
- Reducing other complications
- Avoiding high follow-up costs

The advantages over conventional therapies are so considerable and unmistakable that the patients' acceptance is overwhelming. Consequently, bills are settled readily. In Germany, the HealOzone treatment is offered as a private service only and is invoiced according the effort and expenses involved in the treatment of the individual patient.

Conclusion

HealOzone is real progress in dental therapy but it also requires fundamental rethinking on the part of the dentist. The procedure adds to the complexity of dental therapy while offering enormous advantages with regard to the patient's comfort and the preservation of tooth substance. It is not a remedy for every problem, it actually is complex and sophisticated dentistry. But having understood its potential, it is a great tool with a broad spectrum. In our view, it represents a big step into the future of dentistry. In our surgery, we would not want to do without it anymore, as it opens up new horizons, but we have also learned that:

Nothing becomes simpler. Preserving natural tooth substance requires great effort and cost!

Fissure caries without visible enamel collapse (cavity)

1. Cleaning with ProphyFlex
2. Diagnodent measurement >30: Go to step 7 or 8
3. CSI assessment >1: Go to step 7 or 8
4. X-ray: Bite wing
5. Documentation
6. Photo, if necessary
7. Cautious setting up of the discolored area of the fissure (area of the Diagnodent reading) with fissurotomy burr (SS White Burs)
8. Alternatively, careful opening with air abrasion/ Rondoflex
9. CSI (CSI is simpler and more accurate here); documentation
10. Checking for surface hardness, with sharp probe; documentation
11. 0_3 application: 40–60 sec.; documentation
12. Reductant 5 sec.
13. Cervitec application; dry carefully
14. Care kit, instruction to patient: Put paste into the cavity, ensuring absolute absence of biofilm

Follow-up: 4 weeks:

1. Cleaning with Prophyflex
2. Diagnodent measurement not very meaningful; only useful as additional information
3. Visual and clinical checkup; sharp probe: soft consistency, dull surface
   - If necessary, cautious excavation of the soft caries surface
   - 0_3 application: 40 sec., and another checkup after 4 weeks

4. Visual and clinical check; sharp probe: hard consistency, shiny surface
   - SÄT and micro-composite filling with flowable composite

Advantage: Maximum preservation of hard substance, compared to conventional filling therapy

Fissure caries with cavity in the enamel (D4)

1. Cleaning with ProphyFlex
2. Diagnodent measurement
3. CSI assessment >3
4. X-ray bite-wing
5. Documentation
6. Photo, if necessary
7. Further opening with turbine, if necessary
8. Cautious excavation of the soft surface
9. 0_3 application: 60 sec.
10. Reductant 5 sec.
11. Cervitec application; dry carefully
12. Insert wad pellet with reductant
13. Care kit, Instruction: Put paste into the cavity, ensuring absolute absence of biofilm

Follow-up: 2 weeks

1. Cleaning with Prophyflex
2. Diagnodent measurement hardly meaningful in such cases; only to be used as additional information
3. Visual and clinical checks
   - Sharp probe: soft consistency, dull surface: go to a
   - Hard consistency, shiny surface: go to b
   - a. 0_3 60 sec. and recall after 2 weeks
   - b. 0_3 40 sec.; continue with 5
4. reductant
5. Multi-layer composite filling

Approximal caries with approximal enamel wall intact

1. Clinical assessment; Diagnodent inaccurate in this application
2. X-ray: bite wing
3. Small trepanation in the area of occl. enamel surface above the caries
4. 0_3 application: 60–80 sec.
5. Introducing reductant into the cavity
6. Cervitec application
7. Care kit; instruction: Put paste into the cavity, ensuring absolute absence of biofilm

Follow-up: 2 weeks

1. Cleaning with Prophyflex
2. Diagnodent measurement hardly meaningful in such cases; only to be used as additional information
3. Visual and clinical checks
   - Sharp probe: soft consistency, dull surface: go to 4
   - hard consistence, shiny surface: Finish from point 4
4. 0_3 application: 60–80 sec.

Finish

1. Cleaning
2. Visual and clinical checks
3. X-ray bite wing; decision: Progression or remission
4. 0_3 application: 20 sec.
5. Closing the trepanation opening with flowable composite

Approximal caries with defective enamel wall (cavity D4)

1. X-ray bite wing
2. Removing the soft caries surface; neatly display approximal enamel
3. Clinical assessment
4. Building up the missing approximal wall with composite up to just under the approximal strip of the neighboring tooth
5. 0_3 application: 60–80 sec.
6. reductant
7. Cervitec
8. Insertion of wad pellet with reductant
9. Care kit; instruction: Put paste into the cavity, ensuring absolute absence of biofilm

Follow-up: 2 weeks

1. Cleaning with Prophyflex
2. Diagnodent measurement hardly meaningful in such cases; only to be used as additional information
3. Visual and clinical checks
   - Sharp probe: soft consistency, dull surface: go to a
   - hard consistency, shiny surface: go to b
   - a. 0_3 60 sec.; recall after 2 weeks; insert wad pellet with reductant
- b. 0_3 40 sec.; with finish point 6

Finish

1. Cleaning
2. Visual and clinical checks
3. X-ray: bite wing
4. 0_3 application: 20 sec.
5. Multi-layer composite filling

Root caries

1. Cleaning with Prophyflex
2. Diagnodent measurement
3. CSI assessment
4. Documentation,
5. X-ray, if necc.
6. Photo if necc.
7. Careful removal of soft surface
8. 0_3 application: 40–60 sec.
9. Reductant
10. Cervitec
11. Care kit; Instruction: Put paste into the cavity, ensuring absolute absence of biofilm

Follow-up: 2 weeks
1. Cleaning with Prophyflex
2. Diagnodent measurement >30<30
3. sharp probe soft hard
4. 0_3 application: 40 sec.
5. Cervitec

Finish
1. Cleaning with Prophyflex
2. Visual and clinical checks
3. X-ray: bite wing
4. 0_3 application: 20 sec.
5. Multi-layer composite filling
6. Cervitec application

Caries profunda (with and without clinical complaints), prior to the integration of crowns, partial crowns or inlays and the provision of composite fillings
1. Anesthesia if necc
2. CP: Excavation with rose burr, SmartPrep (SS White Burs), Carisolv
3. 0_3 application: 40 sec.
4. Integration of filling

Advantage: Spontaneous abatement of minor complaints, sterilization of residual caries (present in 80% of cases), sterilization of infected dentin tubuli, Minimization of postoperative hypersensitivities

Endodontics combined with medicinal inlay
1. Irrigation with slight drying of the canal. Recommendation: There should be only one occl. trep.
2. 0_3 application: 60 sec.
3. Med
4. Provisional closure

Root filling
1. Canals prepared completely
2. 0_3 application: 60 sec.
3. Root filling

Advantage: Avoiding postop. complaints, sterilization of the cavum and coronal canal segments, sterilization of apical canal segments with cups not proven yet. Reduction of the risk of reinfection of the root filling from coronal, provided the filling does not leak.

Sensitive tooth necks
1. Cleaning with rubber cup and polishing paste
2. 0_3 application: 20 sec.
3. Duraphat or Cervitec application

Advantage: Sterilization of the infected dentin tubuli

Enamel and dentin fractures
1. Preparation of the cavity
2. 0_3 application: 60 sec.
3. Filling or partial crown

Advantage: Reduction of sensitivity, sterilization of the fracture region

HealOzone Study

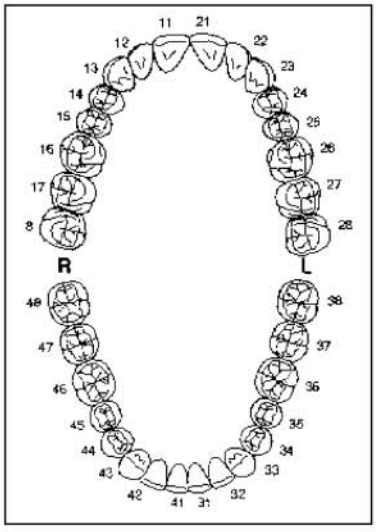
Name:_____ Pat. No.:_____

Initial photo no.:_____

Final photo no.:_____

KV: _____
Elmex Fluid
Duraphat
Fluor Protector
Cervitec
Care kit

Prophylaxis patient: Yes No
MH: good average poor



Clinical Severity Index - CSI	
5	Lesion requires filling therapy, infected dentin extends 1 mm or more into dentin.
4	Lesion requires filling therapy, infected dentin extends less than 1 mm into dentin.
3	Lesion requires therapy with a preventive composite filling; enamel caries extends to the enamel-dentin boundary.
2	Lesion requires fissure sealing; caries limited to enamel.
1	Healing lesion, defined as infected, demineralized dentin or decaying enamel in the process of remineralization; remineralization not completed. The discolored (white) enamel (visible when dry) is recovering.
0	Remineralized lesion; remineralization of the formerly infected dentin complete and finished. No further clinical infection. Remineralized enamel not showing white discoloration when dry.

Initial diagnosis – caries therapy						Checkup 1		Checkup 2		Final check			
Tooth	Surface	CSI	Date	DD	Sec	Date	DD	Date	DD	Date	DD	CSI	Therapy

Action taken	Tooth	Sec.	Date	Postop. complaints / comment
Endodontics				Yes No
Preparation				Yes No
Tooth neck sensit.				Yes No
P				Yes No
				Yes No

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CHAPTER 4.5

The Clinical Experience in a Private General Dental Practice in Italy

Giovanni D. Megighian

I have established my dental practice in Verona since 1989. The office is in the down town area. The population I serve belongs mainly to a high social economical level. This fact reflects the epidemiological findings that the demand of services is mainly orientated on remake of previous dental works in the adult age group, and minimal invasive and aesthetic dentistry in the younger age group.

The mean DMFT in the dentate population visiting my practice is 1,9 in the age group 12 to 35, and 5.3 in the age group 35 to 75 years old. The oral hygiene level is very high. Periodontal diseases are mainly connected with general health problems, and only few patients suffer of severe periodontal disease as a consequence of poor oral hygiene.

In such scenario the demand for preventive dental services is very high. The concern of our patients in an early diagnosis of dental disease is very high. In an internal audit on a sample of 150 patients, 92% were concerned of early diagnosis, and 93% would rather have minimally invasive treatments than wait and have later in time conventional treatments.

This change of attitudes in our clientele is partly consequence of new communication strategies such intra oral cameras and informed consent, and partly the consequence of a changed perception of health in the population. Awareness and freedom of choice for the self leads the attitudes of our patients.

Another important aspect of the population visiting my dental office is the high percentage of old and old-old patients. In the urban area of down town Verona population over 65 years is 25% of the whole population (Bollettino, 2001). This aspect of the population residing in down town Verona reflects the general trend of aging population in Northern Italy. The medical training and the post graduate in Gerodontology I achieved with Prof. Robin Heath at the University of London, as a consequence, are for me very useful.

My work today is connected with the work of other Medical Consultants, Psychotherapists and Physiotherapists, and this because of the awareness that health and healing are threatened by risks we are exposed to. The protection to counter balance those risks is the aim of treatment. Therefore it is necessary that diagnosis discloses all risks, expressed and hidden, and treatment offer the protection against those risks.

In our practice, dental diagnosis is oriented by three major aspects: 1) the analysis of structural changes in the oral environment; 2) the oral reflection of the unbalance in metabolic function; and 3) the oral reflection of emotional unbalance on neurovegetative homeostasis. Therefore treatment is the planning of a protection scheme which consists on structure, metabolism and neurovegetative homeostasis.

Recently we have introduced a systemic psychological approach to oral diseases, according to the constellation technique of Dr Bert Hellinger (1996). It offers an incredible opportunity for the patients to understand the psychosomatic involvement in the genesis their conditions. The demand of prevention from our clientele has operated also a change in the dental materials we use. At present we try to perform a complete metal free dentistry and utilize non toxic and biocompatible materials.

In order to comply with the needs and demands of our clientele, I have achieved the Master at the Forum

Odontologicum of Lausanne with Prof. Sami Sandhouse and I am completing a Post graduate in Homotoxicology at the Scuola di Omeopatia Clinica e Discipline Integrate.

Change in paradigm

Greene Vardiman Black, the father of modern Dentistry said that "a sharp explorer should be used with some pressure and if a very slight pull is required to remove it, the pit should be marked for restoration even if there are no signs of decay" (Black, 1924). Today we are also aware that caries is regularly found beneath a seemingly intact enamel surface (Chan, 1993). The primary goal of operative dentistry is to maintain primary oral health, defined as the absence of disease of the teeth, periodontium, and mucosa (Lutz and Krejci, 1999).

The greatest change in the way I see my work has been operated by technology because of the wider possibility of observation. The possibility to perform an early diagnosis of dental lesions and the assessment of risks, produced a shift in the offer of dental services which matches with the demand from the patients. The change in the dental paradigm is to me the awareness that any pathology has an early rise that can be recognized and successfully treated before tissue disruption. The goal of operative dentistry for primary oral health could no longer remain an unreachable ideal.

In the psychoneuroimmunological concept (Ader et al, 1991), diseases are generated by the unbalance of immune system as a consequence of a process in which central nervous system affects via neurovegetative pathways the endocrine system. The unbalance at these different levels generates the structural changes in anatomical structure of organs and apparatuses. Therefore the treatment at a metabolic level is successful if no tissue disruption has taken place yet. This is the rationale of dietary control in preventive dentistry, and it is also the rationale for the use of ozone. Ozone is therefore effective in the remineralisation of early lesions not because it alters the dental structure, but as it alters the metabolic processes taking place in and around tissues.

This concept, which changes our perspective of conservative dentistry, has proved to be very promising. The challenge to me is to accept to treat dental caries as a metabolic disease, at a very early stage, with non-invasive methodologies, and not as a cancer. If dental caries are conceited as a metabolic process, then the bi-directionality of the process, expressed by remineralisation and demineralisation of dentin and root cement, is the phenomenon with which future dentistry has to deal (Kidd and Banerjee, 2001).

Some Colleagues argue that stained grooves are not carious lesions, and the most skilled of them add that they characterize grooves in fillings, inlays and crowns with such stains. The facts are different. CRA clinical data show that almost all stained pits ands fissures of non-smokers are carious (Christensen, 1999). To assure primary oral health all lesions have to be treated at the earliest stage interacting with pathogenic metabolic pathways.

Introducing ozone in the dental practice

Our experience in early diagnosis and minimally invasive dentistry concepts and technologies (intra oral cameras, dyes, DIAGONOdent, saliva tests, air abrasion, Carisolv, fissurotomy burs, glass ionomers, flowable composites etc.) and the attitude of our patients towards preventive dentistry has made the encounter with Ozone a real *coup de foudre*.

Prof. Edward Lynch from Queen's University Belfast, presented his work on remineralisation of root caries with ozone at the annual meeting of ECG European College of Gerodontology in December 2001 in London. I was the President of ECG that year, and during the gala dinner we had a long conversation. As a consequence once back home I ordered a HealOzone prototype.

I have been extensively using the HealOzone machine for more than one year, and in the following pages I will describe my and my patients' experiences. The fields of utilization of HealOzone in my practice are the following:

1) Treatment of deciduous teeth lesions
2) *Au lieu* of sealants at the eruption of permanent dentition and as prophylaxis in population at risk of rampant carious lesions
3) Treatment of primary pits and fissure carious lesions (PFCLs)
4) Treatment of primary root carious lesions (PRCLs)
5) Lesion sterilization before placing an inlay

6) Lesion sterilization before placing a filling
7) Treatment of sore lesions.

Methodology application

The utilization of ozone for every day use has been standardized in order to obtain coherent data susceptible of statistical analysis. Every value is registered on a data base which performs the analysis of samples as much as we increase the number of patients treated with HealOzone. This has allowed us to participate in the multicentric study promoted by Prof. Lynch. As a baseline, any deciduous and primary lesion is evaluated following this methodology:

- ☐ clinical classification index, (Ekstrand, 1998,modified by Holmes and Lynch 2001)
- ☐ video camera at 40 X
- ☐ cleaning of the surfaces with air abrasion using alumina oxide with 27,5 micron at 2.5 PSI in PFCLs
- ☐ cleaning of the surfaces with nylon brush in primary root carious lesions
- ☐ standard readings using qualitative laser fluorescence with DIAGONOdent® (KaVo, Germany).

All Patients receiving ozone are recalled after 30, 60 and 90 days to check the remineralisation. The Studies that follow represent our clinical experience on selected groups of patients.

Intra-oral camera

Since their first introduction in the dental field, Intra Oral Cameras have been marketed with the concept that they improve communication between patients and dental staff. We have been taught that 83% of all learning is visual and that live action is better than stills.

In my clinical experience, intra oral camera is also essential to dentists and Hygienists to see the signs of disease and to perform a clinical diagnosis.

Fluoridation has changed the number and type of lesions we encounter in our work. The new model of carious lesions has been widely illustrated in literature (Paterson, 1991) since the early 90s. Clues of presence of decay in grooves and smooth surfaces depend more on direct visual analysis than traditional tools as X rays and probe (Ismail, 1997). Since I believe that the luckiest of patients is the one whose carious lesions are stopped at the earliest stage, the use of intra oral camera becomes primary for a correct diagnosis, coupled with laser fluorescence and other risk indicators such as ph measurement of stimulated saliva.

Cleaning the surfaces

To have reproducible DIAGONOdent readings all dental surfaces have to be cleaned from chromogenic and non chromogenic proteins deposits and food debris each single time. In my practice I use air abrasion spray at low pressure (2.5 PSI). The same results can be achieved using bicarbonate spray. In primary root carious lesions we use a nylon brush to gently clean the surface, in hard, leathery and soft lesions.

DIAGONOdent

DIAGONOdent readings have a range from zero to 99. Higher the score, higher the gradient in mineralisation through the dental tissues, which indirectly witnesses for the presence of demineralisation and decay (Lussi et al, 1999; Shi et al, 2000). The monitoring of the effect of ozone is performed registering a standard value for each tooth prior the ozone treatment and for controls, mapping on computerized imaging system the exact spot . Further readings are performed at intervals of 30, 60 and 90 days on the very same areas. The value of each reading is recorded as *unchanged* if no variation occurred in respect to the standard initial reading; as *decreased* if the value is lower than initial reading; as *increased* if the value is higher than the initial reading.

In my clinical practice a DIAGONOdent score of 10 or higher is considered a carious lesion.

Ozone treatment, severity Index and Ozone exposure

When evaluating treatment needs for PFCLs, it has showed useful to match DIAGONOdent readings with clinical examination using intraoral camera at 40X.

Table 1: Clinical Severity Index (Ekstrand, 1998, modified by Holmes and Lynch 2001)

Index	Assessed Treatment Needs	Tx (sec)
1	Lesion requiring drilling and filling (define this as deemed to have infected dentine where clinical infected demineralisation of the underlying dentine is deemed to be present)	40 seconds with O_3
2	Lesion possibly requiring drilling and filling (defined as possibly deemed to have infected dentine where clinical infected demineralisation of the underlying dentine is possibly considered to be present)	30 seconds with O_3
3	Lesion requiring a pharmaceutical approach but not drilling and filling (defined as deemed to have infected demineralised dentine which is reversing and getting smaller. This scenario is where clinical remineralisation of the underlying dentine is considered to be in the process of remineralising the demineralised dentine but is not yet complete)	20 seconds with O_3
4	Lesion arrested (defined as deemed to have had infected dentine which reversed and where clinical remineralisation of the underlying dentine is considered to be complete, with no infection remaining in the dentine	10 seconds with O_3

In our clinical experience we developed the scheme as shown in Table 2.

Table 2: Clinical Severity Index, DIAGONOdent Scores and Ozone exposure in PFCLs

Severity Index	DIAGONOdent Scores	Ozone Exposure
1	>31	40''
2	21–30	30''
3	16–20	20''
4	10–15	10''

In deep lesions exposure time 30'' per mm. of depth.

It is important to underline that laser fluorescence, reproducible and sensitive, does not supply information about the extension of the lesion under examination (Tam and McComb, 2001). Our experience is that DIAGONOdent is technique sensitive, and the clinical classification is the only quantitative method to assess and monitor carious lesions.

Ozone treatment and safety – the HealOzone Kavo device

Some Authors underline the fact that early diagnosis can lead to false positives and consequently to unnecessary treatments that damage dental tissues. Non-invasive treatments certainly solve the problem, since no damage to dental tissues is induced by ozone.

Risks inherent to the use of ozone are those of its high oxidising activity. Exposure related risks for concentration higher than the recommend FDI and EU dosage are well documented in literature. They are pulmonary oedema, chronic respiratory diseases, respiratory failure, inflammatory reactions and ocular irritation.

The level of ozone released by the appliance is high, up to 2,200 ppm. Potential risks related to ozone have been taken into due consideration in the engineering of the device. The ozone delivery system works under vacuum and it is followed by aspiration and reductant liquid flow. This creates absolute safety conditions for both the patient and the Operator (Baysan and Lynch, 2001).

Limitations in Ozone treatment

Ozone acts by contact. Lesions that cannot be reached by the ozone flow, or where the silicon cup does not seal the surface cannot be treated. Limitations are interproximal lesions, hidden root caries and hardly accessible surfaces in general.

Clinical trials

Four clinical trials are presented in this chapter. They represent part of our experience in clinical observations of the effects of ozone. They focus on:

1) Deciduous teeth 24 subjects – 48 lesions
2) Ozone *au lieu* of Sealants 5 subjects – 20 lesions.

3) Primary fissure carious lesions PFCLs 98 subjects – 352 lesions.
4) Primary root carious lesions 18 subjects – 30 lesions.

Statistical analyses

Statistical analyses of the data were obtained by paired Student t-tests to determine differences between test and control groups, with the threshold of significance chosen at 0.05. Means and standard errors were also recorded.

Techniques

At the end of this chapter some techniques of using ozone are illustrated and discussed. They are:

1) Mapping DIAGONOdent values.
2) Apply ozone on natural crowns and roots.
3) Treating cavitated lesions with ozone and filling.

Deciduous teeth treatment with ozone

Treatment of deciduous teeth lesions is challenging, since an increased consciousness of the risks involved in introducing possibly allergenic or toxic material in children mouth is expressed by parents and carers. Ozone seems to offer an encouraging solution.

Material and Methods

A Sample of 48 lesions in 24 young patients aging 3 to 10 years have been selected. Males and females participated to the study. It was necessary that primary carious lesions on deciduous molar, premolar and canine teeth were present in at least two teeth accessible to diagnostic procedures. The lesions should have had four walls of sound enamel. Clinical assessment was performed using a probe. Lesions with stickiness at probing were excluded from the study.

Teeth must show DIAGONOdent readings higher than 10. It was necessary that the subjects were reliable concerning the attendance to recall appointments. Exclusion criteria were absence of primary carious lesions on deciduous molar, premolar and canine teeth, carious lesions with pulp exposure and presence of severe gingival disorders. Antibiotic therapy for angina and respiratory tract infectious diseased during trial would exclude the subject from the trial.

Each subject presented two initial deciduous teeth lesions. One has been treated with HealOzone for 40 seconds, and one lesion has been left untreated as control. All lesions have been monitored over time. Treated lesions have been monitored after 1, 2 and 3 months. Control (untreated)lesions have been monitored only at 1 and 2 months. After the 2 months reading, increased QLF values (83% n=20) in almost all the control lesions minus four of them, compelled to perform ozone treatment. The remaining 4 control lesions have been monitored at 3 months.

Results

In the study group (lesions treated with ozone), at 1 month QLF values decreased in 67% of lesions (n=16), increased in 25% of lesions (n=6), remained unchanged in 8% of lesions (n=2). At month 2 QLF values decreased in 83% of lesions (n=20), increased in 13% of lesions (n=3), remained unchanged in 4% of lesions (n=1). At month 3 QLF values decreased in 88% of lesions (n=21), increased in 4% of lesions (n=1), remained unchanged in 8% of lesions (n=2).

In the control group (lesions untreated), at 1 month, QLF values decreased in 25% of lesions (n=6), increased in 58% of lesions (n=14), remained unchanged in 17% of lesions (n=4). At month 2, QLF values decreased in 17% of lesions (n=4), increased in 83% of lesions (n=20). As the control lesions were rapidly worsening, all 20 lesions which showed increased QLF readings were treated with ozone. The remaining four lesions in the control group showing decreased QLF reading have been monitored at month 3. Three of them showed increased QLF readings compared to the initial value, and one had increased reading compared to the initial value.

Discussion

Only one work has been published at present on the effect of ozone on deciduous teeth. Data do match with our clinical experience. If data should be confirmed by further studies, ozone might become the choice in the treatment of early deciduous teeth lesions, avoiding the use of filling materials.

Table 3: Deciduous Teeth Trial – Sample Group at 1 Month.

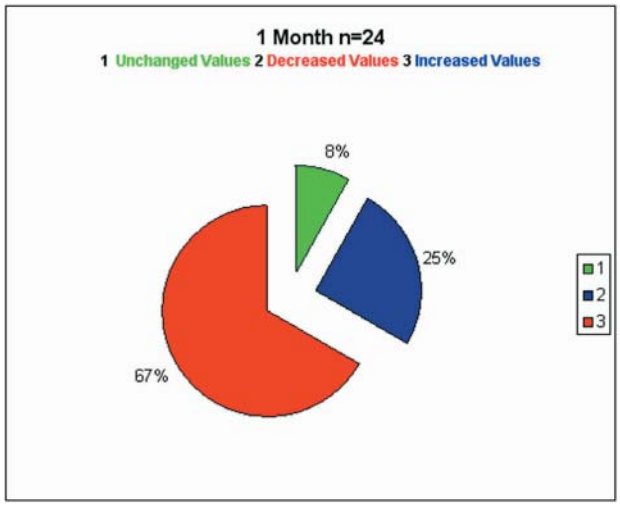


Table 4: Deciduous Teeth Trial – Sample Group at 2 Months.

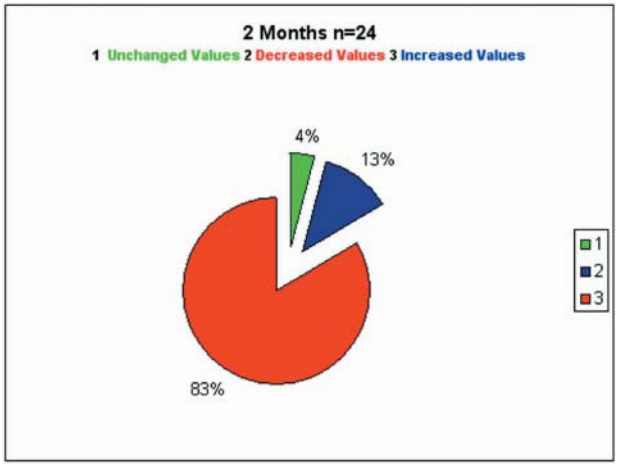


Table 5: Deciduous Teeth Trial – Sample Group at 3 Months.

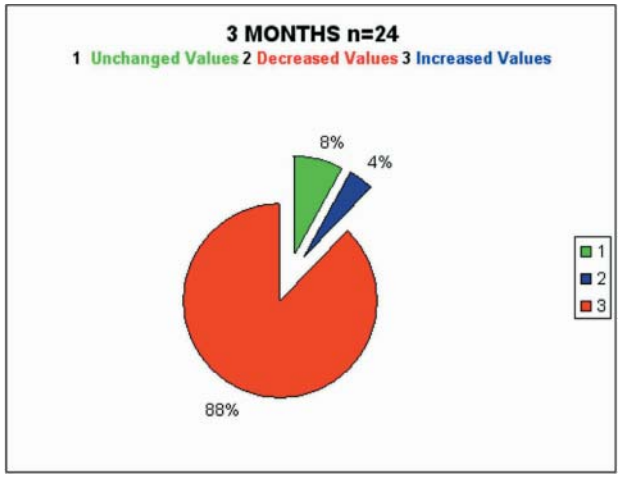


Table 6: Deciduous Teeth Trial – Control Group at 1 Month.

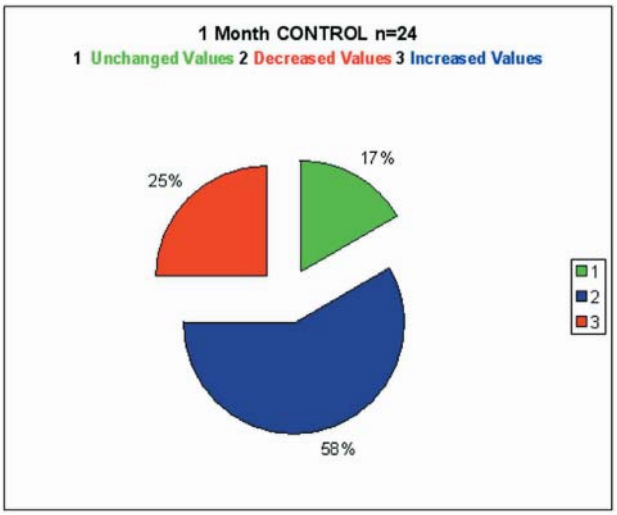


Table 7: Deciduous Teeth Trial –Control Group at 2 Months.

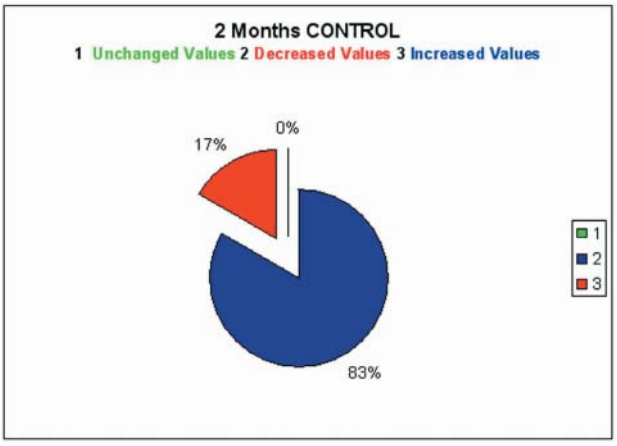
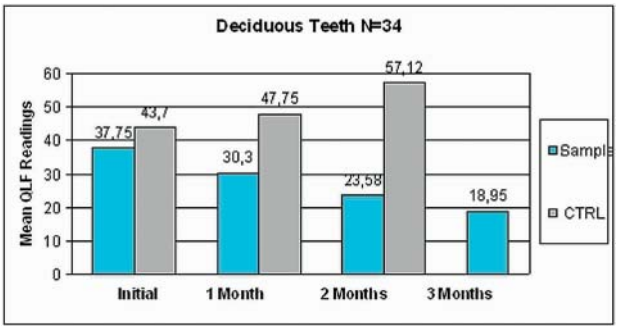


Table 8: Deciduous Teeth Trial – Mean DIAGNOdent Readings.



Ozone *au lieu* of Sealant in erupted molars in kids

Sealing occlusal grooves in recently erupted first and second molars is a diffused preventive practice. Sealing resins prevent bacterial attack on maturing enamel. Ozone seems to give protection to the newly erupted teeth, creating an ecologic niche on grooves for aerobial micro flora, and thus allowing the maturation of erupted enamel to take place.

Material and Methods

A Sample of 20 lesions in 5 young patients aging 6 to 13 years have been selected. Participation criteria to the study were the following. Males and females took part in the study. It was necessary that grooves with clinically suspected demineralization were present in at least four first and second molars accessible to diagnostic procedures in each patient. Clinical assessment was performed using a probe. Lesions with stickiness at probing were excluded from the study.

Teeth must show DIAGONOdent readings greater than 10. It was necessary that the subjects were reliable concerning the attendance to recall appointments.

Exclusion criteria were presence of cavitated carious lesions on molars, and presence of severe gingival disorders.

Each subject presented four teeth whose groove were clinically selected for sealing. Three of them have been treated with HealOzone for 40 seconds, and one lesion has been left as control. All lesions have been monitored over time. Treated lesions have been monitored after 1, 2 and 3 months. At the end of the study control lesions have been treated with ozone and monitored.

Results

In the study group, at 1 month QLF values decreased in 73% of lesions (n=11), increased in 20% of lesions (n=3), remained unchanged in 7% of lesions (n=1). At month 2 QLF values decreased in 80% of lesions (n=12), increased in 13% of lesions (n=2), remained unchanged in 7% of lesions (n=1). At month 3 QLF values decreased in 87% of lesions (n=13), increased in 0% of lesions (n=0), remained unchanged in 13% of lesions (n=2).

In the control group, at 1 month, QLF values decreased in 20% of lesions (n=1), increased in 20% of lesions (n=1), remained unchanged in 60% of lesions (n=3). At month 2, QLF values decreased in 0% of lesions (n=0), remained unchanged in 40% (n=2), increased in 60% of lesions (n=3). At month 3, QLF values decreased in 0% of lesions (n=0), remained unchanged in 40% (n=2), increased in 60% of lesions (n=3).

Discussion

The use of ozone in primary preventive dentistry is certainly a very exciting opportunity. Very little studies have been performed, but the experiences collected in the last two years do justify the hopes that acting on the metabolic process of demineralisation of dentine might prove a successful and non-invasive approach. The very limited sample of this test nonetheless demonstrates the efficacy of ozone. It has to be stressed that clinical results should be evaluated longitudinally.

Ozone in the treatment of Primary Pits and Fissure Carious Lesions (PFCLs) in Permanent dentition

Material and Methods

A Sample of 352 lesions in 98 subjects aging 22 to 57 years have been selected. Young and adult subjects have been selected for this study presenting occlusal primary lesions in permanent dentition, and conforming to the criteria of participation to the study.

Males and females, aged over 12 participated to the study.

It was necessary that primary fissure carious lesions PFCLs were present in any tooth of permanent dentition, with exclusion of third molars, in at least two teeth accessible to diagnostic procedures. Teeth have been classified with the Clinical Gravity Index Group 1–3, or they must show DIAGONOdent readings greater than 10. It was necessary that the subjects are reliable concerning the attendance to recall appointments.

Exclusion criteria were absence of primary fissure carious lesions PFCLs as defined in participation criteria, presence of advanced periodontal disease. Clinical classification index, (Ekstrand, 1998, modified by Holmes and Lynch 2001) has been utilized to classify lesions prior to treatment. Video camera at 40 X has been used on both sample and control lesions. Classification has been compared with DIAGONOdent readings.

Table 9: Ozone *au lieu* of Sealant Trial – Sample Group at 1 Month

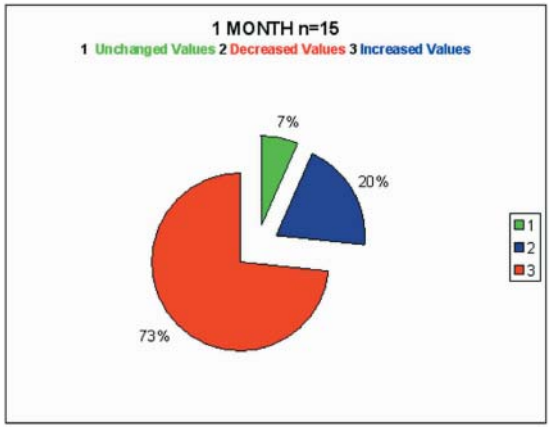


Table 10: Ozone *au lieu* of Sealant Trial – Sample Group at 2 Months

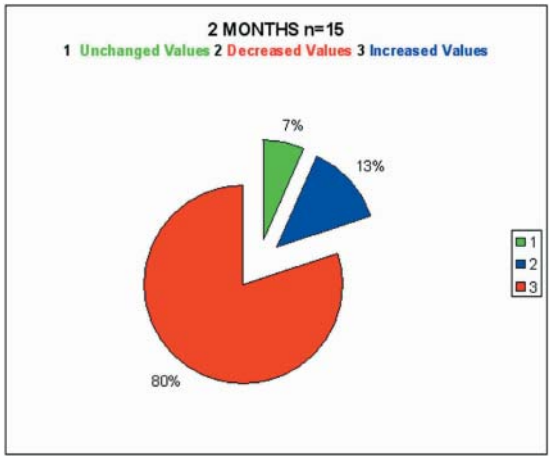


Table 11: Ozone *au lieu* of Sealant Trial – Sample Group at 3 Months

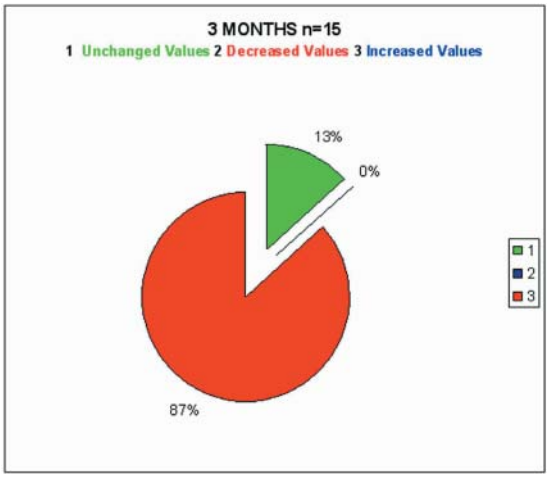


Table 12: Ozone *au lieu* of Sealant Trial – Control Group at 1 Month

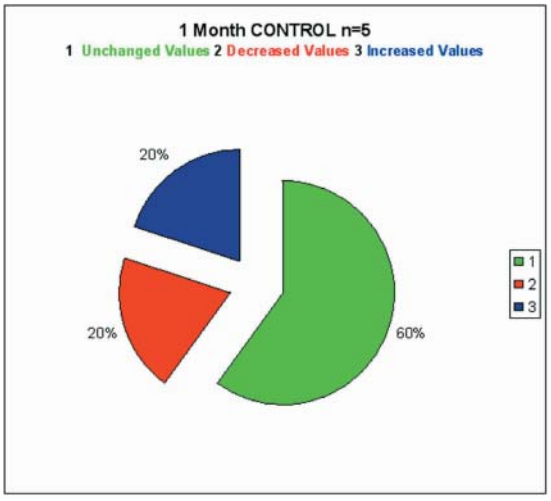


Table 13: Ozone *au lieu* of Sealant Trial – Control Group at 2 Months

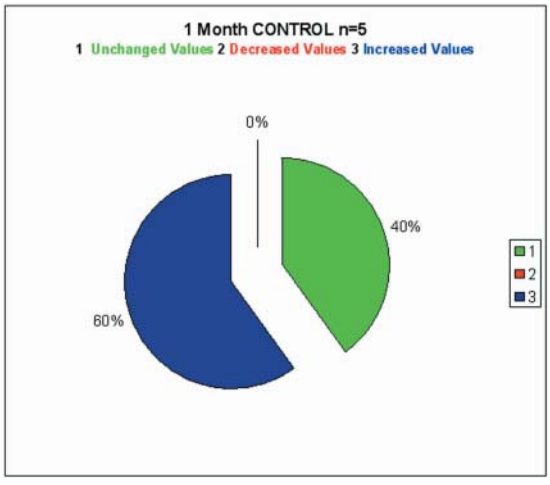
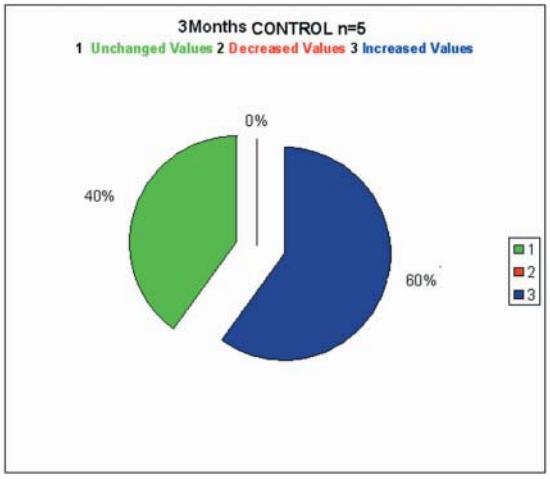


Table 14: Ozone *au lieu* of Sealant Trial – Control Group at 3 Months



After the clinical classification, and the cleaning of the surfaces with air abrasion using alumina oxide with 27,5 micron diameter, standard readings using qualitative laser fluorescence QLF with DIAGONOdent® (KaVo, Germany) have been performed. Half of the lesions have been treated with ozone (HealOzone unit; KaVo, Germany), the remaining half has been left as control. The amount of ozone exposure is related to the CSI and DIAGONOdent values as in Table 2. All lesions have been monitored over time. Ozonized lesions have been monitored after 1, 2 and 3 months. As the control lesions were rapidly worsening, all 176 lesions which showed increased QLF readings were treated with ozone before 90 days.

Results

In the study group, at 1 month QLF values decreased in 83% of lesions (n=146), increased in 11% of lesions (n=19), remained unchanged in 6% of lesions (n=11). At month 2 QLF values decreased in 90% of lesions (n=160), increased in 5% of lesions (n=8), remained unchanged in 5% of lesions (n=8). At month 3 QLF values decreased in 92% of lesions (n=163), increased in 5% of lesions (n=8), remained unchanged in 3% of lesions (n=5).

In the control group, at 1 month, QLF values decreased in 16% of lesions (n=28), increased in 68% of lesions (n=116), remained unchanged in 16% of lesions (n=29). At month 2, QLF values decreased in 6% of lesions (n=10), remained unchanged in 13% (n=22), increased in 81% of lesions (n=144). At 1 month the mean QLF readings in the study population decreased from 13.522 to 10.18. In the control group the mean QLF readings increased from 16.88 to 18.5. At 2 months the mean QLF readings in the study population decreased from 10.18 to 6.517. In the control group the mean QLF readings increased from 18.5 to 20.8 At 3 months the mean QLF readings in the study population decreased from 6.517 to 6.25.

Table 21 shows sample lesions classification according to the Clinical Severity Index.

Initial values refer to the CSI prior to ozone exposure.

Discussion

Early diagnosis of primary pits and fissure caries is of great importance in children and adults because of the rise of a new model of carious lesion (Paterson, 1991)

Table 21: Clinical Severity Index in the Sample population

INDEX	Initial	1 Month	2 Months	3 Months
1	0	0	0	0
2	12	7	2	2
3	7	11	3	1
4	157	168	171	173

which is difficult to diagnose with the traditional methods as oral radiographs and probe (Islam, 1997; Ricketts et al, 1997). Low sensitivity to visual, probing and bitewing examination leads to a significant number of teeth with dentinal caries being undetected (Luzsi, 1993).Lesions have a natural history of deepening into dentine leaving a macroscopically undamaged enamel surface. Minimal mineral loss prevents X-rays to show evidence of decay (Christensen, 1996), and no macroscopic cavitation shows no probe stickiness. Systems using indirect light fluorescence have been demonstrated effective in the clinical diagnosis of decays in permanent (Pinelli et al, 2002; Lussi et al, 2001) and in deciduous dentition (Attrill and Ashley, 2001).

Clinical reproducibility has been confirmed *in vitro* (Lussi et al, 1999). It has been observed that such methods are superior to oral radiography in the detection of occlusal lesions (Shi et al, 2000). Moreover it has been correctly underlined as such methodology, reproducible and sensitive, does not supply information about the extension of the lesion under examination (Tam and McComb, 2001). Our experience is that Laser fluorescence is technique sensitive, and the values do correspond to the clinical classification only if a thorough cleaning of the tooth surface from stains and salivary proteins has been properly done. If the reading of DIAGONOdent has no distortions given by the presence of stains or extrinsic protein deposits, then the values can be scored according the Severity Index Classification. Clinical classification is preferable when performed using an intra-oral video camera.

The relevant aspect of such a diagnostic methodology relies in the possibility to identify metabolic processes' dynamics taking place in dental tissues. What is made evident and available in every day dental practice is that dental decay is not a process of destruction of dental tissues, but the destruction is the consequence of an early detectable dynamic metabolic process whose development can be indirectly monitored (using i.e. DIAGONOdent), and then confronted with a clinical framework.

Table 15: PFCLs Trial – Sample Group at 1 Month

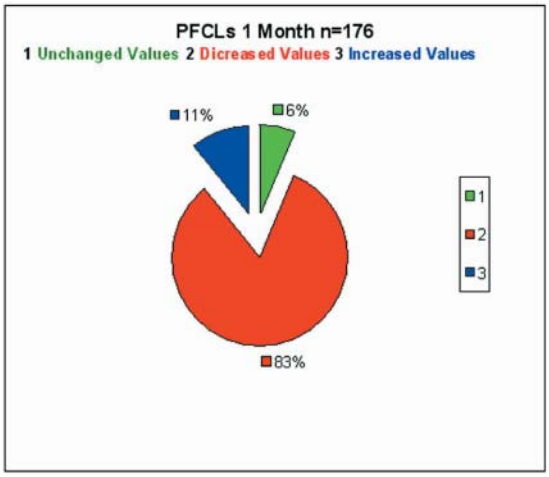


Table 16: PFCLs Trial – Sample Group at 2 Months

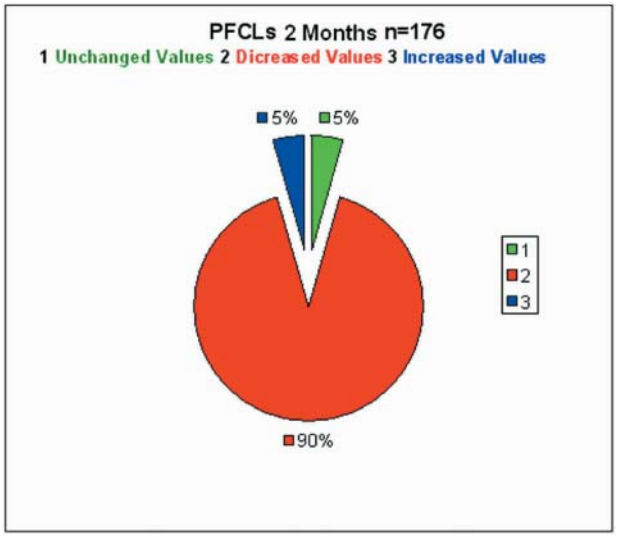


Table 17: PFCLs Trial – Sample Group at 3 Months

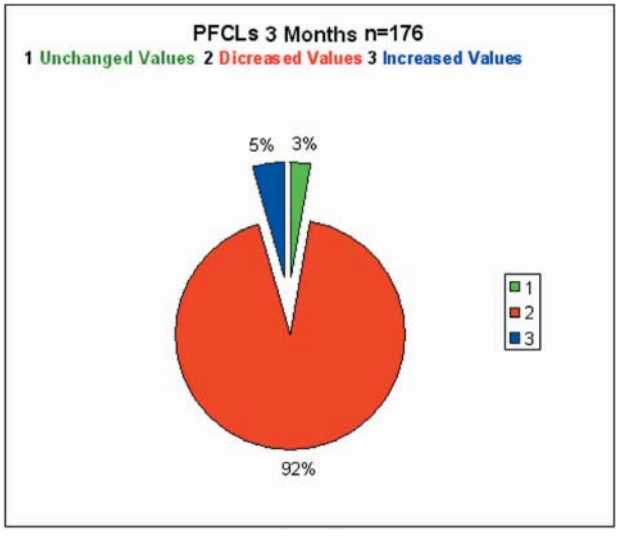


Table 18: PFCLs Trial – Control Group at 1 Month

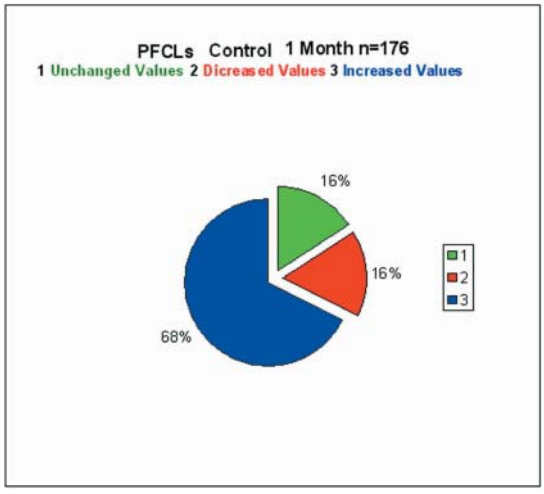


Table 19: PFCLs Trial – Control Group at 2 Months

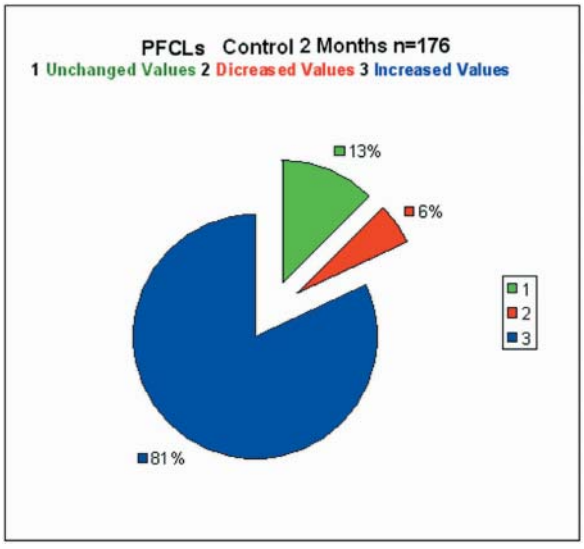
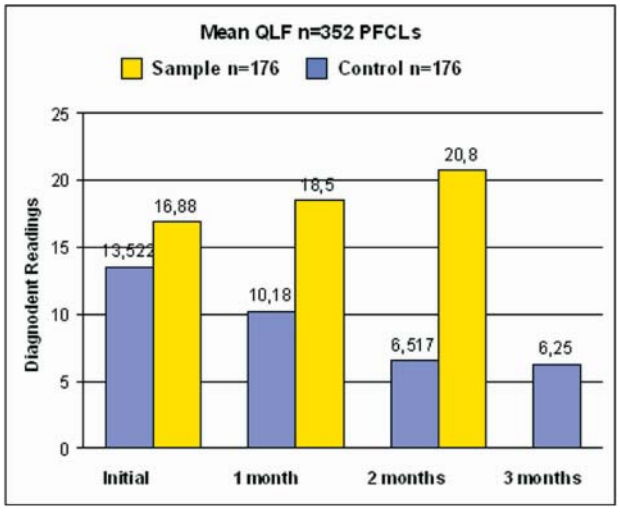


Table 20: PFCLs Trial – Mean DIAGONOdent Reading



The use of ozone in the remineralisation therapy of primary occlusal lesions gives much greater advantages in comparison with demolitive and also minimally demolitive methodologies .

I would stress three major advantages: no pain at all, rapidity and effectiveness. It is a non-invasive treatment, which means that micro burs or air abrasion are no longer needed. Since we know that a preparation greater than 1/4 the inter-cuspal width reduces the inter-cuspal strength by up to 50% (Mondelli et al, 1980), early diagnosis and non-invasive treatment stops the progression of remaking fillings. And costs are significantly reduced.

Primary Root Carious Lesions (PRCLs) treatment with ozone

Treatment of PRCLs with ozone has been the pioneering study which validated the effectiveness of such technique (Baysan et al, 2000; Baysan and Lynch, 2002; Lynch et al, 2001). In everyday clinical practice if is difficult to monitor the results, as many of these lesions have difficult access, and those easily accessible are in many cases not aesthetic for patients. Even though we have been extensively using ozone to remineralise root lesions prior to reconstruction with fluid composites or veneers, or simply leaving the lesion to remineralise in the oral environment, we could perform only limited clinical trials and controls are not available.

Material and Methods

A Sample of 30 primary root carious lesions in 18 patients aging 55 to 82 years, males and females, have been selected. It was necessary that primary root carious lesions on molar, premolar and canine teeth were present in at least two teeth accessible to diagnostic procedures. The lesions should be classified as hard, leathery and soft according to Lynch. Clinical assessment was performed using a probe. Teeth must show DIAGONOdent readings greater than 10. It was necessary that the subjects were reliable concerning the attendance to recall appointments. Exclusion criteria were absence of primary root carious lesions on molar, premolar and canine teeth, carious lesions with pulp exposure and presence of severe gingival disorders.

Each subject presented at least two primary root lesions. All have been treated with HealOzone for 40 seconds. No lesions could be left as control. All lesions have been monitored over time, and DIAGONOdent readings registered after 1 and 3 months.

Results

At 1 month QLF values decreased in 77% of lesions (n=23), increased in 13% of lesions (n=4), remained unchanged in 10% of lesions (n=3). At 3 months QLF values decreased in 90% of lesions (n=26), increased in 7% of lesions (n=2), remained unchanged in 3% of lesions (n=1).

Table 22: PRCLs Trial – 1 Month

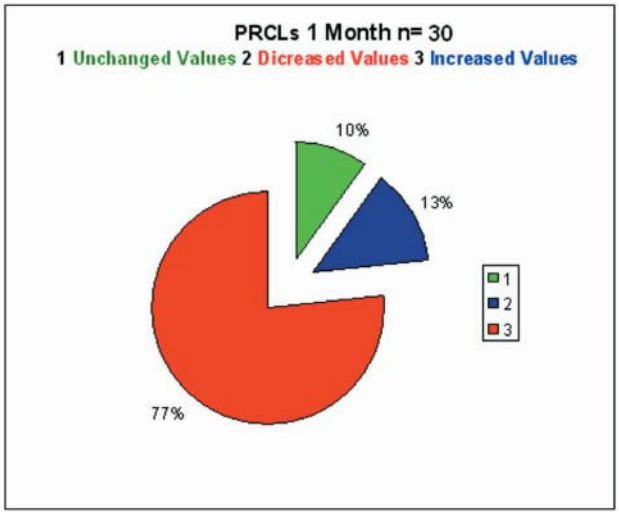
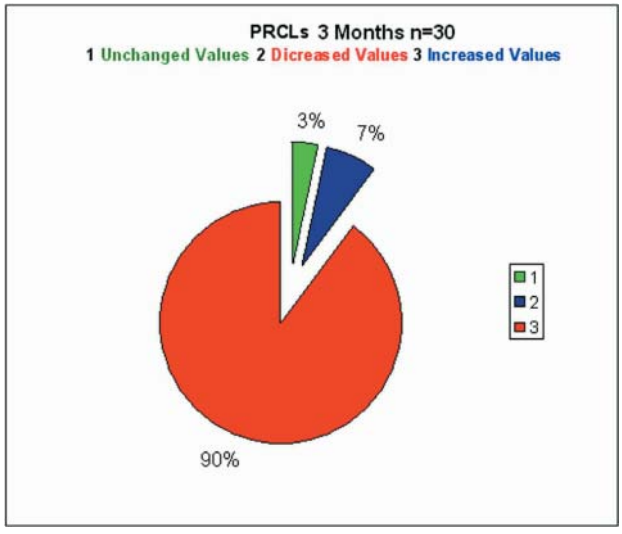


Table 23: PRCLs Trial – 3 Months



Discussion

Treatment of primary root carious lesions has been the great challenge of geriatric dentistry (Beighton et al, 1993). Studies demonstrated that consistency of the lesion, discoloration and bacterial activity were not directly correlated (Schupbach et al, 1995). Therefore a diagnosis and the consequent course of action (treatment or not) has always been controversial (Lynch and Beighton). The same studies demonstrated also that any root lesion is reversible and associated with remineralisation if antibacterial methods are applied (Lynch, 1996). Studies on the effectiveness of ozone on PRCLs have demonstrated its tremendous effects in removing organic debris enhancing remineralisation (Baysan et al, 2001).

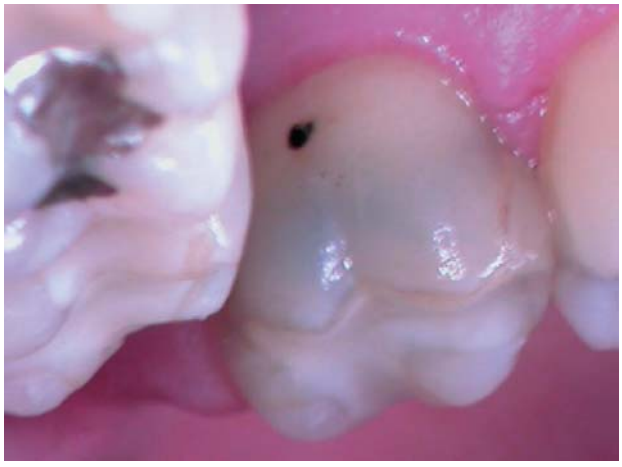
Techniques

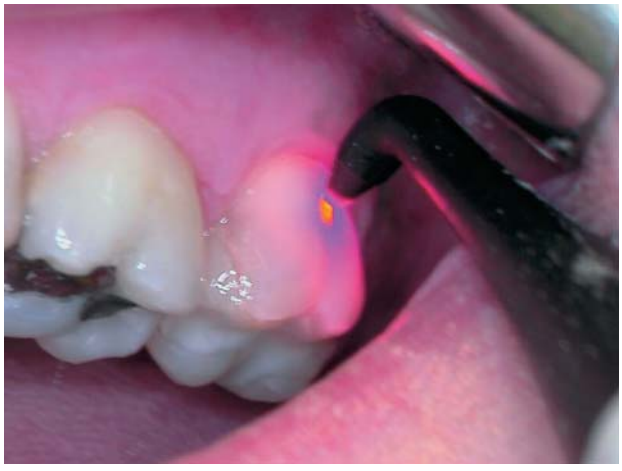
Treating Primary Carious Lesions using DIAGONOdent and HealOzone

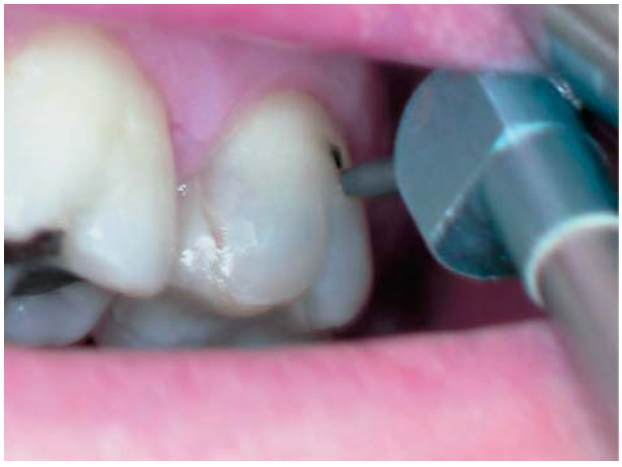
In our experience treating primary carious lesions with ozone is a five steps procedure. Step one is the clinical inspection with magnification (Picture 1). Step two is the cleaning of fissures using air abrasion or bicarbonate and then rinsing with air-water syringe (Picture 2). Step three is the measurement with DIAGONOdent (Picture 3). Step four is the classification according to the Clinical Severity Index and the QLF readings (see Table 2). Once the lesion has been classified, step five is exposure to ozone (Picture 4). The steps are summarized in the following flow chart.

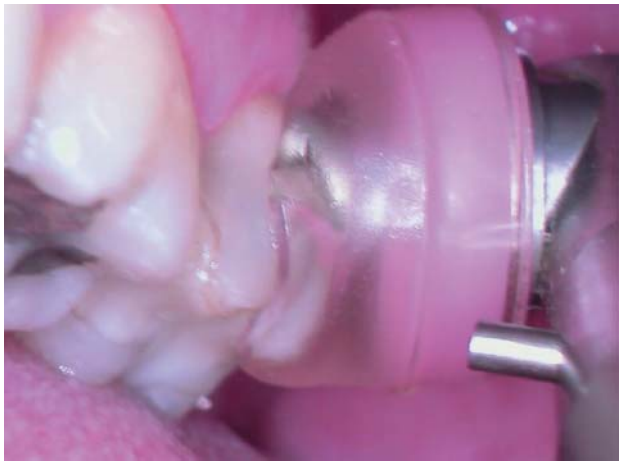
5 Steps Flow Chart for treating Lesions with Ozone

Inspect ☐ ***Clean and Rinse*** ☐ ***Measure*** ☐ ***Classify*** ☐ ***Treat*** ☐









Apply ozone on natural crowns and roots

Ozone produced by HEALOZONE acts when in contact of the tooth surface. Sometimes this is not always feasible, due to the anatomy of teeth which defies the diameter range of silicon cups provided by the manufacturer. We suggest two tips: using your own finger to obtain seal, and reconstruct or create seal for the cup using flowable composite without etching and applying adhesive. In picture 2 some help from a rightly placed finger is illustrated. Lattice gloves are damaged by ozone. So it is recommended to dispose gloves after the procedure if further treatment is to be done. In picture 3 and 4 the use of flowable composite is suggested to overcome some anatomical obstacle to seal the silicon cup.

Treating cavitated lesions with ozone and filling

The study of Mertz-Fairhurst's group provides a long term proof that infected dentine left underneath sealed restorations does not progress (Mertz-Fairhurst et al, 1998). Surprisingly there is no scientific evidence that infected dentine should be removed (Kidd and Banerjee, 2001). Destruction of bacterial agents in carious lesions using ozone stops the progression of the demineralisation process. With adequate dosage of ozone, the infected layer of dentine becomes disinfected, and the affected dentine (demineralised dentine with fewer pathogenic micro-organisms (Massler, 1967)) is subject to remineralisation. These observations provide the rationale for a non-invasive technique consisting in an ozone exposure of infected dentine as long as the estimated depth of the lesion radiographically or clinically determined. The exposure time we use are a ratio of 30 seconds of ozone for every 10 tenths of millimetre of infected dentine's depth. In doubt, I remind that *melius est abundare quam deficere* (it is better to have plenty than to have little). Once the lesion is sterile, a filling can be placed provided that it can be sealed to a 1 mm wide sound enamel. I am confident that new studies will be performed to validate the technique.

Conclusions

Ozone has demonstrate effective in treating carious lesions at a very early stage. It is my believe that its main field of action is the maintenance of primary oral health. To achieve this goal two things are necessary. The first is to teach the new scientific paradigm at Dental Schools and in continuous education programs. The latter is to inform the public that a new strategy is available which can stop the progression of dental caries at very early stage. And both parties, dentists and public, should develop a common ground where dentists act more as therapists and patients are more responsible for their well-being.

Companies in the dental sector also should understand that the change in the scientific paradigm makes dental therapists and patients equal in the decision process. Therefore a greater attention to the needs and demands of the public should be considered in their marketing strategies.

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CHAPTER 4.6

Ozone: an Adjuvant For Long-Term Success in Restorative Dentistry

Liviu Steier & Gabriela Steier

Minimal invasive therapy is the declared goal of today's dentistry: saving tooth substrate (undermined enamel and caries affected dentine) even at the expense of leaving bacterial penetration.

The following dental diseases are classified as of bacterial origin:

1. Dental decay
2. Periodontal diseases
3. Pulpal infections

Scientific research has documented optimal disinfection procedures using a gas used for the last 100 years to eliminate bacteria – ozone! The author considers that ozone, using the HealOzone system, should be a routine step during all treatment procedures on teeth in dental practice to-day and really is applying science and evidenced based dentistry to clinical procedures. Patient responses to this treatment modality have been excellent, and the clinical results of ozone have matched this excellence.

The HealOzone unit (KaVo GmbH, Germany) brings the well-documented properties of ozone gas to dentistry. In the early days, HealOzone was utilized only for the reversal of incipient dental decay with great results. Since then, thousands of dentists have been using the HealOzone with superb success in numerous applications in dentistry including:

1. Reversal of incipient caries.
2. Prevention of pulpal infection in deep caries affected dentine.
3. Disinfection adjunct factor during root canal treatment.
4. Disinfection of cavity preparations prior to restoration
5. Treatment of cervical sensitivity
6. Prior to all fissure sealant placement
7. Bleaching teeth
8. Combined with other approaches to manage caries such as the atraumatic restorative technique.

Of course, the tremendous potential and the excellent results of the above applications of HealOzone support preventive clinical dentistry. The near future will bring more and more indications turning Ozone into the GOLD STANDARD of dental disinfection.

A search of the literature reveals articles questioning the long-term outcome of bacteria included in adhesive procedures. Others question the adhesive potential of demineralised dentin. It is almost unanimously accepted that Black's "Extension for Prevention" is obsolete. In the search for non-invasive procedures which preserve the most possible natural tissue, the biggest persisting question is regarding the outcome of bacteria.

"Less effort has been placed on incorporating what is known about the pattern of caries progression and how it relates to caries removal or excavation. Although the reaction pattern of the pulp-dentin organ is quite different in terms of the nature of active (rapid-progressing) and arrested (slow-progressing) lesions, no widespread major distinction has been made regarding the different restorative treatment approaches in these situations" (Bjorndal, 2002).

"In the Scandinavian countries a stepwise excavation procedure has traditionally been used on deep carious lesions, particularly in the primary dentition (Magnus-

son and Sundell, 1998), but also in permanent teeth, in order to reduce the risk of causing exposure of the pulp." (Bjorndal and Larsen, 2000).

Treatment of Caries

The goals of treatment are to reduce the causative microbiota and contributing risk factors to halt the caries decay process and stimulate remineralisation. Caries activity can be subdivided according to the rate of tooth demineralisation. If demineralisation stops, a caries lesion is regarded as "arrested". Conversely, if the caries lesion is "active", it can be either slowly or rapidly progressing (Bjorndal and Darvann, 1999; Murray et al, 2002).

"Pulpal reactions to resin based composite restorations placed using an acid – etched technique show a close relationship between bacteria at the tooth – restorations interface and the degree of pulpal inflammation" (Mjör, 2002).

"Results acquired revealed that O_3 treatment gave rise to the oxidative carboxylation of the electron donor pyruvate (generating acetate and CO_2 as products), and the oxidation of the volatile sulphur compound precursor methionine to its corresponding sulphoxide" (E Lynch, A Baysan, CJ Silwood, M Grootveld).

Nothing can be more disappointing in clinical dentistry than middle to long-term failure, especially if the dentists have struggled for perfection. It happens that at the 4- or 5-year recall, direct placed composite restorations start to look unaesthethic as marginal discoloration comes from the tooth. A publication Lars Bjorndal wrote in 2002 attracted me. He discussed "rapidly progressing lesions" and "slowly progressing lesions".

"early arrest of dentine caries is not necessarily related to an operative treatment approach, as the dentinal reactions at this stage are not developing as an isolated phenomenon, but are still determined by the superficial cariogenic plaque covering the outer enamel lesion." (L. Bjorndal, 2002).

This raised some questions. Does this mean a shift of paradigm? As soon as we started researching Heal-Ozone the answers were around. Ozone could be the previously missing link in the chain. Could this mean "Preserving partially demineralised dentine, even in infected status" was the issue. But how can the dental practitioner distinguish "caries affected dentine" from "caries infected dentine".

The research of Yoshiyama et al revealed:

"Our results suggest that the resins can infiltrate into porous caries – affected dentine matrices and into thin zones of caries – infected dentine. This does not mean not leave leathery demineralised dentine but allows a more conservative approach" (Yoshiyama, F.R. Tay, J. Doi, Y. Nishitani, T. Yamada, K. Itou, R.M. Carvalho, M. Nakajima, and D.H. Pashley, 2002).

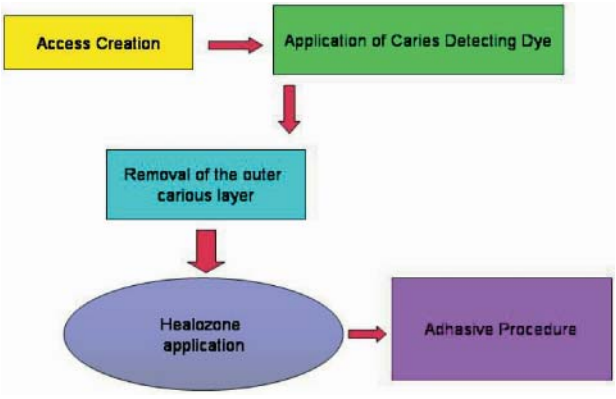
I monitored Edward Lynch and his research team in this concern, who found that just 10 seconds of treatment with ozone eliminated 99% of the micro-organisms; over 70% of the lesions studied reversed or improved, and the remainder did not progress.

There were concerns regarding the integration of Ozone in operative dentistry because of a possible polymerisation inhibition by the oxygen radicals. However, it has been shown that there is no adverse effect on bonding to enamel or dentine (Hussey DL, Armstrong C and Lynch E, 2002).

Takao Fusayama introduced the first caries detecting dyes. These differentiated carious dentin in distinct layers:

1. outer layer
2. inner layer.

Fusayama mentions that just the outer layer of carious dentine is (seems to be) infected by bacterial invasion and stainable using a caries detector. As an objective method to define dentin in need of removal, the use of the Caries Detector (J. Morita) is advocated by the author (Takao Fusayama, 1993).



Flowchart indicating the incorporation of HealOzone into the treatment protocol of the author.

Treatment procedure step-by-step

Pulp protection in deep cavities

Every cavity preparation should be ozone treated using HealOzone for 20 seconds prior to restoration. The use of this step prior to restoration has led to the elimination of any sensitivity associated with posterior composite resin restorations.

The use of ozone has permitted the author to be more conservative in his cavity preparation on the pulpal floor.

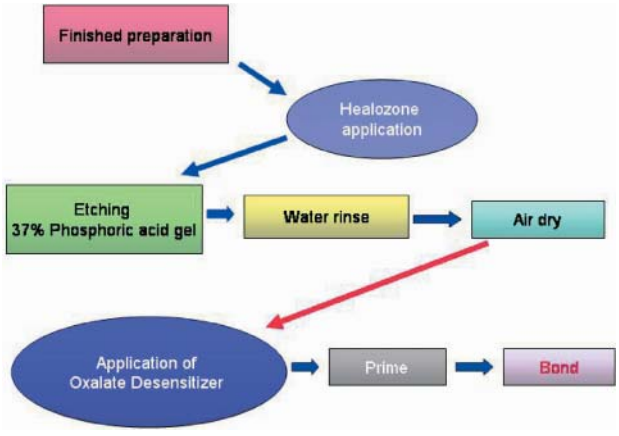
Deep cavities, with very thin layers of remaining pulpal dentine, receive a special dentin protection by application of a layer of resin – modified glass ionomer cement. An excellent material is GC Fuji II LC Improved, which is always applied after conditioning the dentin for 20 seconds with 10% polyacrylic acid solution – GC Dentine Conditioner.

Enamel–Dentin Conditioning

The author advocates the Total–Etch technique using phosphoric acid-application of primer (hydrophilic resin and adhesive resin=bonding agent). My preference is a fourth generation adhesive like Bond–It (Pentron), or Optibond FL (Kerr).

Desensitizer as re-wetting agent

Resin containing oxalate desensitizer (MS – Coat, Sun Medical CO. Ltd., Shiga, Japan) is used to re-wet the collagen after etching with phosphoric acid–rinsing and air drying (F.R. Tay, D.H. Pashley, Y.F. Mak, R.M. Carvalho, S.C.N. Lai, and B.I. Suh F., 2003).



Flowable Composite

A good choice for a flowable composite is Flow–It ALC (Pentron) with its 70% micro-hybrid filler loading (excellent alternative: Point 4 Flow and Revolution II, Kerr).

"Although the increased permeability of acidic adhesives to water is probably responsible for their improved fluoride release, water sorption by hydrophilic and ionic resin monomers within both the hybrid layer and the adhesive layer may contribute to the degradation of resin-dentin bond strength over time" (R. Tay, D. H. Pashley, 2003).

As a solution to overcome the above problem Tay and Pashley suggest covering the adhesive with a thin layer of flowable composite.

Composite

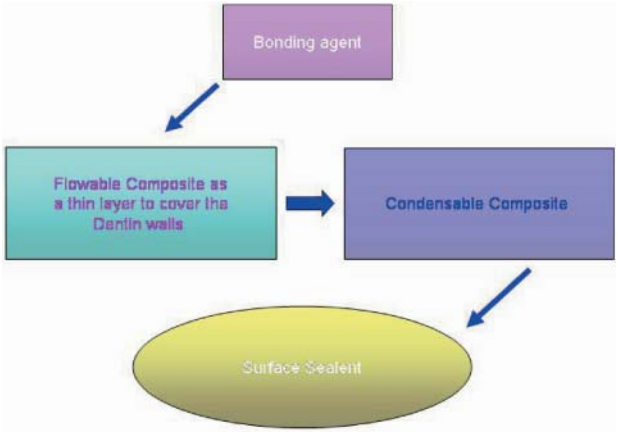
The proximal wall and the occlusal surface of direct composite restorations are performed with Nano Hybrid Composites. An excellent material is Simile (Pentron).

The author incrementally packs the resin, starting to rebuild proximal walls first – then sealing gingival margins/approximal box/occlusal first layer using Flow–It ALC, all placed under the orange filtered light of the Xenon light source fixed on the 3.6 Zeiss magnification loupes. This technique makes sure that a Class II decay is transformed to a much simpler to handle Class I defect, offering better visibility and accessibility. Resin increments never exceed 2 mm. Matrix bands used are removed immediately after performing the proximal wall. If a danger of gingival bleeding persists, the wedge is immediately replaced for better bleeding control.

Each cusp is added alone and is not cross-linked. The material is cured with a curing lamp. This way the configuration factor for each cusp will be 0.5 as just the walls (the horizontal and the outer vertical) are bounded. It is known that the lower the C-factor, the lower the shrinkage stress.

Surface Sealer

A surface sealer is applied when the restoration is cured. A short 5 second etching with a 37% phosphoric acid gel is followed by a brush application of a surface sealant (e.g.: Protect – It, Pentron).



Case number 1

The patient aesthetically unsatisfied with these two alloy fillings asked for replacement. After discussing the different treatment options he requested direct composite restorations.

The voluminous restorations indicated at the beginning of the treatment the extensive loss of tooth structure. To maintain as much tooth structure as possible it was decided to apply Ozone after completing the excavation procedure.

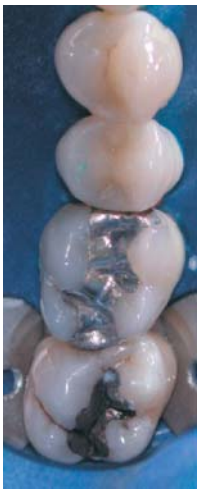


Fig. 1–1

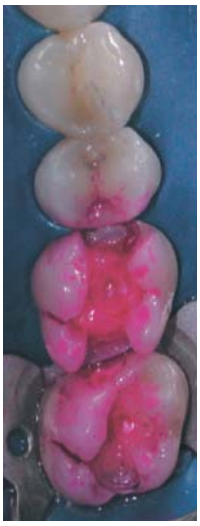


Fig. 1-2

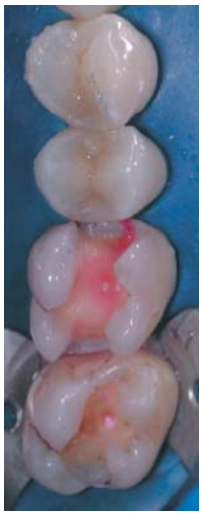


Fig. 1-3

Figure 1-1: Preoperative view of two molars with alloy restorations and marginal gaps.

Figure 1-2: Application of Caries Detector.

Figure 1-3: After copious water rinse, the remaining discoloured dentine was also removed. At this point the ozone was applied using the 8 mm application cap to obtain the necessary vacuum.

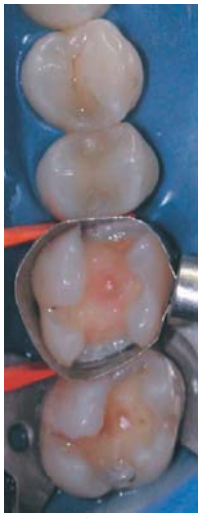


Fig. 1-4

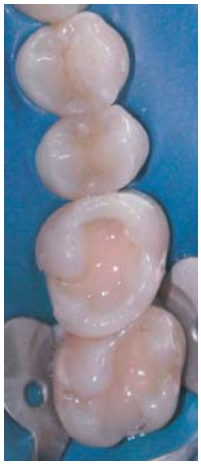


Fig. 1-5

Figure 1-4: Tofflemire matrix retainer and classical bands secured in place by wooden wedges.

Figure 1-5: The proximal walls were restored first. With the completion of the proximal walls the matrices were immediately removed.

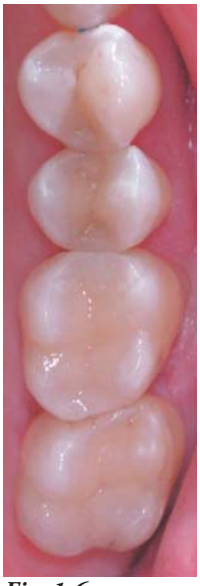


Fig. 1-6

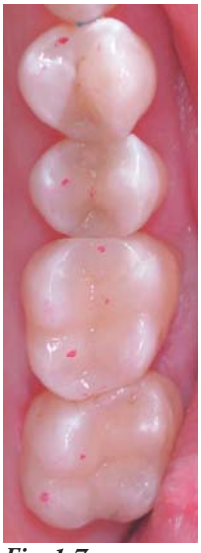


Fig. 1-7

Figure 1-6: The completed restorations – occlusal view.

Figure 1-7: Detecting the proper interocclusal relation using a 6 microns occlusal foil.

Case number 2

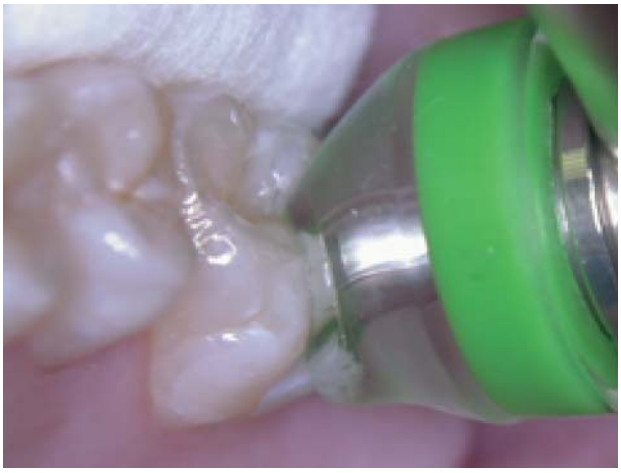


Figure 2-1: Preoperative occluso–buccal view.

A 27-year-old female was detected with caries at these two lower molars at her routine recall. The first molar presented with an insufficient fissure sealing with marginal decay. The second molar showed pit and fissure decay. To confirm diagnosis DIAGNOdent measurements were performed. A value of 85 was recorded.

The patient agreed to accept direct composite restorations procedure executed with modern Nanohybrid and ozone application.

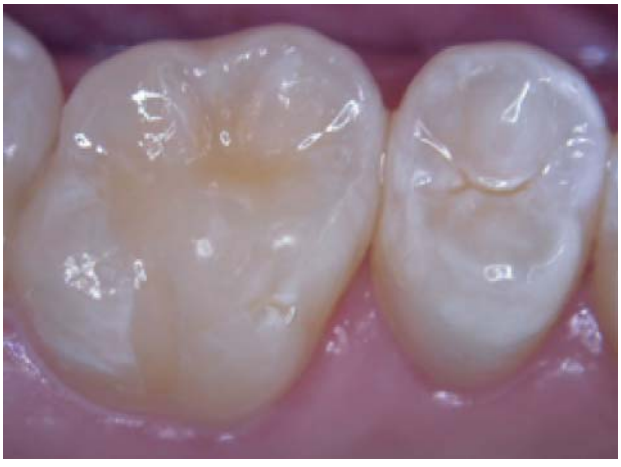


Figure 2-2: The caries affected dentine was very extensive.

After removal of infected dentine ozone was applied for 40 seconds. The direct adhesive restoration was than performed.

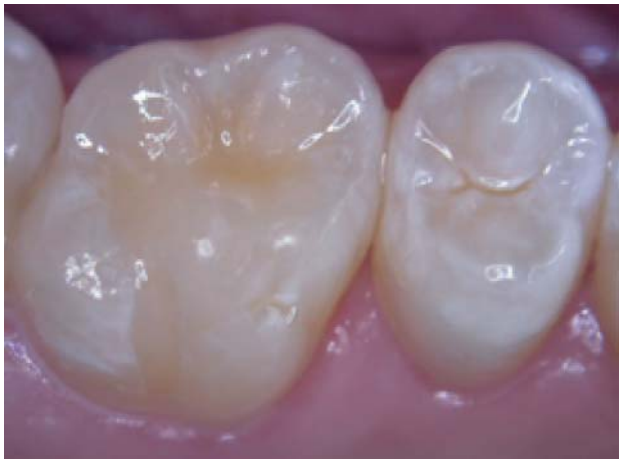


Figure 2-3: Occlusal view of the finished restorations.

Case number 3

Fig. 3-1

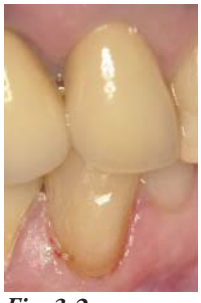


Fig. 3-2

Fig. 3-3

Figure 3-1: Preoperative view.

Figure 3-2: Occlusal view before ozone application.

Figure 3-3: The adhesive restored tooth integrity.

Routine bitewing radiography indicated in this case a gap in the proximal wall of this lower molar. Secondary decay was recorded. The insufficient alloy and the infected dentine substrate were removed. To maintain a considerable dentin wall thickness at minimal risk of bacterial penetration Ozone was delivered before starting the adhesive procedure.

Case number 4

This 22-year-old young lady had sealants placed 8 years ago. Using the DIAGNOdent values of 68 were measured, indicating a need for conservative therapy.

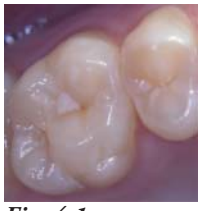


Fig. 4-1

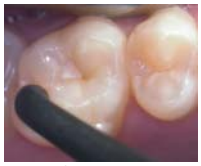


Fig. 4-2

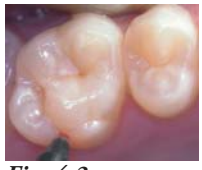


Fig. 4-3

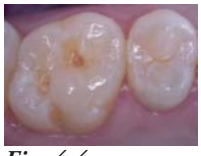


Fig. 4-4

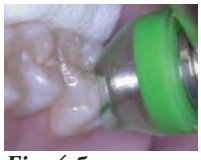


Fig. 4-5

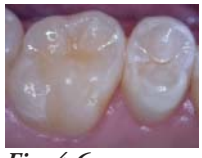


Fig. 4-6

Figure 4-1: Preoperative view of an upper molar with multiple caries portals.

Figure 4-2: DIAGNOdent measurements on the occlusal surface.

Figure 4-3: DIAGNOdent measurements in the lingual fissure.

Figure 4-4: The extent of decay was deep in the coronal to apical direction. Uniting the cavities would lead to the removal of a large amount of tooth structure.

Figure 4-5: Ozone was applied to each of the cavities for 40 seconds before adhesive procedure.

Figure 4-6: Picture illustrating the pleasant aesthetic result.

Case number 5

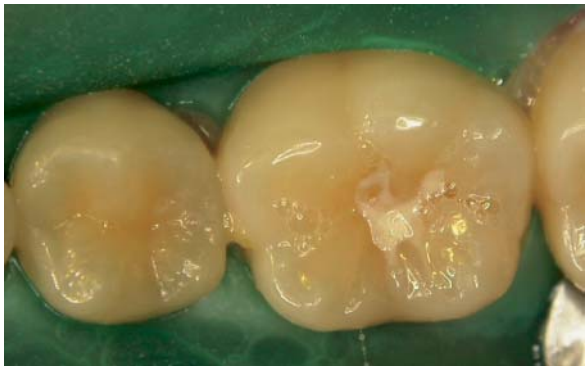


Figure 5-1: Occlusal view of a delaminated fissure sealing with secondary decay.

A non invasive decay removal protocol was applied to this tooth. To assure a good long time result Ozone disinfection for 40 seconds was used before starting the adhesive procedure.

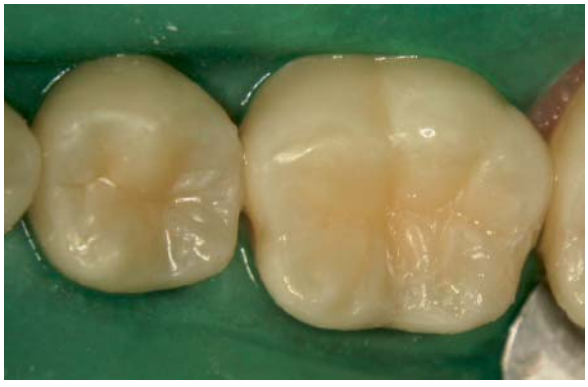


Figure 5-2: The re-established occlusal relief assures the functional integrity.

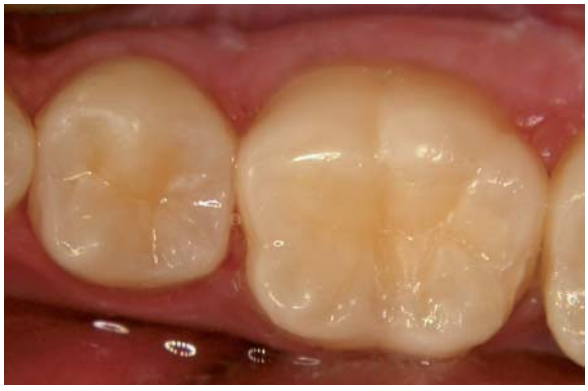


Figure 5-3: Occlusal view of the performed direct composite restoration.

Case number 6

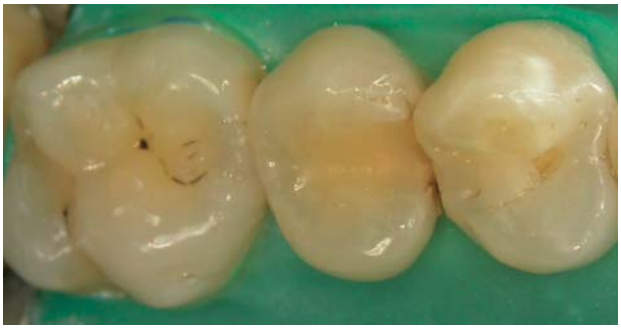


Figure 6-1: The premolars had failing restorations. The molar showed pit and fissure decay. Laser fluorescence diagnostics performed with the DIAGNOdent indicated the need for treatment.

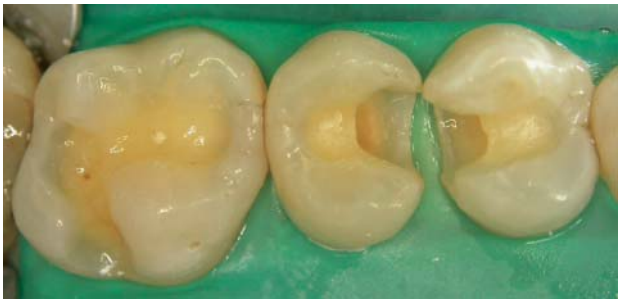


Figure 6-2: Clinical situation after caries removal.

At this moment the Ozone disinfection was carried out. A 40 seconds application of HealOzone was performed.

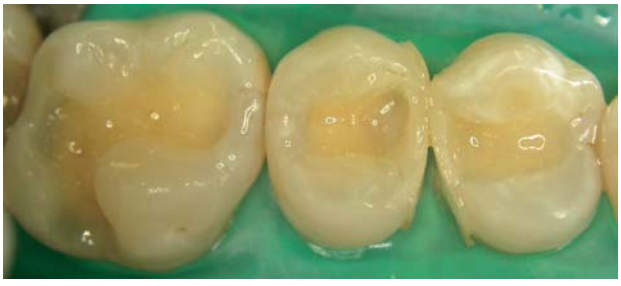


Figure 6-3: Rebuilding the proximal walls of the premolars is the first step. Removing the matrices offers a better sight and access.

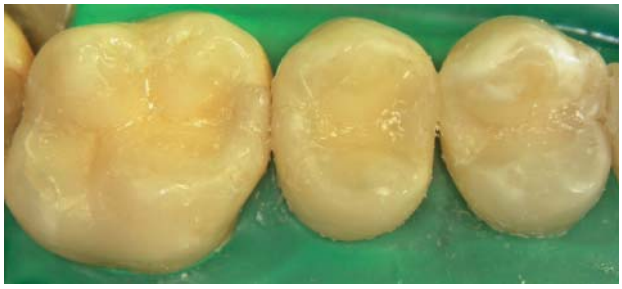


Figure 6-4: Occlusal view of the completed restorations.

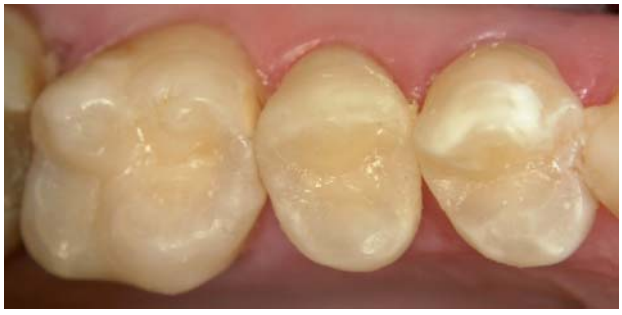


Figure 6-5: A six month recall picture demonstrating no modification at the restorations.

Case number 7

Fig. 7-1

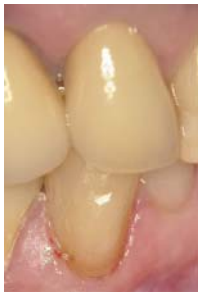


Fig. 7-2

Fig. 7-3

This 57 years old patient was concerned about the unaesthetic discoloration in a previous restored canine.

Case number 8

A 38 years old female patient is presenting onlays.

The restorations were placed 12 years ago. Tooth 26 presented with a discoloration. DIAGNOdent measurements indicated values of 76.The patient was concerned about the discoloration and asked for treatment.

After superficial excavation Caries Detector was used. Picture number 3 shows the discoloration after copious water rinsing. The above mentioned protocol was applied using ozone.

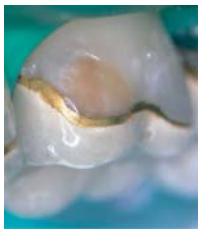


Fig. 8-1

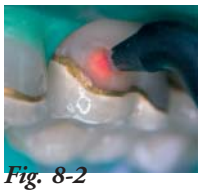


Fig. 8-2

Fig. 8-3

Fig. 8-4

Fig. 8-5

HealOzone was applied for 40 seconds before starting adhesive manoeuvres.

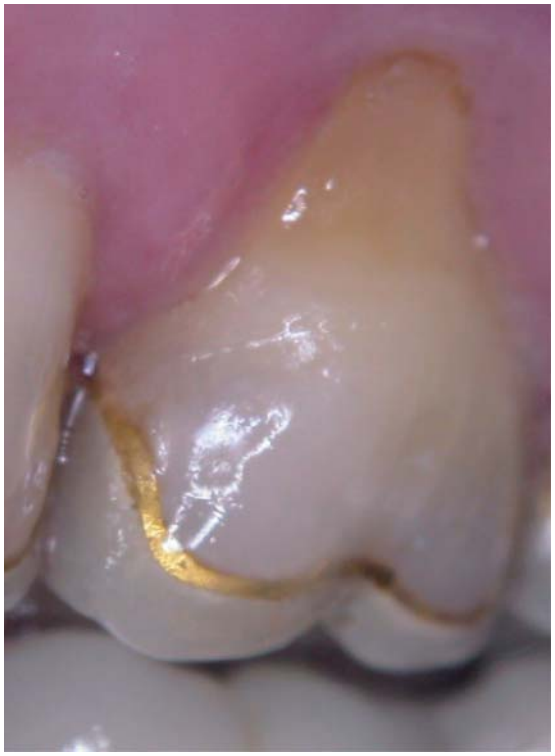


Figure 8-6: The restoration is completed.

Case number 9

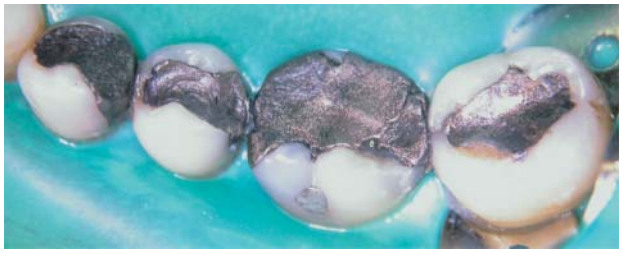


Figure 9-1: Preoperative view of old alloy restorations.

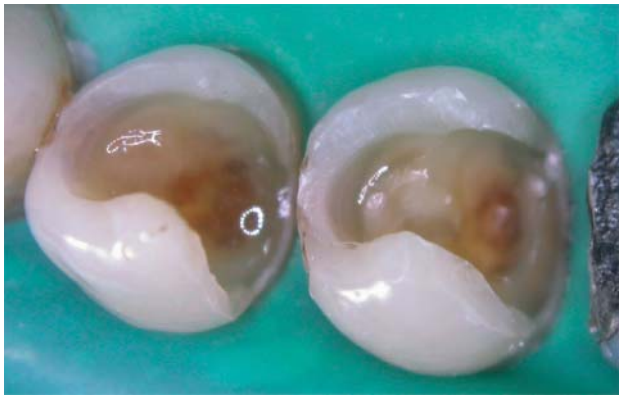


Figure 9-2: Clinical situation after the removal of the insufficient restorations. At this moment ozone is applied to the cavity for 40 seconds.

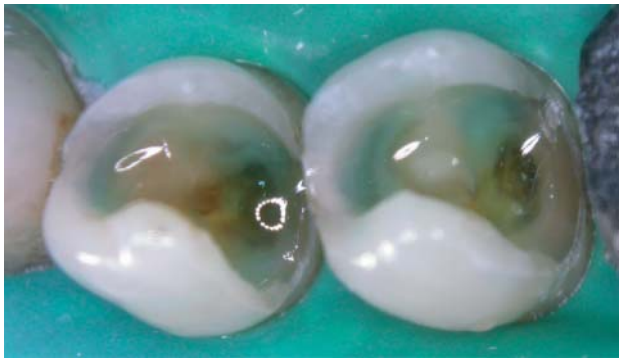


Figure 9-3: Twenty seconds application of 10% polyacrylic acid solution as cavity cleaner – GC Dentine Conditioner follows the HealOzone application.

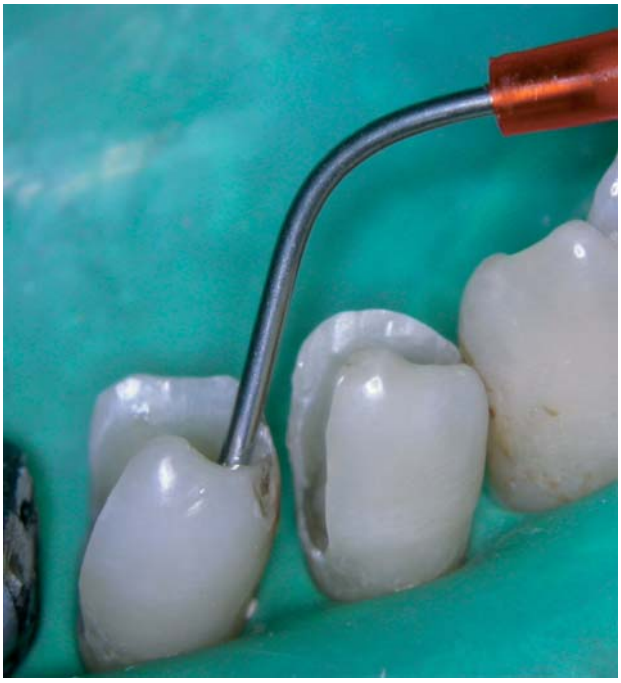


Figure 9-4: GC Fuji II LC Improved is applied as a liner.

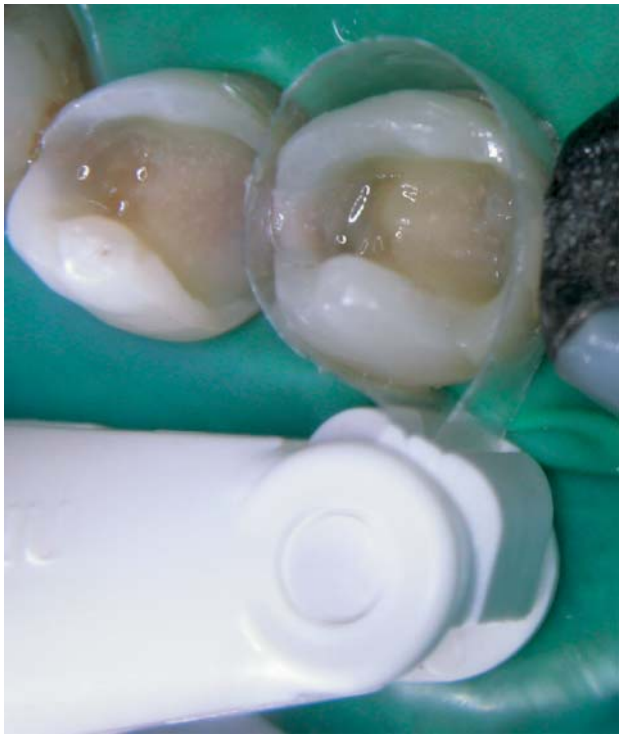


Figure 9-6: The restored proximal wall.

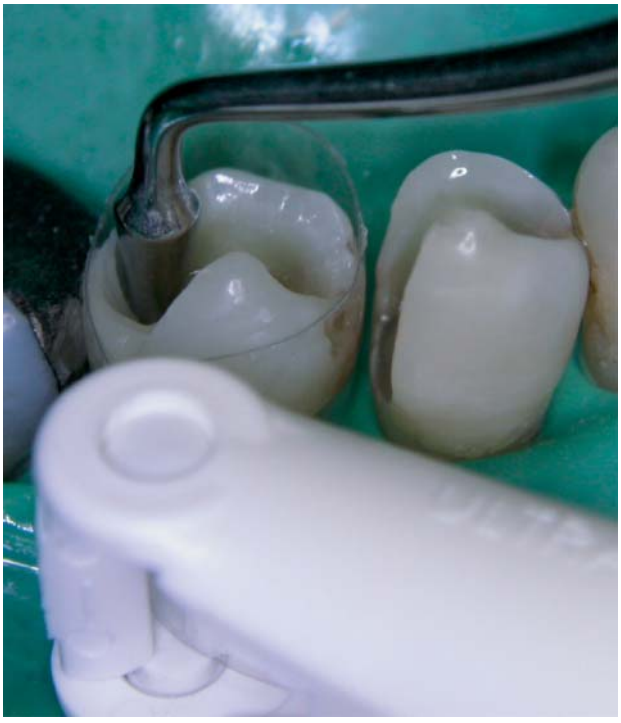


Figure 9-5: After the application of a transparent matrix Omnimatrix (Ultradent) the restoration starts with the rebuilding of the proximal wall.

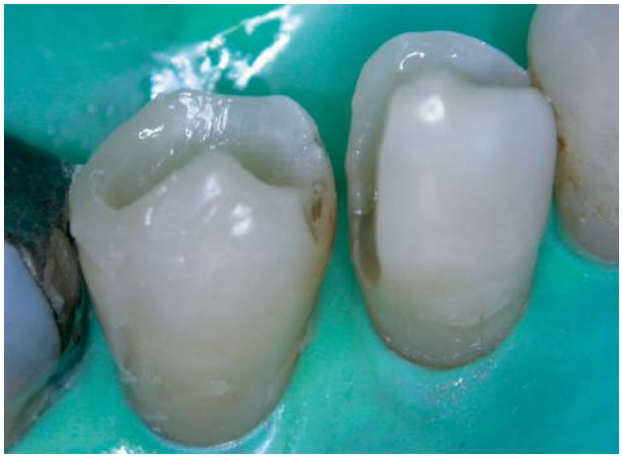


Figure 9-7: The matrix is removed to accomplish the remaining class I cavity. It is always easier to build up the occlusal relief of a class I cavity. For this the transfer of the class II to a class I cavity is the first step of treatment.

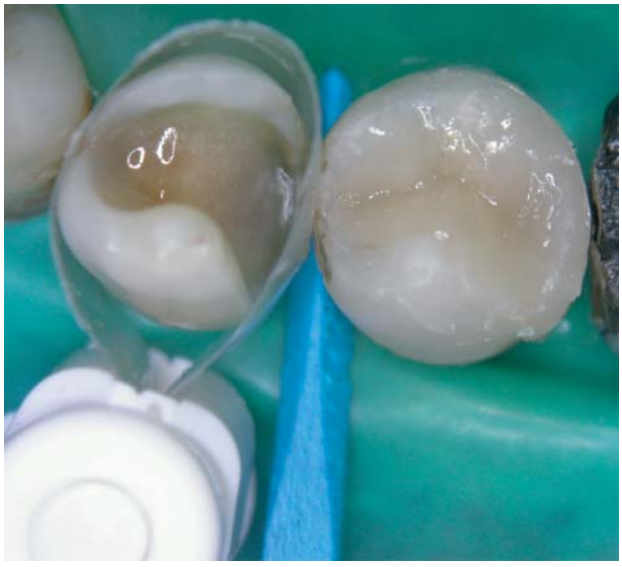


Figure 9-8: The transparent matrix is applied for the second tooth.

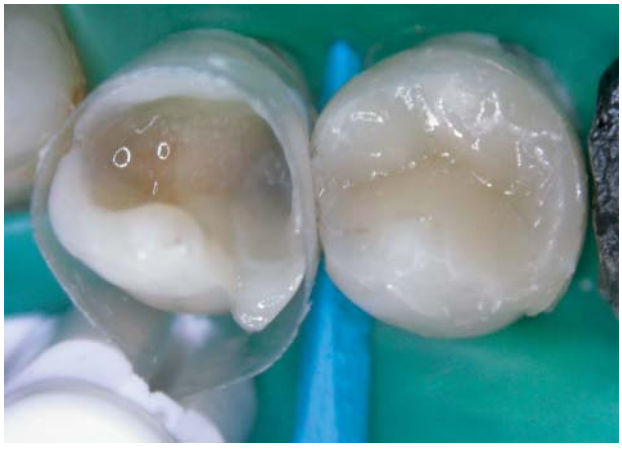


Figure 9-9: The proximal wall is being restored.

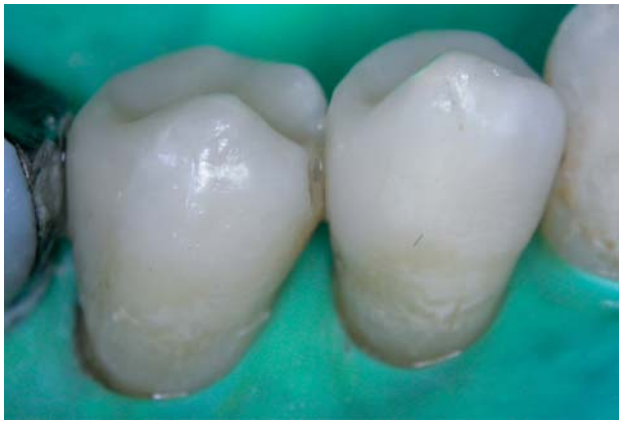


Figure 9-10: The two completed restorations.

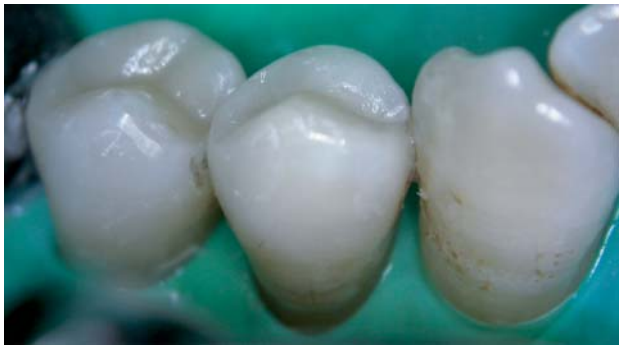


Figure 9-11: Occlusal view of the two completed restorations.

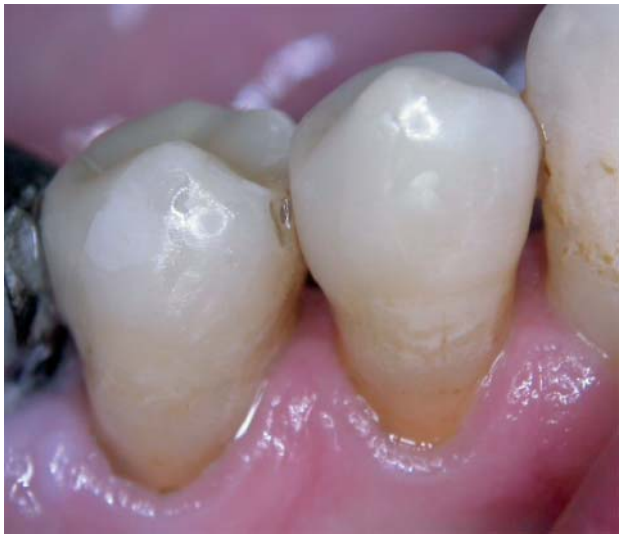


Figure 9-12: Clinical situation after removal of rubber dam.

Case number 10

A 37 years old young lady came into the office for routine check up. Clinical inspection recorded two failing composite restorations.

The treatment options were discussed with the patient. The treatment alternatives were:

- Direct composite restoration executed in layering technique.
- Composite inlay in different techniques (direct – indirect).
- Inlay executed in other dental materials (ceramic – gold).

The patient opted for the less invasive alternative: the direct composite restoration.

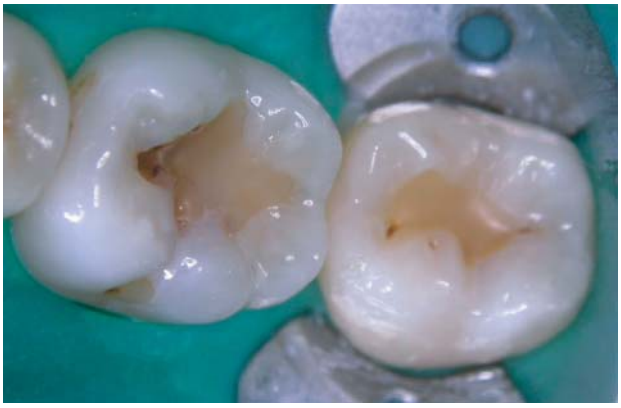


Figure 10-1: Occlusal picture featuring the delaminated and discoloured restoration showing also strong wear loss.

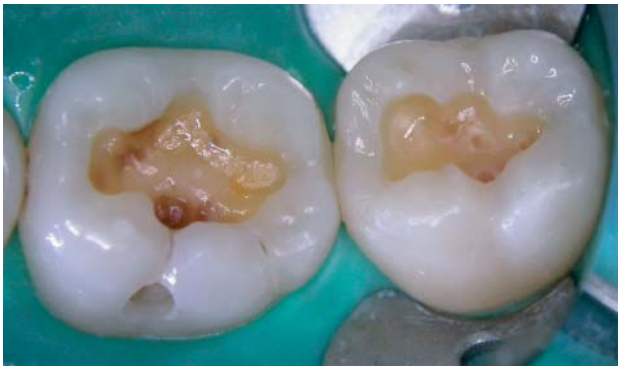


Figure 10-2: After removing the restoration a more intensive image of the leakage could be identified. After completition of excavation, a 40 seconds application of Ozone using the HealoOzone device (Kavo) is performed.

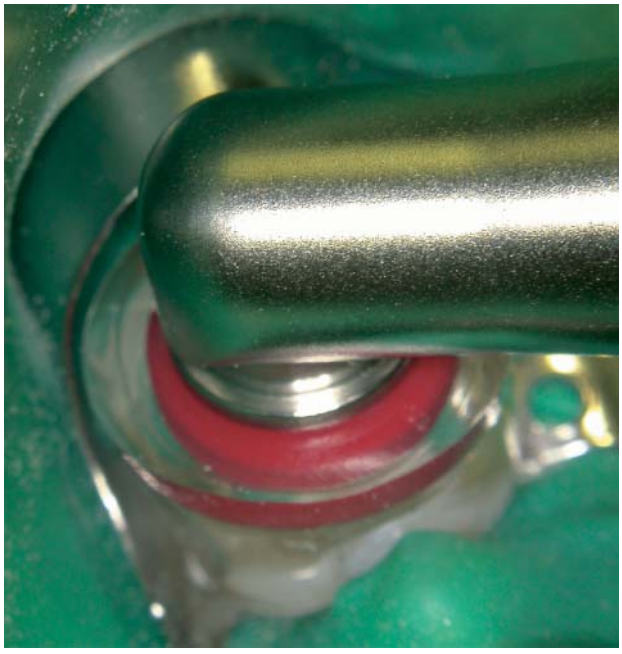


Figure 10-3: The HealOzone applied to the tooth.

The treatment protocol for adhesive restorative procedure is not altered after the ozone application. Etching (total etch technique using 35% phosphoric acid) and bonding (Nano – Bond, Pentron) are performed as described by the manufacturer (Pentron).

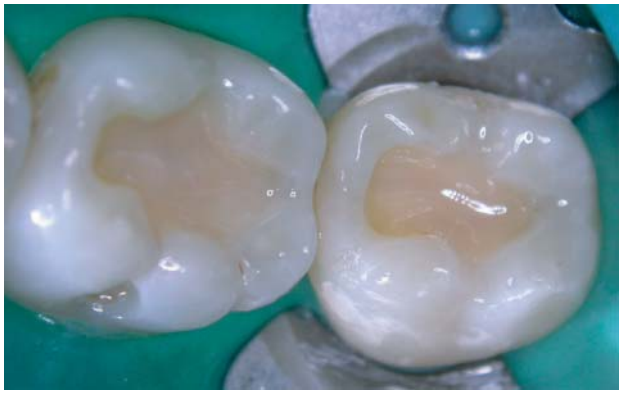


Figure 10-4

The dentine wall is covered with a layer of flowable (Flow – It ALC, Pentron) and light cured.

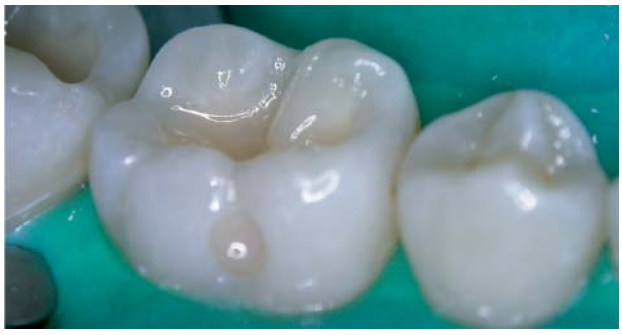


Figure 10-5

Simile (nanohybrid condensable composite, Pentron) was used to rebuild the occlusal morphology. Each cusp is placed separately after a precise analysis of the exact place and extension. It is mandatory to avoid extensive finishing procedures – therefore it is necessary to add precise fitting amount of material.

Here the author started with the mesio – lingual cusp. The shape of the rebuilt cusp tried to mimic the form of the lingual cusp of the premolar.

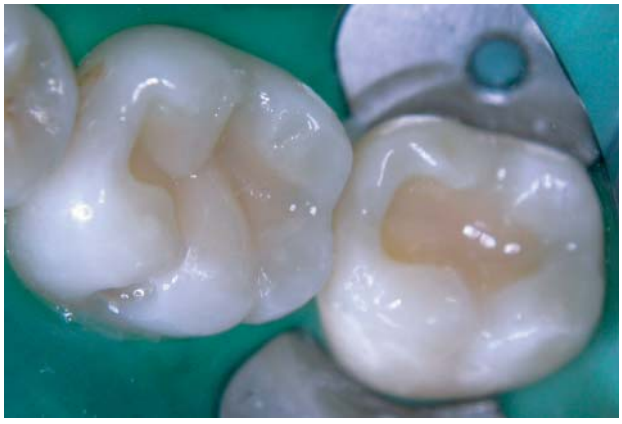


Figure 10-6: After light curing the opposite cusp was placed.

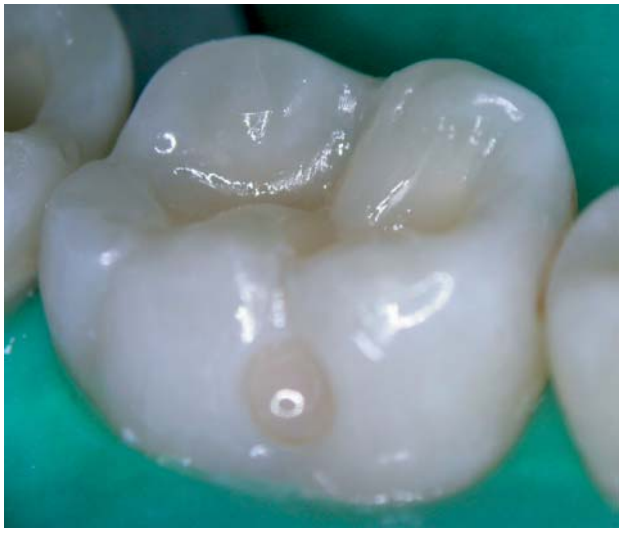


Figure 10-7: The mesiobuccal cusp is in place. The complexity of the shape can be seen in this picture. The dimensions of each cusp are already respecting the peripheral contour of the enamel walls. The enamel walls will always end as the highest relief in the new occlusal surface.

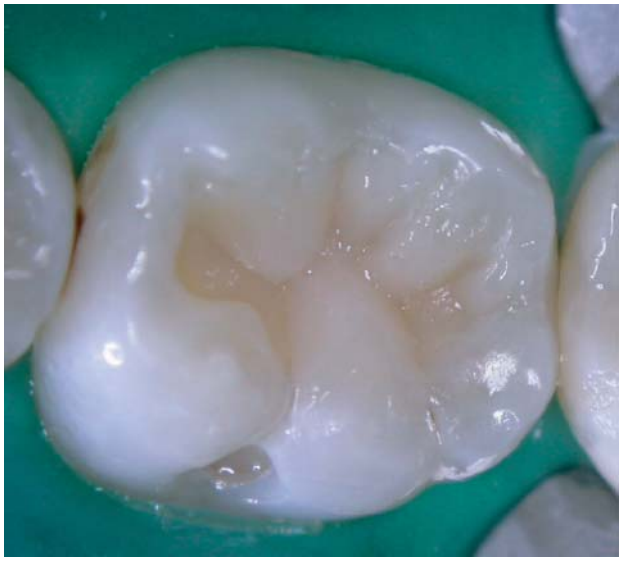


Figure 10-8: Occlusal view of the restored disto – lingual cusp. In this particular case the cusp is slightly split.

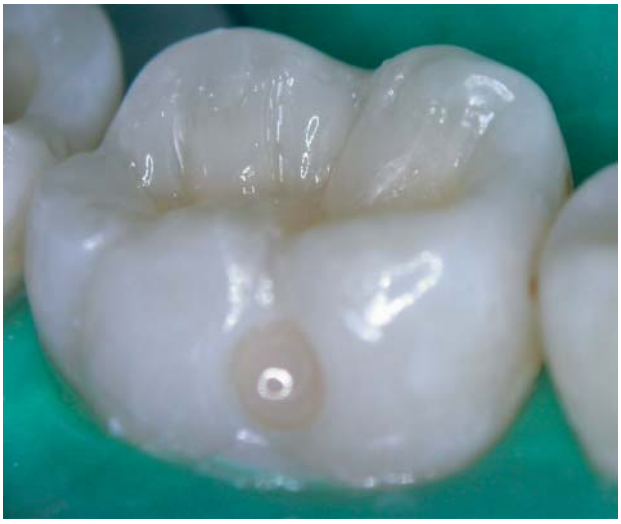


Figure 10-9: Occlusal view of the rebuilt lingual cusps.

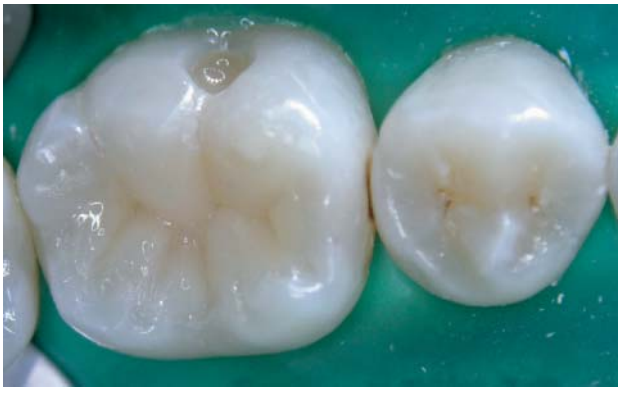


Figure 10-10: The finished restoration is very similar to the adjacent premolar.

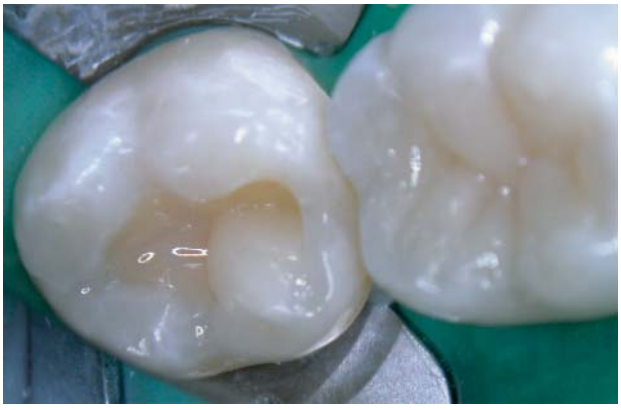


Figure 10-11

The second molar is restored following the same criteria.

The mesio-lingual cusp is the first one to be placed.

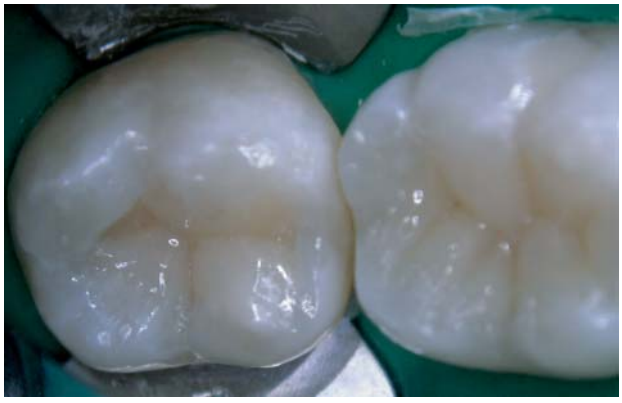


Figure 10-12

Three cusps are in place. Shape, size and extension of the last cusp are defined by the remaining free place, the position and 3 dimensional orientation.

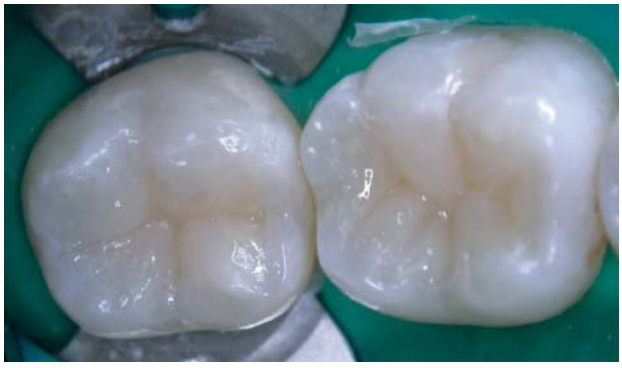


Figure 10-13

The two occlusal restorations are ready. There is no need to remodel, reshape or readjust the restorations. A surface sealing using Protect – It (Pentron) is the last step before removing the rubber dam.

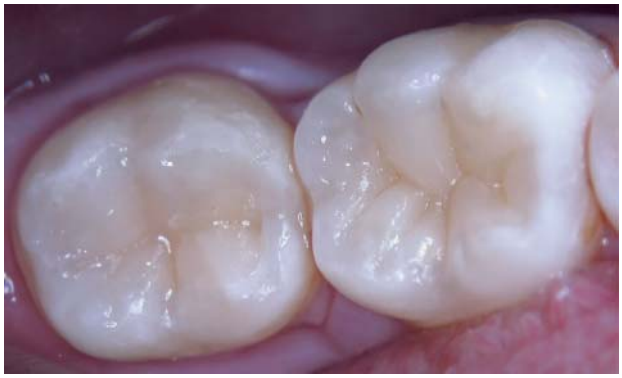


Figure 10-14: The finished case.

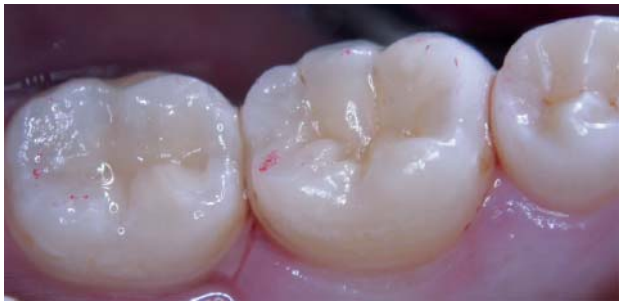


Figure 10-15

The occlusal check up indicated a very satisfactory clinical situation.

Case number 11

These two premolars were retreated according to the established protocol.

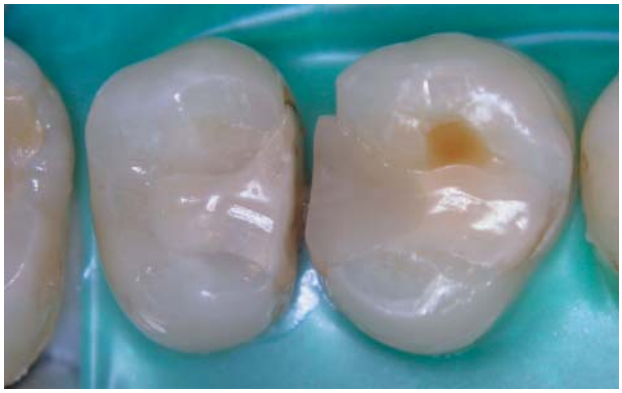


Figure 11-1: Clinical view of two composite restorations. Please note the insufficient marginal adaptation, marginal fracture, discoloration, delamination and partial new decay.

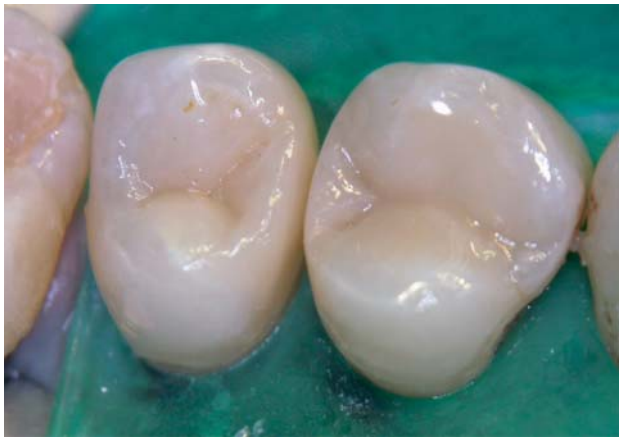


Figure 11-2: Both premolars restored with direct composite restoration after ozone treatment.

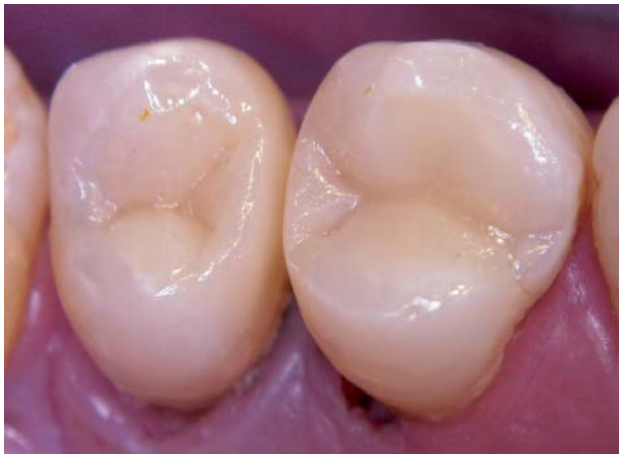


Figure 11-3: The restored occlusal morphology.

Case number 12

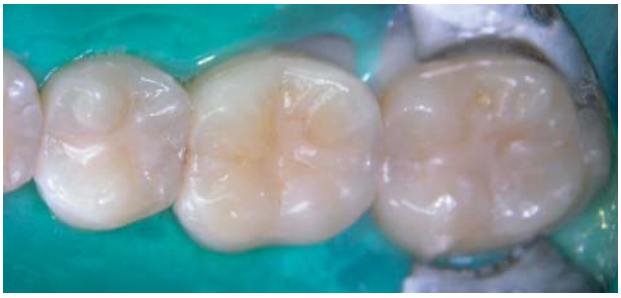


Figure 12-1: Incipient fissure decay.

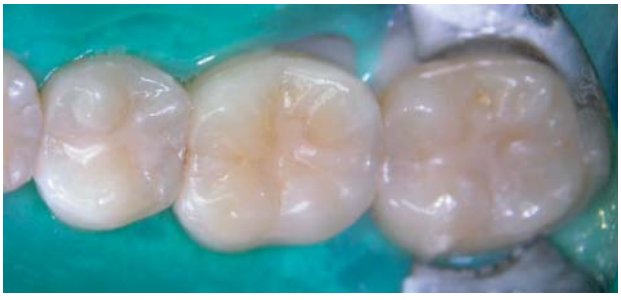


Figure 12-2: The decay has been removed.

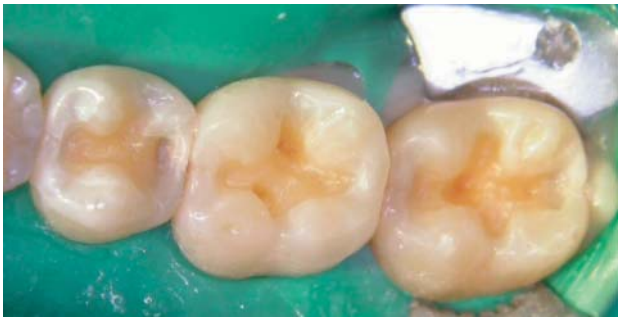


Figure 12-3: After 40 seconds of Ozone application the restorations have been performed using a Nanohybrid composite (Simile, Pentron).

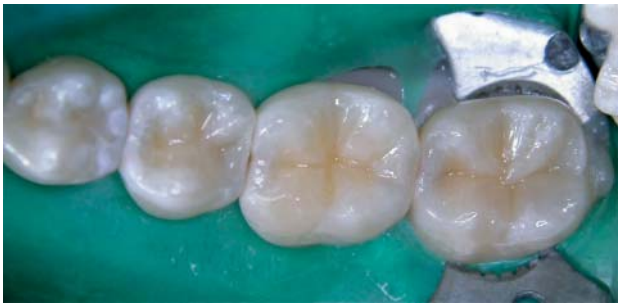


Figure 12-4: The clinical situation after rubber dam removal.

Case 13

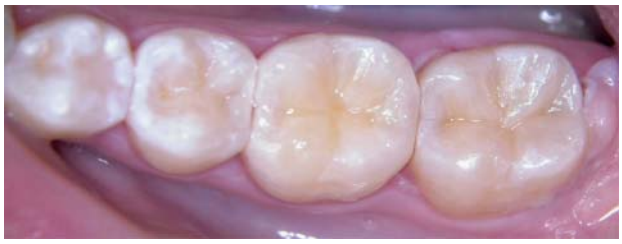


Figure 13-1: Preoperative direct view.

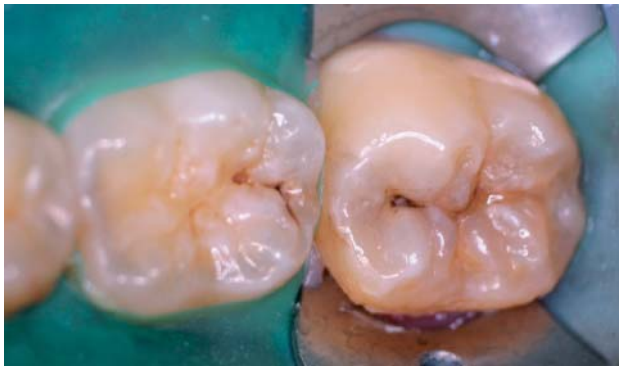


Figure 13-2: The DIAGNOdent value measured in the fissure.

Figure 13-3: Clinical situation after decay removal.

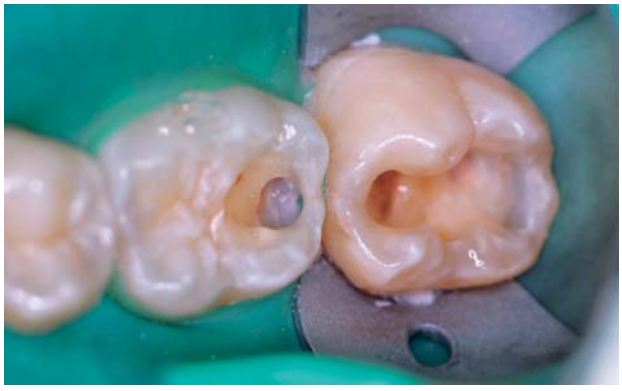


Figure 13-4: Ozone application for 40 seconds.

Fig. 13-5

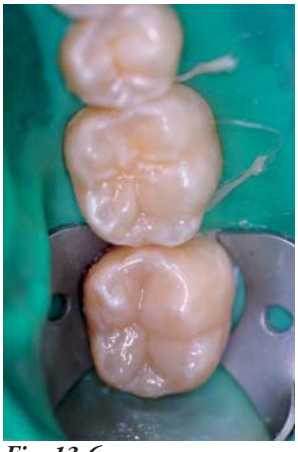


Fig. 13-6

Figure 13-5: Direct picture showing the restorations in place.

Figure 13-6: Clinical situation after rubber removal.

Conclusions

Integrating HealOzone therapy into daily restorative treatment, has complimented adhesive procedures. Post-operative sensitivity after ozone treatment using the techniques described in this chapter, are associated with highly predictable results.

It is estimated that the whole ozone/restorative procedure is delayed by less than 2 minutes, presenting a very acceptable time prolongation. The pulpal protection afforded by a more conservative excavation approach saves time and trauma.

The tooth structure preservation as a result of Ozone integration is very promising for the future.

After more than three years of HealOzone application, positive conclusions can be drawn. Future recall of the cases so far presented should continue to prove the efficacy of ozone application as an adjuvant in restorative dentistry in my clinic, leading to more predictably successful results.

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CHAPTER 4.7

Ozone Application in Root Canal Disinfection

Liviu Steier & Gabriela Steier

Sodium hypochlorite (NaOCl) is today the most common and accepted irrigation solution currently used for chemo-mechanical preparation of root canals. It has been proved that "Ozone kills *all Enterococcus faecalis* when its concentration in suspension is 10^6 and lower, even at exposure times of *only* 10 seconds" (Chang et al, IADR 2003 Goteborg).

The author has been using Ozone delivered from the HealOzone unit (KaVo) for the past two years. All teeth have received Ozone treatment as described in this chapter. The clinical results achieved using the HealOzone have been excellent, with not a single failure recorded during this two-year period. In previous years, the author noted a number of failures, especially in patients who had presented with apical abscesses. The technique used during these past two years has been to combine Ozone with NaOCl during chemo-mechanical root canal disinfection. Ozone has dramatically improved the predictability of root canal therapy, and even can shorten the time required. As a result, the HealOzone has made the one session root canal treatment an acceptable and predictable treatment technique.

1. Ozonizing the NaOCl makes NaOCl into an even more effective oxidant.
2. Ozone oxidises the cell walls of the micro-organisms and destroys them.
3. Ozone speeds up the dissolution activity and reduces the time required for routine root canal therapy

The Ozonated NaOCl acts:

- as a lubricant for instrumentation
- can flush loose debris from root canals
- as a promotor, clearing and dissolving both vital and non-vital tissue

Part of the mechanism of chemical removal of organic tissue by the ozonated NaOCl is achieved by the release of hypochlorous acid, which reacts with insoluble proteins to form soluble polypeptides, amino acids, and other by-products.

This Ozonated NaOCl clearly has important properties:

- antimicrobial effect
- tissue dissolution capacity
- acceptable biologic compatibility

Ozonated sodium hypochlorite acts as an organic and fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol, which reduces the surface tension of the remaining solution.

Ozonated sodium hypochlorite also neutralises amino acids forming water and salt. With the exit of hydroxyl ions, there is a reduction in pH. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent, and combines with the protein amino group forming chloramines. Ozone, hypochlorous acid ($HOCl^-$) and hypochlorite ions (OCl^-) lead to amino acid degradation and hydrolysis.

The chloramines interfere in cell metabolism. These strong oxidants have antimicrobial actions inhibiting bacterial enzymes leading to an irreversible oxidation of the sulphydryl (SH) groups of essential bacterial enzymes.

Oxidation promotes irreversible bacterial enzymatic inhibition. This enzyme inactivation can be observed in the reaction of ozone with amino groups (NH_2^-) and an irreversible oxidation of sulphydryl groups (SH) of bacterial enzymes (cystein). It is generally accepted that oxidation due to ozone induces the destruction of cell

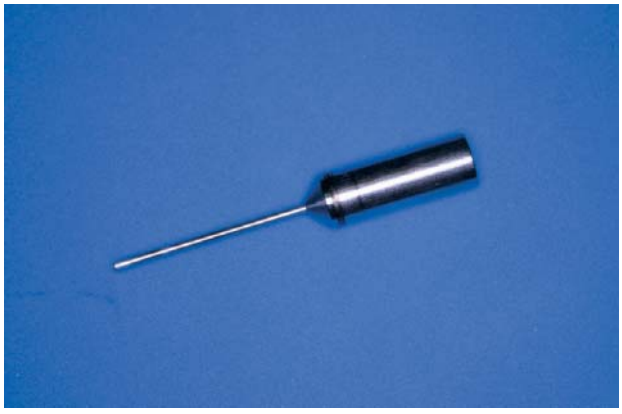
walls and cytoplasmic membranes of micro-organisms, and that differences in the sensitivity to ozonated water are probably due to differences in the structure of their cell walls (Yamayoshi et al, 1993).

There is evidence to prove that various factors interfere with pulp tissue dissolution:

a) quantity of solution related to organic tissue
b) contact surface
c) action time
d) solution volume
e) mechanical agitation
f) solution temperature
g) solution concentration

Ozonated 1.25% sodium hypochlorite can dissolve pulpal tissue.

The Healozone device for root canal Ozone



The prototype of the needle (Kavo) to enhance the application of Ozone into root canals

The needle is mounted to the Healozone handpiece

The ready-mounted Healozone (Kavo) handpiece to be used for Ozone insufflation

All the root canal obturations shown in this chapter are typical examples where Ozone was used combined with modern adhesive obturation techniques – Real Seal (SybronEndo), Epiphany (Pentron).

The ozonisation occurs at different stages of the root canal preparation.

Clinics

Case 1

This 24-year-old patient presented with acute tooth pain. Objective diagnostics indicated the pulp was affected and the need for a root canal treatment.

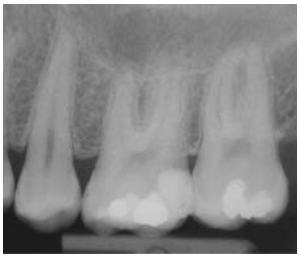


Figure 1-1: Tooth 26 needed a root canal treatment.

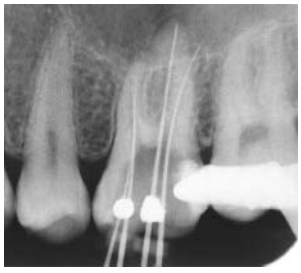


Figure 1-2: Radiographic length control confirmation.

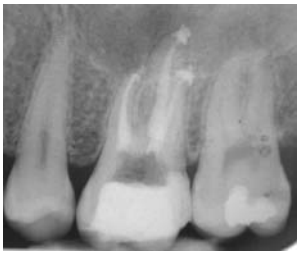


Figure 1-3: Before the obturation Ozone was applied into the canals for 40 seconds. Using warm vertical condensation procedure the canals were obturated.

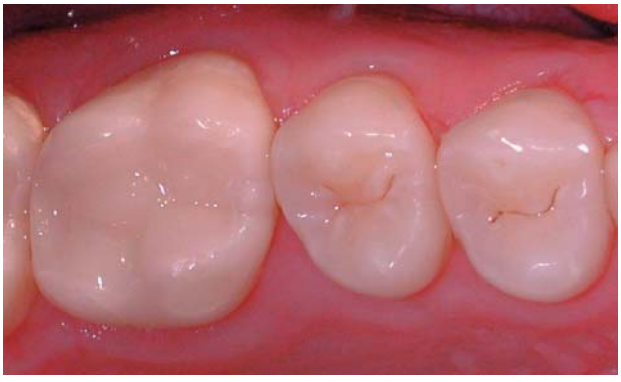


Figure 1-4: Occlusal view of the direct composite restoration performed.

Case 2

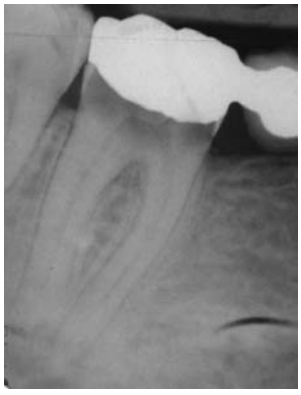


Figure 2-1: Preoperative radiograph.

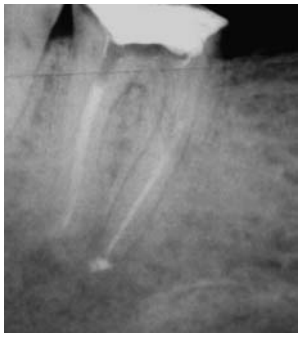


Figure 2-2: Postoperative radiograph.

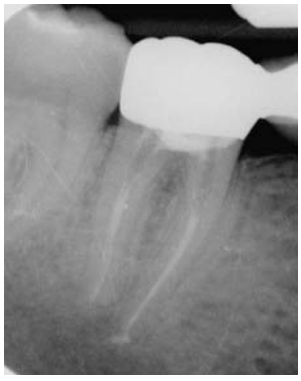


Figure 2-3: 8-month recall radiograph showing the healing of the periapical lesion.

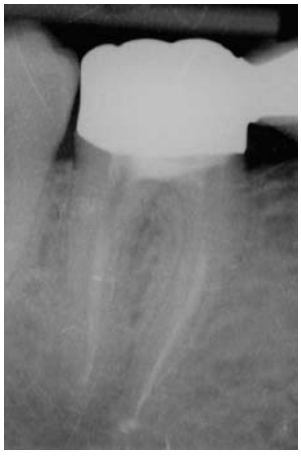


Figure 2-4: 15-month recall radiograph showing the continuation of the healing process.

Case 3

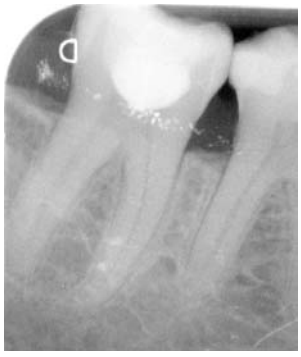


Figure 3-1: Preoperative radiograph.

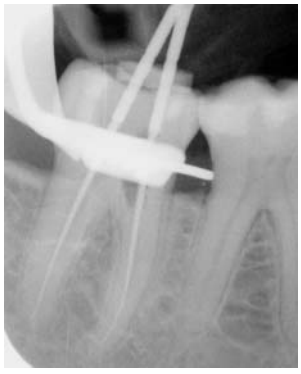


Figure 3-2: Radiographic length confirmation.

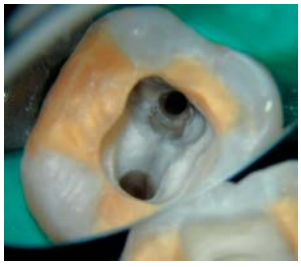


Figure 3-3: Mirror image showing the straight line access to the canals.

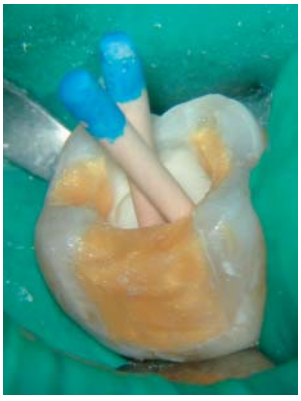


Figure 3-4: The Resilon points *in situ* (Epiphany, Pentron).

Figure 3-5: Primer is applied into the root canal using a Microbrush.

Figure 3-6: Excess primer is removed using a paper point.

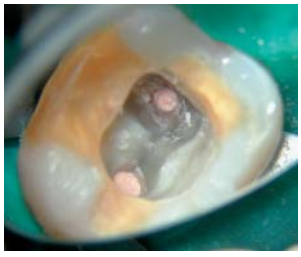


Figure 3-8: Mirror image showing the performed backfill.

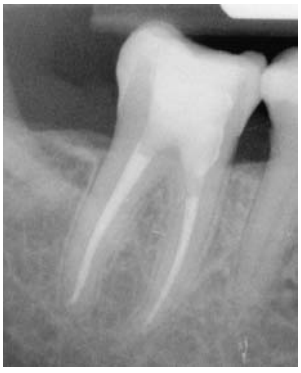


Figure 3-9: Postoperative view of the root canal obturation.

Case 4

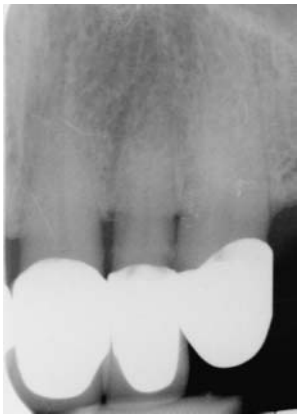


Figure 4-1: Preoperative radiograph.

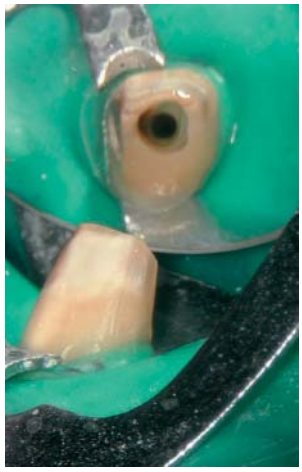


Figure 4-2: The previous restoration was removed. Image showing the coronal access.

Figure 4-3: The canal is being obturated using the squirt technique-injecting warm Resilon into the canal.

Figure 4-4: The heatable System B tip is introduced into the canal and the material is condensed apically *ad modum* continuous wave of condensation.

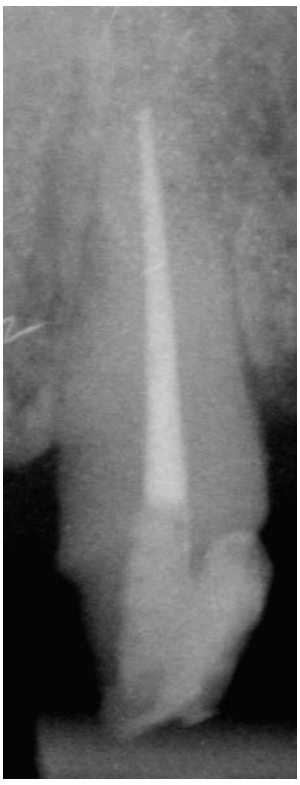


Figure 4-7: Postoperative view of the root canal obturation of tooth number 21.

Case number 5

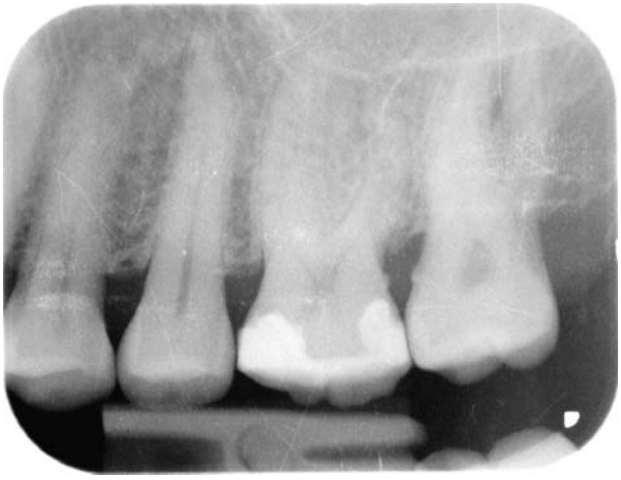


Figure 5-1: Preoperative radiograph.

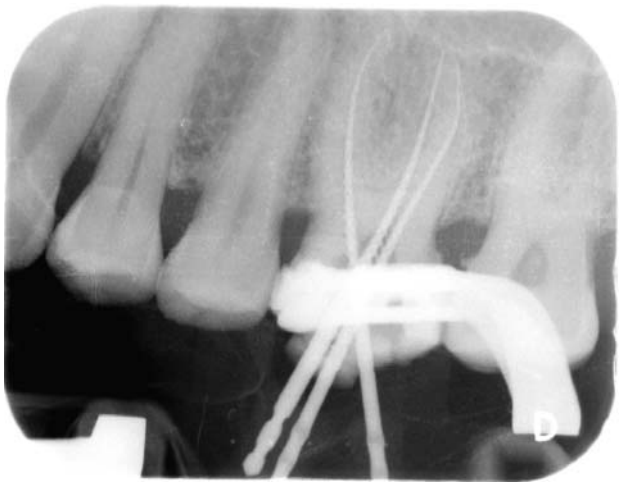


Figure 5-2 :Radiographic confirmation of the root canal length.

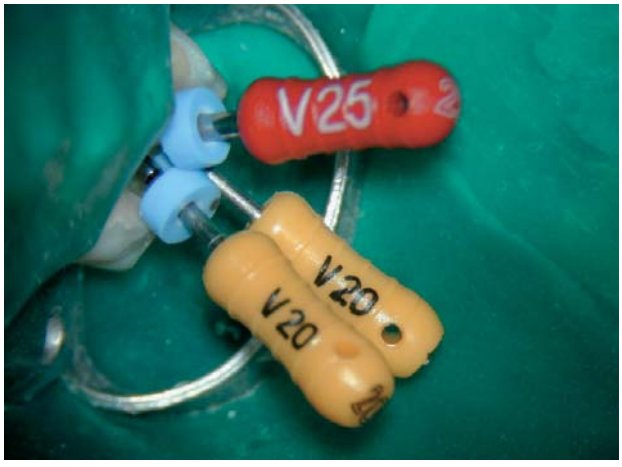


Figure 5-3: Direct picture of confirmation of the root canal length.

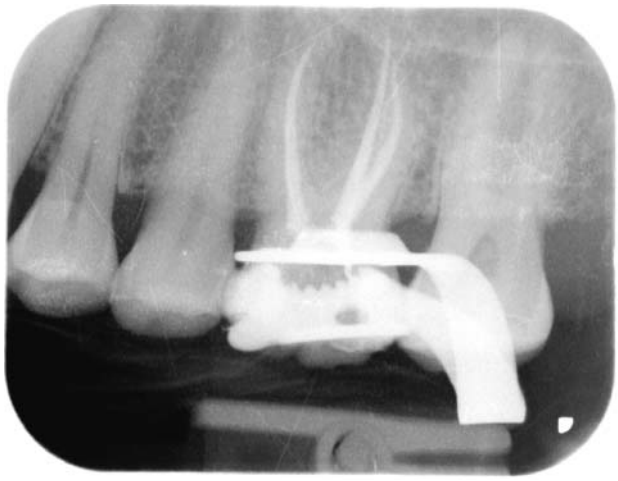


Figure 5-4: Postoperative radiograph of the performed root canal obturation.

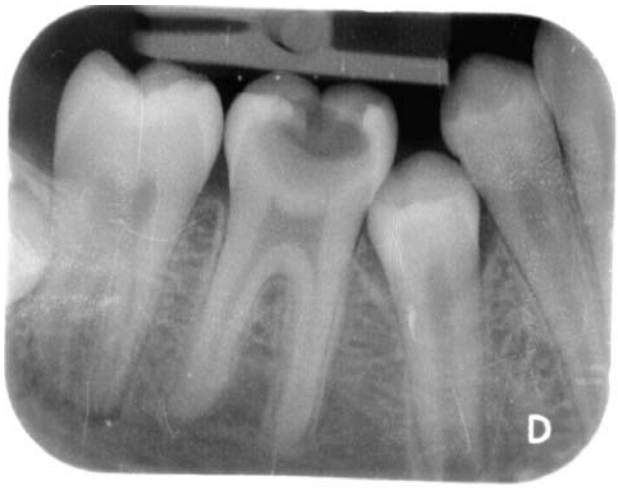


Figure 6-1: Preoperative radiograph.

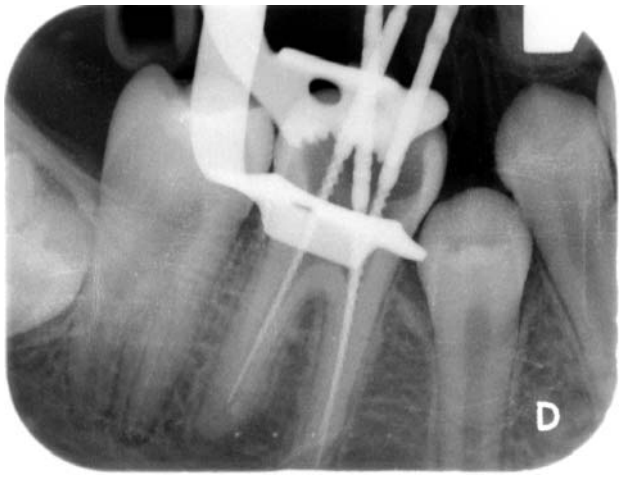


Figure 6-2: Radiographic confirmation of the root canal length.

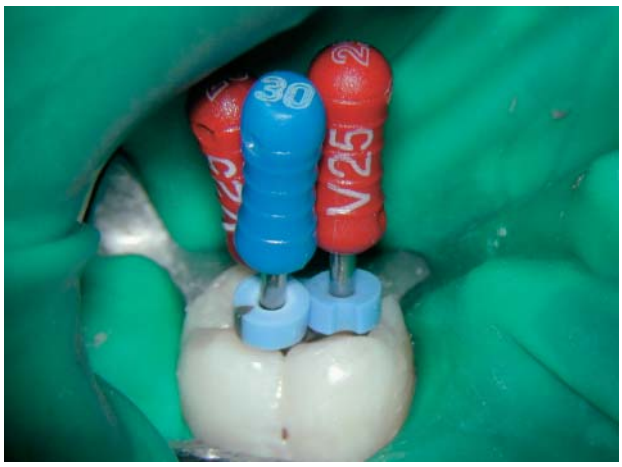


Figure 6-3: Direct picture of confirmation of the root canal length.

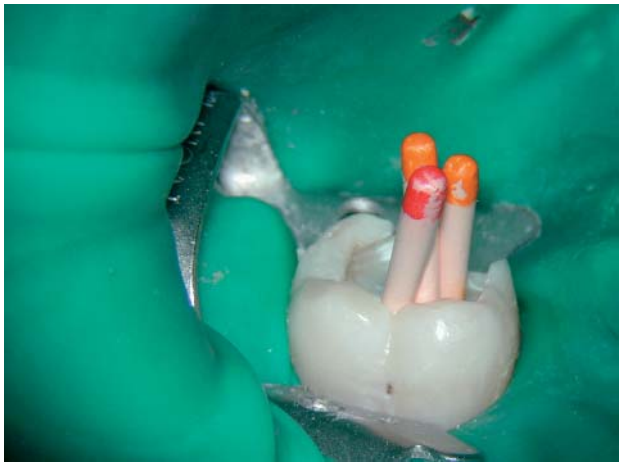


Figure 6-4: Direct view of the Resilon master cones in place.

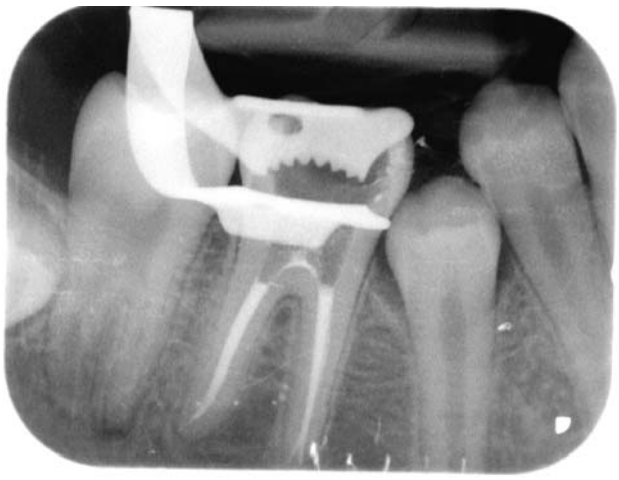


Figure 6-5: First postoperative radiograph of the root canal obturation.

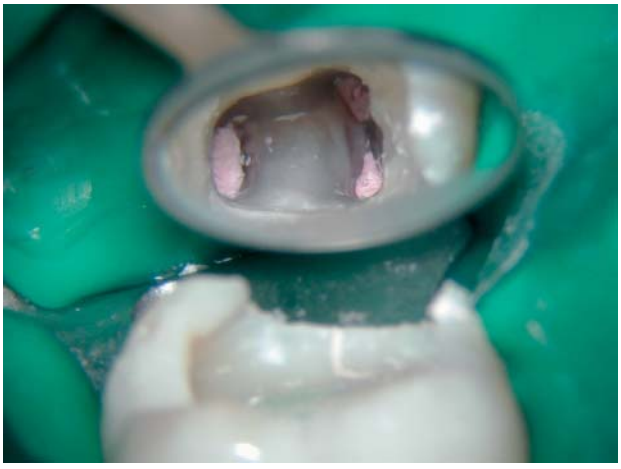


Figure 6-6: Mirror picture of the root canal obturation.

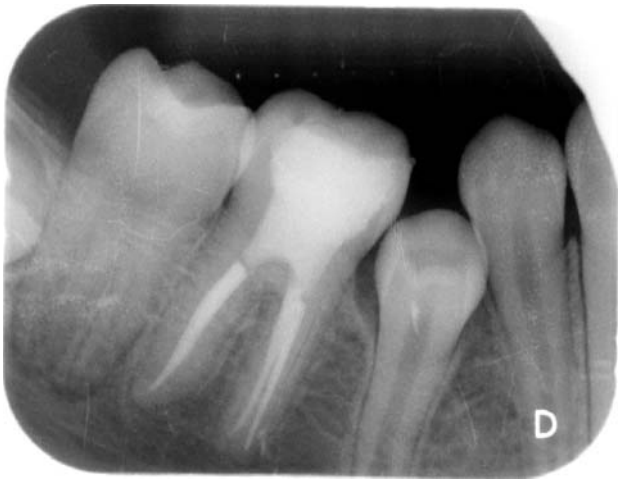


Figure 6-7: Radiographic control of the completed root canal obturation.

Case number 7

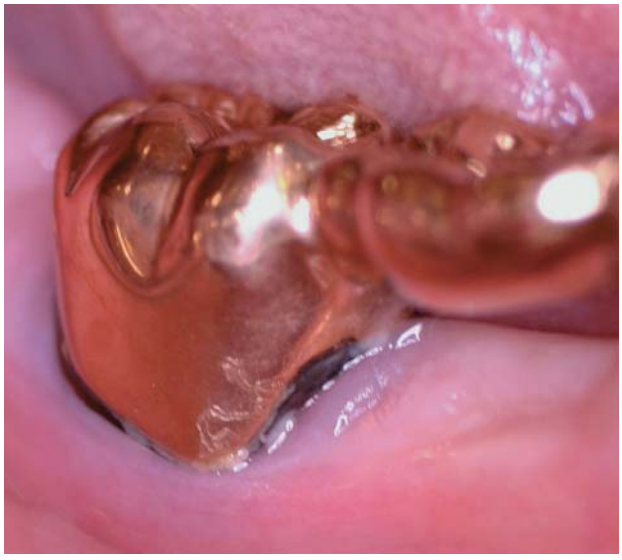


Figure 7-1: Direct clinical picture demonstrating the insufficient marginal fit of the prosthetic restoration as well as the dramatic discoloration of the tooth.

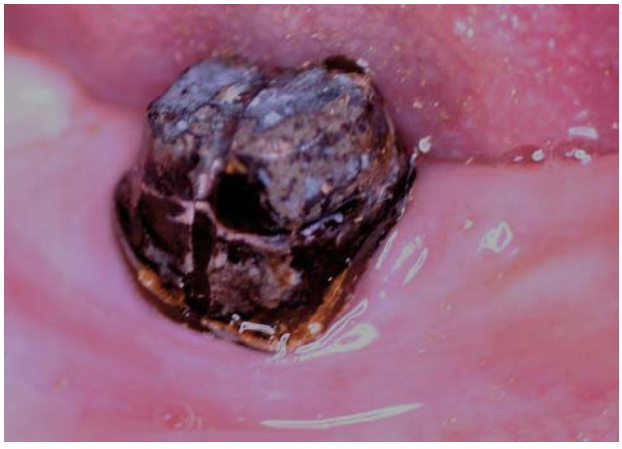


Figure 7-2: The prosthetic restoration removed reveals a strong discoloration by corrosion caused by an alloy build up under a high precious crown.

Figure 7-3: Two screwed in and cemented intracoronal posts have been uncovered after the alloy removal.

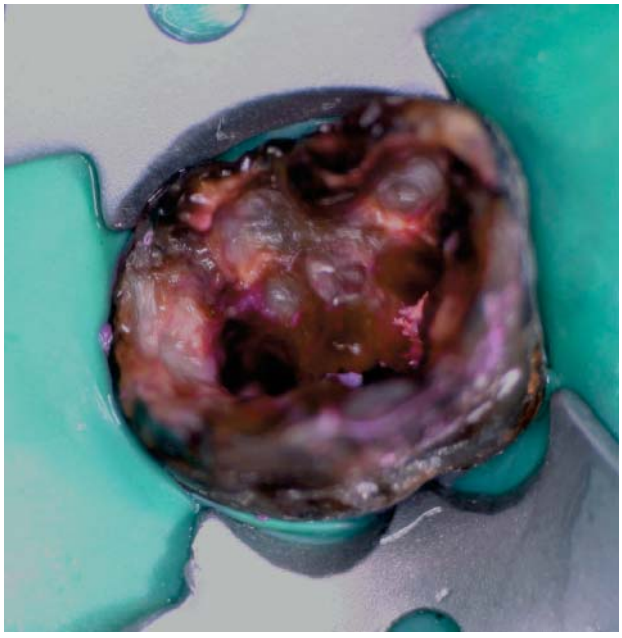


Figure 7-4: Once the posts were carefully removed the Caries Detector is used to precisely identify decayed tooth structure.

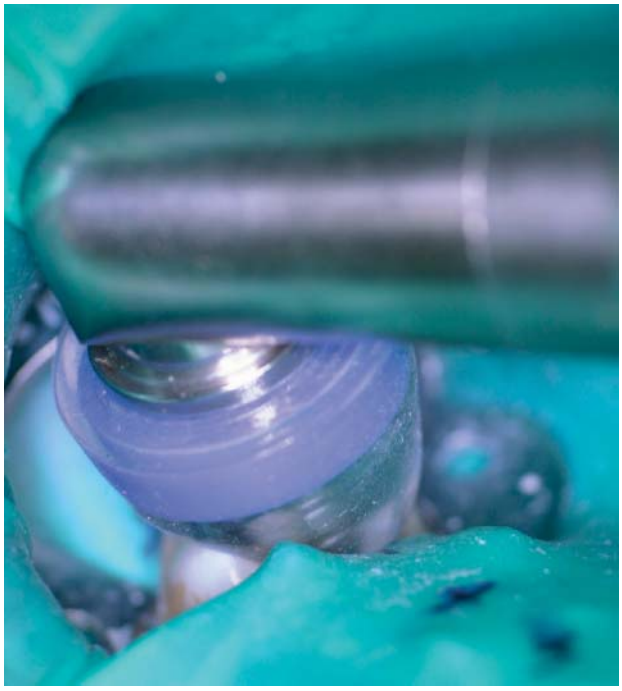


Figure 7-5: Ozone (Healozone, Kavo) is bubbled into the root canals previously irrigated with NaOCl.

Figure 7-6: To properly select the Resilon cones (Real Seal, SybronEndo, Epiphany, Pentron) Thermafill Verifiers (Dentsply) are used to gauge the deep shape of the root canal.

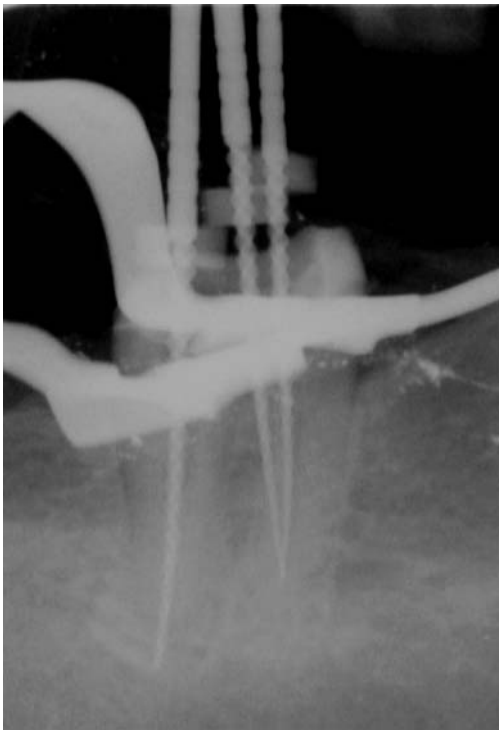


Figure 7-7: Radiographic confirmation of the working length.

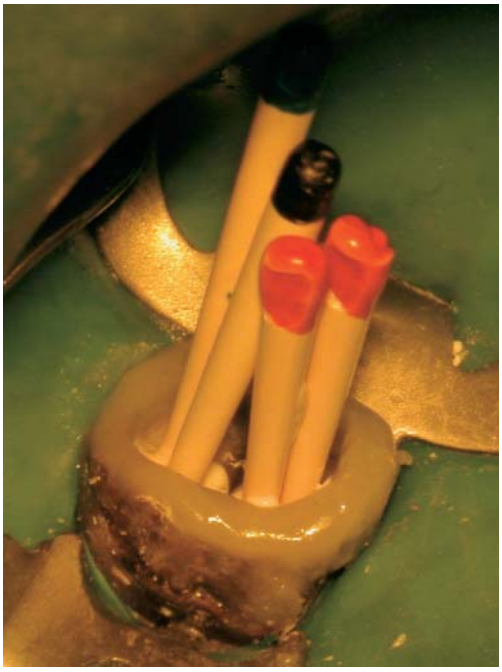


Figure 7-8: Coronal leakage has been proven to be one of the major causes for long-term root canal failures. Conventional root canal sealers do not adhere to the dentinal walls or to Guttapercha. Replacing Guttapercha by Resilon and introducing a methacrylate sealer (Epiphany, Pentron or Real Seal, SybronEndo) has dramatically improved the success rate of root canal obturation, subsequent to the use of the Healozone.

Figure 7-9: Mirror image to prove the perfect intracoronal preparation for the following adhesive build up procedure. The endodontic sealer (Real Seal, SybronEndo; Epiphany, Pentron) is dual setting. Using a light-curing device to start the polymerisation effect in the superior millimeters of the root canal obturation allows the immediate and consecutive placement of the adhesive build up restoration.

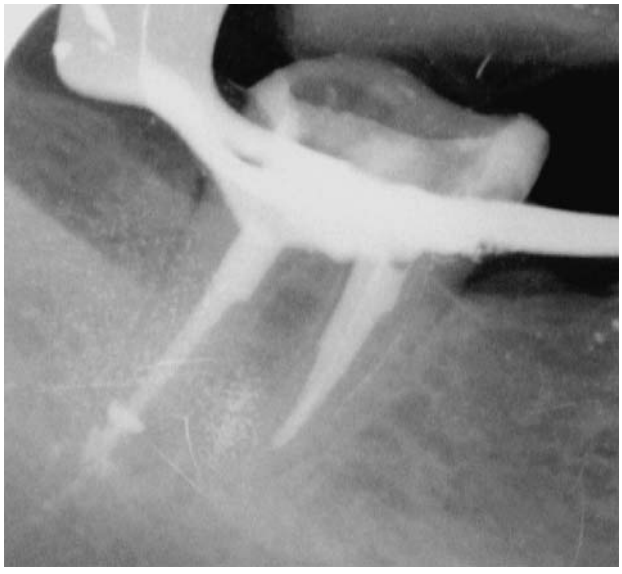


Figure 7-10: Control radiograph of the completed root canal obturation before proceeding to the light-curing of the sealer.

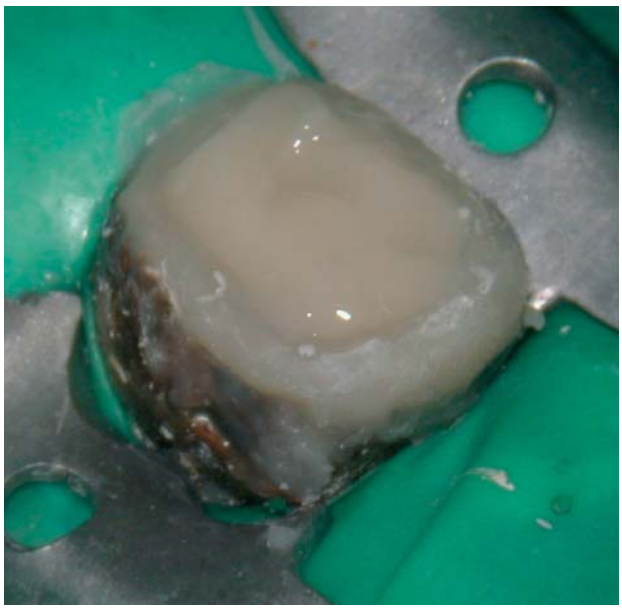


Figure 7-11: Direct picture of the completed build up procedure using a glass fibre reinforced resin cement (Build-It, Pentron).

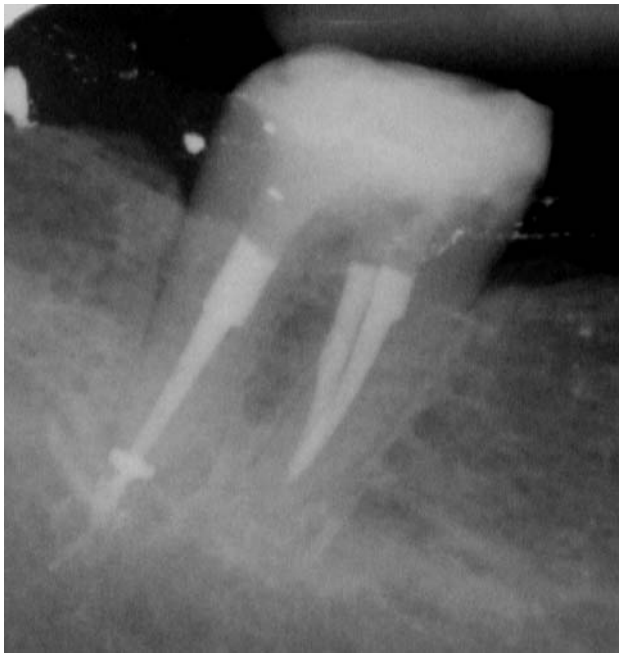


Figure 7-12: The radiograph showing the root canal obturation complimented by the adhesive build up procedure. The previous cylindrical post places are now hermetically filled up by the root canal obturation material. The distal root shows some apical extrusion, probably associated with a lack of an apical constriction.

Case 8

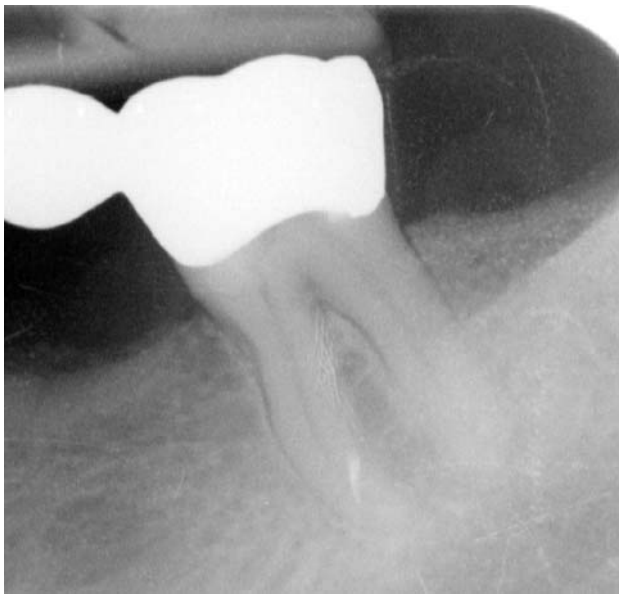


Figure Fig: 8-1: Preoperative control X-ray.

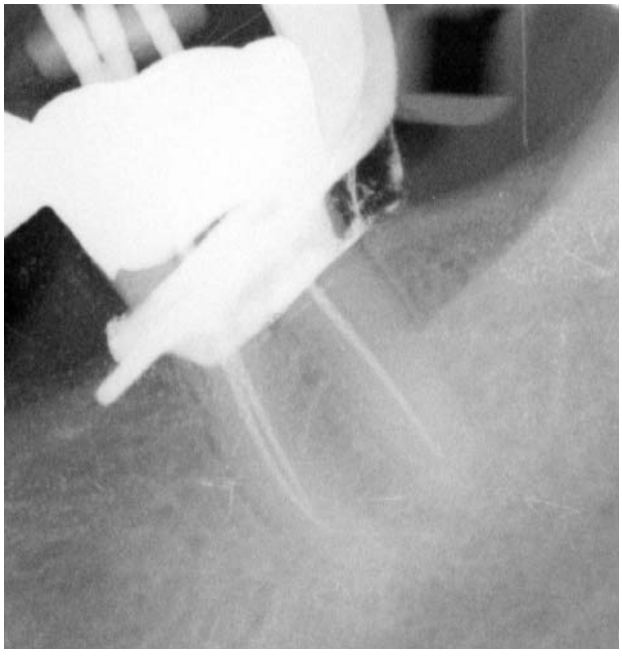


Figure Fig: 8-2: Radiographic confirmation of the root canal length.

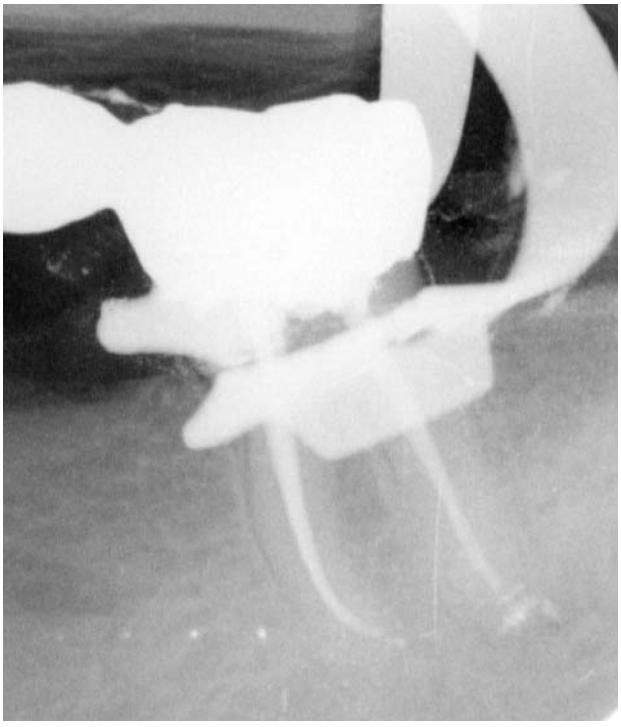


Figure 8-3: Immediate postoperative control X-ray of the performed obturation.

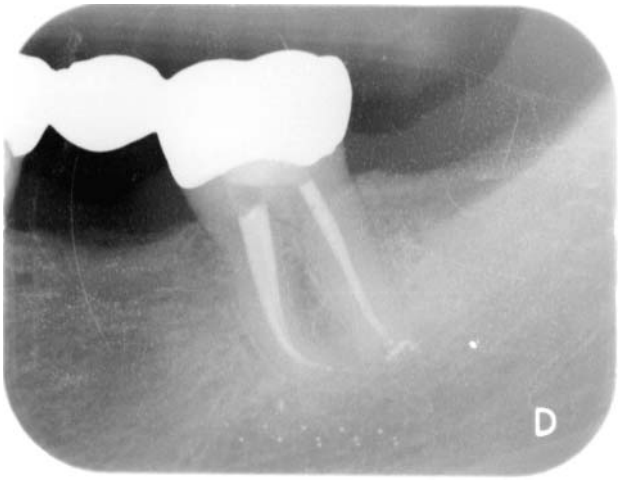


Figure 8-4: Radiographic 12-month recall.

Conclusions

The introduction of Ozone in routine root canal disinfection has reduced the required action time of NaOCl. Even teeth presenting apical, with apical lesions, could on the whole be successfully treated in one session. Ozonated NaOCl offers a tremendous time benefit making root canal disinfection predictable.

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CHAPTER 5.1

The Implications of Using Ozone in General Dental Practice

Newton Johnson, Julian Holmes & Edward Lynch

The detection of dental decay has conventionally been dependent upon direct visual assessment of the dried tooth surface under good illumination, tactile assessment on open dentinal caries using a ball burnisher, occasionally using a dental probe to help clean debris within fissures, clinical experience and the use of dental radiography. Studies have shown considerable variation between clinicians in the detection of dental caries. Recent additions to assist the accurate detection of dental caries include the DIAGNOdent (KaVo GmbH, Germany), caries detector dyes and digital radiography.

Pre-1980s, a sharp-tipped explorer, of various design, was used to 'probe' all accessible tooth surfaces for detection of any defects. It was thought correct to apply pressure on the explorer to detect any 'sticky' areas. The degree of 'stickiness' of an area was subjective and in general a sticky pit or fissure indicated a carious area that required conventional treatment. This comprised mechanical removal of the sticky area until crisp, hard tissue remained, and the restoration of the subsequent defect by some form of filling material.

In the 1980s the dental profession acquired a better understanding of the caries process. It became known that an early carious lesion is in a constant state of balance between the loss of dental mineral as a result of the acids produced by plaque micro-organisms, and the re-precipitation of mineral from oral saliva. With this knowledge came the realisation that aggressive use of sharp probes into early lesions would actually disrupt the dental tissue and create a physical cavity, causing more harm than good. Advice followed that an explorer should be used as gently as possible, merely acting as a tactile sensor for the detection of roughness, rather than being pressed into suspicious areas to see if the instrument would 'stick'.

What Changes Have Been Made in Detecting Dental Caries in Recent Times?

Front surface reflecting mirrors have provided a less distorted and sharper image of the dental tissues. There have been advances in conventional dental x-ray films providing good images with a reduction of x-ray exposure times. Clinicians have been investing in the use of magnification loupes that are a welcome advance as they can magnify the field of view by 2.5 to 4.0 times, facilitating a superior view compared with the naked eye. Intra-oral imaging systems, such as the intra-oral camera have also been introduced. Such dental cameras have become easy to use and relatively inexpensive. They are capable of producing excellent colour images of tooth surfaces. Such images can be magnified and printed as hard copies. This technology allows fine visual examination of tooth surfaces and is also a great educational tool as the patient can be included in the assessment of the area under investigation.

Digital technology has allowed advances in camera technology and digital radiography has allowed virtually instant x-ray images to be examined on the computer screen. Digital radiography has also brought a substantial reduction of x-ray dosage. Digital technology allows for computer enhancement of images that can help reveal lesions clearly.

The profession has also seen the use of various caries

detector dyes and some oral diagnostic tests to detect carious potential in the oral cavity.

In spite of these advances, accurate detection of carious lesions remains subjective and studies have demonstrated accuracy in the diagnosis of carious lesions is an ongoing problem (Chan DCN, 1993; Al-Sehaibany, White & Rainey, 1996).

Failure to accurately detect early carious lesions may often result in the individual concerned requiring extensive restorative help at a later stage when the lesion has progressed to a point where gross cavitation of the tooth occurs, or symptoms of pain arise. At this point the restorative treatment has become technically demanding, time consuming, potentially uncomfortable for the patient and has considerable financial implications for all concerned. Implications for the patient, if they are paying for their dental care, or for the nation, under a state-funded system.

Are There Any Advances to Help the Clinician Be More Objective In the Detection of Caries?

The introduction of a system capable of detecting early carious lesions with a high predictability and a new technology capable of the eradication of the carious process at an early stage, together with the therapeutic use of agents to encourage remineralisation of the lesion would be welcomed.

In the specialist dental research centres, the Electrical Caries Monitor (ECM), by Lode Diagnostics, Holland, is used to measure the depth of carious lesions. However, the ECM has considerable disadvantages for use in general dental practice. It is expensive and the repeat measurements required for reliable results are very time consuming. In its present form, the ECM does not appear ideally suited to a busy general practice setting, but it is a useful research tool.

The 'DIAGNOdent' instrument (KaVo, Germany), has been available to the profession for the past few years. This instrument is relatively inexpensive and is fairly straightforward to use in a busy general practice setting. Details about the DIAGNOdent are discussed in the Chapter from Prof Lussi, but a summary of use for general practice is presented here.

The DIAGNOdent's handpiece 'shines' a laser beam at the tooth surface and into the fissure or suspect area. The instrument measures the fluorescence of the structures in the path of the beam. It is essential that the area under investigation is as clean and dry as possible. Research has shown that the most effective method of cleansing the surface, in particular the occlusal fissures is by air polishing (Clifford, 2003; Clifford, 2004). This is a powerful jet of water, air and sodium bicarbonate in combination. There are several varieties of dental air polishing units available. The unit chosen by the ozone research teams in the UK is the 'PROPHYflex' (KaVo, Germany). This unit was shown to be effective in removing all surface debris, biofilm and stains.

After simple calibration, the DIAGNOdent scans the surface under investigation. The unit emits an audible tone throughout the measurement. The display screen on the unit provides a reading of a value from 09 to 99. The higher pitch of the audible tone indicates a larger area of decay. This is also displayed as a higher number on the display screen. It is also highly useful in terms of patient response. The patient can easily understand the higher the readout figure, the worse the lesion is. Equally the increasing pitch of the audible tone captures their attention!

The DIAGNOdent does have some shortcomings. The readout figure has a maximum value of 99. A lesion reading 99 could be significantly worse than 99 and on this arbitrary scale, it could well represent a read out of 150 or 200, or higher. A further problem is that re-mineralised enamel can be of a darker colour and that would produce a false reading on the unit. Care would also be required if part of the fissure had a tooth coloured restoration, that had the property of fluorescence, which could also give a false reading.

The DIAGNOdent readings would need to be carefully recorded on a record card, or on a computer and ideally the area examined mapped out. Sequential readings over time are essential for the monitoring of the progress of the lesion. The use of an intra-oral camera is a considerable help in mapping out the lesions. Clinical photographs of the areas in question can be taken and notes of the readings can be added to the photograph.

Understanding the Niche Environment and Dental Caries

Dental caries is the result of microbial infection of a susceptible tooth and the subsequent interaction with

fermentable carbohydrates, which commence the demineralisation by producing organic acids causing loss of mineral and the resultant softening of the tissue. The micro-organisms can have a sheltered environment within the dental tissue and with ample substrate (frequent consumption of fermentable carbohydrates) washing through the lesion the micro-organisms progress with demineralisation and the invasion of the tooth tissue. Since the 19th Century, the best practice in 'treating' decay was physical amputation of softened tissue from the tooth. Conventional treatment of the removal of this diseased tissue is by various physical means, usually, a drill. Over many years dental students were taught engineering principles in cavity design, as clearly exemplified by the work of G.V. Black. The amputated tissue would then be replaced with some form of restorative material, i.e. 'the filling'.

As we move into the 21st Century, the dental profession offers essentially the same form of treatment of dental decay as was practised in the 19th Century: drill and fill or amputation treatment. There have been significant improvement in equipment and technology over the years, but the fundamentals remain the same. Sadly, even with the highest technical and surgical skill, the drill and fill approach involves weakening of the tooth structure, compromising the vitality of the dental pulp. The procedure is also stressful for the patient involving a local anaesthetic, the drilling of the tooth and so on. This form of treatment also has significant cost implications in terms of the dental hardware involved i.e. the equipment, materials, dental staff wages, coupled with the implications of the value of the time involved for the patient to undertake the procedure.

Since the 1960s the 'prevention' movement has seen some success. The use of agents such as fluoride in various forms, to 'toughen' the enamel to help resist the acids produced by oral micro-organisms and the effect of fluoride to help re-mineralise an early carious lesion has been well established. Dietary advice to reduce the frequency of consumption of fermentable carbohydrates and better oral hygiene measures delivering fluoride have all played their part. The use of 'pit and fissure sealants' offered a logical defence of the tooth in terms of artificially eliminating these anatomical 'weak spots', depriving oral micro-organisms from colonising these areas and thereby establishing potential carious sites. The fissure sealing modality is not the success it promised to be. In spite of these preventive approaches, the drill and fill option remains the main method of the treatment of established lesions.

The development of an early carious lesion is associated with the development of an ecological niche of acidogenic (acid producing) and aciduric (acid loving) microflora biofilm. Oral micro-organisms may colonise an enamel pit or fissure, out of reach of a toothbrush bristle. With sufficient fermentable carbohydrates substrate available the micro-organisms may begin the production of various organic acids that can lead to demineralisation of the adjacent tooth structure. This effect is reversed in resting periods between eating episodes many times every day by the bathing of the area with the naturally supersaturated calcium and phosphate rich saliva and the re-precipitation of mineral into the tooth. In cases where too many fermentable carbohydrates repeatedly find their way into the area, the micro-organisms are able to produce acid at a rate where dissolution of tooth is greater than the healing effect of the saliva, resulting in a net loss of mineral. In this latter example the dominant part of the cyclical carious process is demineralisation and the carious process therefore would progress. The acidic nature of the environment within the pit or fissure, encourage further growth of acid loving and acid producing micro-organisms. The cycle repeats over months, even years, until, in the tooth crown, the enamel is breached and the micro-organisms now have the opportunity to begin the dissolution and invasion of dentine.

Early lesions can be arrested if the environment can be altered in favour of a dominance of remineralisation leading the cyclical caries process to reverse. This is not a predictable event, although a marked reduction in the frequency of consumption of fermentable carbohydrates, coupled with excellent oral hygiene frequently delivering fluoride can tip the balance in favour of repair of the lesion.

The alteration of the ecological niche environment of the tooth or lesion, namely dramatically reducing the micro-organisms, and therefore the associated metabolites such as the organic acids, allows the healing of the lesion with a far greater predictability and success. Improved biofilm control with toothpastes, sprays and mouthwashes also enhance the re-mineralisation process, especially combined with Ozone treatment. As mentioned previously, the use of fissure sealants have not resulted in the preventive success they originally promised. It was thought that the sealant would have the effect of cutting

off the food supply to the micro-organisms established in the depths of the fissure and so the progress of any carious activity would cease. This is not always the case. We now know that it is possible for leakage to occur around the margin of the sealant, which can be impossible to detect clinically. With such ingress of fermentable carbohydrate substrate the 'trapped' micro-organisms are able to continue the carious process unchecked. Visual and tactile examination of such teeth may reveal nothing significant. At this stage even good quality bitewing radiographs may fail to reveal any early demineralisation of occlusal carious dentine. Radiographic dentinal caries and its progression in occlusal surfaces was assessed in Dutch 17-year-olds in a 6-year longitudinal study (Poorterman et al, 2003). In this study, the status of 705 occlusal surfaces of first and second molars of 90 17-year-olds was analysed longitudinally in a 6-year follow-up, using a combination of clinical and radiographic information. Between the age of 17 and 23 years, about one third of sound occlusal surfaces developed a new dentinal radiolucency, and over 70% of existing radiolucencies showed progression, both irrespective of the presence of a sealant. In both examination years, almost 20% of the restored surfaces showed signs of a dentinal radiolucency. It was concluded that at the age of 17 occlusal surfaces are still highly susceptible to new dentine caries and further progression of dentinal radiolucencies already present.

The clinical and radiographic judgement of occlusal caries in adolescents has also been examined (Poorterman et al, 2000). In this study, the clinical and radiographic material of two groups of 17- and 20-year-old adolescents, born either in 1970 or in 1976, was compared to study changes in the prevalence of occlusal dentine caries and to determine the additional value of the bitewing radiographs. The first and second molars of 478 participants were included. After clinical and radiographic examination, around 33% of the occlusal surfaces of the 17-year-olds and around 25% of the 20-year-olds exhibited dentine caries. The clinical prevalence of occlusal caries in first and second molars was highly underestimated when compared with the radiographs. In the 1976 group, more sealants were recorded during the clinical examination. On the bitewing radiographs, radiolucencies were found underneath one-half of the sealants of the 17-year-olds and underneath one quarter of the sealants present in the 20-year-olds.

Weerheijm et al quantified the bacterial counts in carious dentine under restorations after 2-year in vivo (Weerheijm et al, 1999). Little was known about the long-term effects of fluoride-releasing materials on carious dentine in vivo. The aim was to investigate the 2-year influence of a resin-modified glass ionomer cement and amalgam on the bacteriological counts of carious dentine that remained under class I restorations in a split-mouth design. The enamel of the carious molars was removed, and the carious dentine was sampled under aseptic conditions just beneath the dentinoenamel junction. The molars were alternately restored with resin-modified glass ionomer cement or amalgam without further removal of carious dentine. The samples were processed for microbiological determination of total viable counts, mutans streptococci, and lactobacilli. The molar pairs of 25 patients were re-evaluated after 2 years using the same clinical techniques and were permanently restored after complete caries removal. Both materials showed a substantial decrease in numbers of total viable counts and lactobacilli of the carious dentine after the 2-year period. Compared to amalgam, the decrease in the numbers of lactobacilli was significantly more pronounced for resin-modified glass ionomer cement. Micro-organisms were detected in 39 out of the 50 molars after the 2-year period (6 resin-modified glass ionomer cement and 5 amalgam were the exceptions). Based on this study using no method to kill the micro-organisms prior to restoration, the authors suggest that complete removal of carious dentine is still the best conservative treatment, irrespective of the restorative material used.

Where individuals have experienced exposure to fluoride from an early age the physical properties of their fluoridated enamel maybe greatly enhanced and may resist early obvious clinical cavitation, in spite of being undermined by soft, carious dentine. In the past 25 years we have seen the phenomenon of "occult caries", or sometimes termed "hidden caries" or a "fluoride bomb" (Al-Sehaibany, White & Rainey, 1996). This is seen clinically where the tooth appears to be totally clinically sound, yet an alarming amount of dentine has been infected. Early carious lesions with infection in dentine are not easy to identify on bitewing radiographs. Sadly, this occult caries progresses and an otherwise sound looking tooth may suddenly collapse revealing a massive cavity; sometimes a radiograph will reveal a seemingly perfect rim of enamel, but a radiolucent lesion beneath which may occupy the entire circum-pulpal dentine.

The introduction of optimum concentrations of

fluoride into drinking water and oral health care products such as toothpaste has reduced dental decay over the past few decades. The concern for the profession is the question of whether we wIll see primary pit and fissure caries becoming a problem later in life. Fluoride in concentrations used in oral hygiene products may be delaying the overall progress of caries rather than arresting the process.

An interesting social change over the past few years is the increasing use of bottled water in the family home. Many health conscious adults feel bottled mineral and spa water offers a healthier option compared with drinking tap water. Some individuals may also prefer to use toothpaste that does not contain fluoride. This may have an impact on their dental caries risk. The preventive use of a novel agent, such as ozone therapy, could offer a great help for such people.

The key to success in helping prevent the spread of pit and fissure primary carious lesions is accurate detection. We have mentioned some of the difficulties faced by clinicians in correct detection. The primary fissure carious lesion accounts for some 70% of all new decay encountered in dental practice. An essential part of the original research into the use of ozone in the treatment of dental caries was to use accurate and reproducible methods to detect and measure the extent and depth of decay.

How Ozone Therapy Can Affect the Ecological Niche Environment

The ability to treat a carious lesion without the need of amputation of the diseased tissue would be one of the greatest achievements in the history of dentistry. Ozone therapy has the potential to move toward this goal. Remineralised dental tissue is far less likely to decay again. The resistance of further decay may be attributed to many factors including a change in the morphology of the dentine, an altered mineral content in the tooth and changed hardness of the tissue all of which contribute to an increased resistance to new acid attack. Mineral re-precipitation within the dentinal tubules can obliterate the tubular structure, thereby rendering it impenetrable to micro-organisms and their acidic metabolites. Furthermore, the higher fluoride and metal ion content of the newly mineralised tissue offers enhanced resistance to future potential acid attack. The provision of ozone therapy facilitates the remineralisation of lesions. Studies show that caries reversal occurs after Ozone treatment. This re-mineralised tissue can then support a restoration, bringing the once diseased tooth back to full function.

In addition to the detection tools described above, a clinician should have at their disposal some form of 'Clinical Index' to assist their assessment of teeth for carious lesions and these are discussed in chapter ??.

It is believed that a number of important changes occur within the carious lesion during ozone treatment. Firstly, any infection by micro-organisms is dramatically reduced. Further, pyruvic acid produced by the micro-organisms is converted to acetate and carbon dioxide. It is also possible that some proteins, which are natural inhibitors to remineralisation, are also reduced and some dentine channels maybe opened. These factors allow the bioavailable minerals from the patient's super-saturated saliva, aided by the re-mineralising rinses, sprays and toothpastes, to remineralise the lesion, penetrating the tissue. This morphological change, together with the inability of the ecological niche of acidogenic and aciduric micro-organisms to re-establish easily, creates a favourable environment to encourage re-mineralisation, rather than to be prone to further decay.

There is a logical argument to consider this treatment modality as a potentially highly powerful prophylactic tool in the routine ozone treatment of erupting dentitions in caries risk children. There are compelling arguments to support the cost-effective use of ozone therapy as a prophylactic agent.

All the research findings, and the action of ozone in dramatically disrupting the micro-organism's ecological niche, offer a new hope to future dental health. If newly erupted teeth were ozone treated, coupled with preventive advice, there should be a significant reduction in the need for future costly restorative care. This has important implications in public dental health terms.

Conventional drill and fill dentistry involves the amputation of a considerable volume of diseased tissue. With ozone therapy and re-mineralisation, only minimal quantities of dental tissue need be removed to facilitate a restoration of the tooth. This makes the restorative treatment much simpler, less time consuming and much more cost-effective. For the patient, it is far more comfortable than drilling and filling.

Prevention of disease has always been the most cost-effective modality for any health care system. Modern dentistry has the prevention of dental disease at its heart. We have seen success in the reduced incidence

and prevalence of dental decay over the past 30 years, but sadly we still see areas where dental decay poses a significant problem in the community. With an improved strategy of oral health education coupled with prophylactic ozone therapy, there are considerable potential benefits in oral health terms.

In general dental practice, the benefits of the utilisation of ozone therapy as opposed to drill and fill would seem clear. Ozone treatment takes a mere 10 to 40 seconds per tooth, and requires a minimum amount of disposable items, such as cotton rolls and so on. Conventional drill and fill treatment can take at least 20 minutes and often a lot longer. It also requires expensive restorative materials.

In England and Wales, the National Health Service figures revealed that for the year 2001/2 some 19.7 million fillings were placed at a cost of £223 million pounds. Conventional fillings weaken the structure of the teeth and the future often brings the need for repeating the restoration as the structure of the tooth/filling break down. The vitality of the dental pulp may also be compromised and endodontic therapy is often required, significantly adding to the cost.

If we postulate that just 45% of these cases could have been successfully treated by ozone then the saving to the National Health Service in England and Wales would have been around £100 million for the single year.

Ozone can now be considered as a realistic alternative management strategy for dental caries and is supported by the published literature. Caries reversal is associated with several factors including the dramatic disruption of microbial infection of the dental tissue and the damage to the ecological niche that supports the acidogenic and aciduric micro-organisms. Shifting the microbial flora in carious lesions to one containing normal oral commensals would predominately allow remineralisation to occur within the lesion.

Patients are astonished by the comfortable nature and the simplicity of the ozone therapy. No local anaesthetic injection, no drills and an appointment time measured in a few minutes. The implications for this new treatment to a general dental practitioner are profound. Ozone therapy has the potential to reduce the cost to a government sponsored dental scheme of restorative treatment. It has unique potential in terms of dental care in developing countries and the poorer regions of the world. For private dental practitioners we have the opportunity to offer patients a totally pain free treatment.

Conclusion

Ozone has been successfully utilised by the medical profession, especially in Russia and Cuba, for around 100 years. The purification of public water supplies in over 6000 cities world wide, including the USA, and the purification of air conditioning units have proven successful over many years. In a nutshell ozone is highly efficient for the elimination of bacteria, fungi and viruses in a variety of situations.

Ozone therapy has the potential to reduce the cost of restorative treatment for both private patients and for government sponsored dental schemes. It offers unique potential for dental health programmes in developing countries and the poorer areas of the world.

Currently, active research is underway into the therapeutic use of ozone in many areas of dentistry, such as endodontics, periodontal therapy and in the treatment of various soft tissue infections. Researchers are examining the beneficial applications of ozone in sterilisation of dental water lines as will be discussed in chapter ??. In fact, in any situation where micro-organisms are producing pathological potential, ozone has the ability to offer a simple and effective solution.

In summing up, the current research is indicating that ozone has proved to be an exciting advance for the dental profession with substantial and far-reaching implications in the delivery of dental care in the 21st Century. Ozone is a powerful anti-microbial agent with the ability to penetrate hard and soft tissues. The benefits of ozone therapy are reduced costs and the potential elimination of dental treatment phobia. Crucially, the profession may now have the ability of treating dental caries without the recourse to the needle and drill.

In the following chapter, the cost implications of the incorporation of ozone therapy in a general dental practice, and its potential in the public dental health setting will be considered.

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CHAPTER 5.2

Cost Implications of using Ozone in Practice

Julian Holmes & Edward Lynch

To date, in the majority of dental practices worldwide, caries detection and therefore the elimination of decay depends on visual recognition with the aid of a mirror, probe and x-ray analysis. Published studies have shown that these traditional systems can be inaccurate. Recent additions to the way the dental profession carry out routine examinations include computerised analysis of digital radiographs, dyes and tests to look for the caries risk of the individual.

If the cost of dental professional's time, a basic examination set of instruments, sterilisation equipment, and a minimum of two bite-wing X-rays is factored for every patient, and set against the cost of fillings required due to the failure to diagnose missed early carious lesions, the financial implications are huge for dental programs financed by any central government or managed care program. Surely the introduction of a system that can detect the early carious lesion reliably, and a technology that can eradicate the carious process at an early stage and start the process of remineralisation, has to be the most cost-effective. It has to be a win-win for both the care system, and the patient involved. An advanced dental care system, coupled with the potential of zero dental disease, as well as keeping a complete dentition to old age in pristine condition, is potentially possible, and it is available now.

The medical profession has used Ozone for some 100 years, with much research being carried out in Russia and Cuba. A search of 'Ozone Medicine Uses' on the Worldwide Web will bring up hundreds of references on the topic. All the information studied showed that the elimination of all bacteria, viruses and fungi is possible by the application of Ozone. Ozone has also been used in commercial applications for over a century, and is now used to purify public water supplies in over 6000 cities worldwide, including the USA. Ozone is also used to eliminate pollution in air supplies in hospitals and other buildings.

21st Century Principles

Dentists leave their places of training as masters in the art of tissue amputation, holding in one had the reliable central suction tip, and in the other, some version of a rotary destruction system. These systems were designed with the basic premise of fast tissue removal, so that the diseased part of the tooth could be removed in a minimum of time. The premise, based on the designs and teachings of the 19th century, was to find firm foundations. It was, and is, a very sound principle of an era that produced engineering feats that still span gorges and rivers around the world today, and those skyscrapers that stand as testimony to those engineering principles of firm foundations. Research and experience has show the dental profession that if these principles were not adhered to, then restorative materials failed as the decay process continued. No wonder that the dental profession has been very wary of any new technology that claims to provide firm foundations, without first removing the soft diseased tissue.

The process of mineral loss from enamel and dentine is well documented and researched. This process of demineralisation is explained by the acid niche theory. Let's briefly review the theory of the development of a carious lesion, as this is crucial to the understanding of the new technologies to be discussed. The 'niche environment theory' is now accepted to explain the process of initial colonisation through to the development of micro-organisms in a specialised niche environment. As the bacteria collect, for instance in a fissure, they produce acid which leads to the loss of mineral content

in the enamel surface. This is known as demineralisation. This is offset to some extent by the balance between demineralisation of the enamel surface by these acids, with remineralisation by the hosts' normal oral environment. As the numbers of acidogenic and aciduric micro-organisms increase, the niche becomes predominately acidic, attracting more acid producing microbial species. Over time a cavity forms by demineralisation and tissue loss, offering a degree of protection for the bacteria during oral care and hygiene sessions. The process of niche development may take many years. The dental profession already knows from previous studies that decay can be reversed in some cases. However, remineralisation on its own is NOT predictable, as research has shown an additional factor is required to make this process of re-mineralisation predictable, by eliminating the micro-organisms that established the niche (Holmes J, 2003). Is this process alone enough? Probably not, as re-colonisation will occur, as the micro-organisms metabolites are still present in the lesion. So the last piece of the puzzle is to find a process that also can denature these metabolites AND eliminate the micro-organisms predictably as well as priming the lesion for remineralisation. Improved oral care and the use of mineral mouthwashes and dentifrices are not enough alone but should be part of any oral health care programme to lead the carious process to have a predominance of remineralisation.

The First Preventive Systems

Some dental practitioners in the UK are old enough to remember the first fissure sealant preventative systems. The dental profession was taught that these fissure sealants should be used on teeth that were most at risk to decay, namely the first permanent molars. At the time of treatment, many of the fissure patterns were already stained and potentially had first stage decay present. The profession in this era had few diagnostic aids that could show the busy general dental practitioner if these stains were stains, or were the result of an active dental decay process.

The protocol used for the placement of fissure sealants in the mid 20th Century was taught as follows; as soon as a tooth erupted, and isolation could be achieved, it should be fissure sealed. As the vast majority of decay occurs in the occlusal surfaces, dentists were encouraged to protect these surfaces as soon as possible. Pumice was used to clean the tooth surface with a bristle brush. This 'cleaned' enamel surface was then acid etched, washed and dried. The first fissure sealants were a two-part resin. A catalyst was mixed with a base, the mixed sealant was then 'floated' onto the prepared surface, and the operator had to wait for it to set. Undergraduates in the mid and late 20th Century were taught that it did not matter if there was incomplete removal of micro-organisms. For once the sealant had been applied, it was postulated that no food substrate could reach any micro-organisms trapped deep within the fissures, so the carious process would halt. Of course, after a great deal of clinical experience and research the profession realises this is not always the case. Research has shown that micro-leakage at the edges can occur with this type of system, and that the bristles could not clean out the fissure pattern totally due to the difference in the large size of the bristles compared to the small dimensions of the tooth surface grooves and fissures. Most of the contaminated debris may be left in situ and some bacteria are unaffected by acid etchants. Many dental practitioners reported cases years later where the decay process has slowly continued, leaving a hollow shell of enamel. This could be explained by micro-leakage around the margins, allowing the ingress of food substrates to the trapped microbial colonies. In this protected niche, the carious process continues unchecked. Periodic visual examination may only show a slightly stained margin to the fissure sealant. It is not until the carious process has progressed considerably into the dentine, that changes on radiographic examination will show the presence of decay.

The use of fluoride containing oral health care products have made the detection of occlusal decay more difficult. The dental profession has been very successful in instituting the treatment of whole populations of areas within countries, and whole countries, by either additional quantities of fluoride in drinking water, and/or by the recommended usage of tooth cleaning pastes, rinses, sprays and gels with additional fluoride. Fluoride can mask vital signs the dental profession look for when trying to detect caries. The problem here is that by the time visible signs on a radiograph can be found, the carious process has progressed well beyond the early lesion, and has become invasive into deeper tooth structures.

So how can the dental profession improve the diag-

nosis of a stain versus an early carious lesion, as this is one of the hardest diagnoses to make? Early occlusal surface decay accounts for up to 90% of all new decay seen in dental practice. Part of the research into ozone technologies was to look at a reliable and reproducible way to measure decay. In the research centres, the ECM unit (LODE Diagnostics, Netherlands) is a very reliable research tool to measure the depth and extent of any carious lesion. Research has shown that when the ECM readings are compared to histological examination of carious lesions, there is a significant correlation between the extent of the lesion, and the readings obtained. The disadvantage of the ECM is cost and time. The ECM takes some 5 minutes to set-up to obtain a reading. For each lesion, up to 5 measurements need to be taken for accuracy. The KaVo DIAGNOdent has been available for over 3 years now and works by 'shining' a laser beam into the tooth surface. The level of decay is expressed as a number on the screen, and as an audible tone. The higher the number and tone pitch, the larger the area of decay. The DIAGNOdent measures the fluorescence of bacteria, and the presence of decay. However, the DIAGNOdent also has significant shortcomings too. For example, remineralised tissue can also be darkly stained, so there can be erroneous values and assumptions. The unit only reports a maximum value of 99, so a lesion measuring 99 could be 99, or 100, or 150 or 200 or higher, and it also will return a value with composites that fluoresce as well as calculus and excessive plaque overlying lesions.

Clean Tooth Surfaces are Essential for Reliable Data

The recommended treatment protocol for assessment with the DIAGNOdent is the thorough cleaning of the surfaces of every tooth before assessment. The ProphyFlex System from KaVo is used to clean the surface thoroughly, ensuring the DIAGNOdent measurement is not from impacted debris or stains. The working tip of the ProphyFlex pushes out a slurry mix of sodium bicarbonate and water under pressure from a standard high-speed turbine outlet, and it is very effective at stain and debris removal. The DIAGNOdent is then used to measure the areas that are suspect. The DIAGNOdent data needs to be recorded, so that at recall, the data can be evaluated for success or failure of the treatment. For practices that are computerised, a custom screen in a computer management system can be designed and used to record this data. If access to an intra-oral camera is possible, clinical photographs can be used to record the tooth, and then notes of the readings can be added into these photographs for each lesion.

All values are noted for future reference. Past dental care may have consisted of minimal cavity preparation, with the use of amalgam, glass ionomer cements, resin bonded composites, or orcimers. In all these cases, a degree of invasive tissue destruction was necessary either to remove the diseased tooth tissue to access firm and disease free tissue as a base for the filling material, or to provide retention for the filling system used. It is estimated that many tons of tooth tissue are drilled or amputated each year which have been shown in the evidenced based dentistry research to be manageable with Ozone alone. This tissue is lost forever, and the tooth compromised for the remainder of its lifetime. This ozone research in the 21st century allows the majority of the early carious tissue to be retained and the disease process eradicated as well as providing a firm foundation for the support of the proposed filling system. And once the carious tissue has remineralised, no research has ever shown that it can subsequently decay again.

Studies showed that carious lesions could be reversed, resulting in hard, remineralised tissue (Baysan, 2000; Baysan et al, 2002; Lynch et al, 2001). This remineralised tissue could then be used to support a filling system. The huge benefit of this research is that diseased tissue does not always have to be amputated. After a short treatment with ozone and a period of time to remineralise, the remineralised tissue is so hard that modern resin bonded fillings, glass ionomers and traditional filling systems can be used to restore the lesion to full function and occlusion.

The Benefits to a Centrally Funded Dental Care Program

The potential benefits to any centrally funded dental care program are huge. Let us look at the savings in time first. The time to treat a lesion with the Heal-Ozone is measured in seconds, not minutes. In initial studies the treatment time was 10 or 20 seconds, depending on the assessment of the lesion (Baysan et al, 2002). Holmes and Lynch in their 2002 study showed

that by modifying the treatment times by varying them between 10 and 40 seconds, the rate of caries reversal could be predictably increased to over 95% (Holmes & Lynch 2002). Holmes, Abu-Naba'A & Lynch modified the Ekstrand Index to allow easy and fast indexing of lesions in a general dental practice environment, and published the Clinical Severity Index (CSI).

The Ekstrand Index has 7 categories as follows:

Table 1a: The Ekstrand Index

0	No or slight change in enamel translucency after prolonged air drying (>5s)
1	Opacity (white) hardly visible on the wet surface, but distinctly visible after air drying
1a	Opacity (brown) hardly visible on the wet surface, but distinctly visible after air drying
2	Opacity (white) distinctly visible without air-drying
2a	Opacity (brown) distinctly visible without air-drying
3	Localised enamel breakdown in opaque or discoloured enamel and or greyish discolouration from the underlying dentine
4	Cavitation in opaque or discoloured enamel exposing the dentine beneath

Table 1b: Clinical Severity Index for pit and fissure carious lesions

Index	Characteristics	Treatment Indicated
6	SEVERITY INDEX 6 Lesion deemed to require drilling and filling (define this as deemed to have infected dentine extending more than one mm into dentine).	Operative intervention and 40 seconds with O3
5	SEVERITY INDEX 5 Lesion deemed to require drilling and filling (define this as deemed to have infected dentine extending one mm into dentine).	40 seconds with O3
4	SEVERITY INDEX 4 Lesion deemed to require drilling and filling (define this as deemed to have infected dentine extending less than one mm into dentine).	30 seconds with O3

Continues next column

Table 1b: Continued from previous column

Index	Characteristics	Treatment Indicated
3	SEVERITY INDEX 3 Lesion deemed to require drilling and filling with a preventive resin restoration (define this as deemed to have enamel caries extending to the amelodentinal junction).	20 seconds with O3
2	SEVERITY INDEX 2 Lesion deemed to require fissure sealing (define this as deemed to have enamel caries confined to enamel and not extending to the amelodentinal junction)	10 seconds with O3
1	SEVERITY INDEX 1 Lesion deemed to be reversing (defined as deemed to have infected demineralised dentine or carious enamel, which is reversing. This scenario is where clinical remineralisation of the underlying dentine is considered to be in the process of remineralising the demineralised dentine but is not yet complete. The frosted enamel in the fissure (visible after drying) will be reducing).	10 seconds with O3
0	SEVERITY INDEX 0 Lesion arrested (defined as deemed to have had infected dentine which reversed and where clinical remineralisation of the underlying dentine is considered to be complete, with no infection remaining in the dentine as well as remineralised enamel with no frosting on drying).	No seconds with O3

The modified Clinical Severity Index (CSI), was developed to assist dental practitioners assess carious lesions and provide a guide to management of the lesions using Ozone. It has become one of the important indices that allow data from multiple sites to be collected, collated and analysed using reproducible criteria that the general dental practitioner can easily use. Over time, the CSI can be further modified to increase the predictability throughout different countries. The use of the DIAGNOdent provides a simple and quick method and a nu-

merical value to record the severity of the lesion. When the DIAGNOdent value and the CSI are used together, the dental profession has a very powerful, but easy to use, tool to first assess a lesion, and then evaluate how this lesion has fared over a period of time.

One area that has caused a great deal of anxiety amongst the early adopters of the HealOzone units has been the treatment of large carious lesions. Typically, these seem to have a DIAGNOdent value of 99 or more. The DIAGNOdent records values from –9 to 99. The screen cannot show values outside this range. For the vast majority of cases this is not a problem. But with the lesion that has a value of 99, the true carious value may be 99, or any other larger value. When "hidden" occlusal pit and fissure carious lesions are detected extending more than 1 mm into Dentine the treatment protocol is to remove the peripheral infected dentine and enamel whilst leaving the slightly softened caries overlying the pulpal floor, and then apply ozone into the cavity for 40 seconds.

One aspect of remineralisation that has the potential to make a huge impact is that once caries arrests, this arrested area has never been shown to be involved in decay again. Studies have shown that the resistance to decay is due to a change in the morphology of the dentine, a change in the mineral content in the enamel, coupled with changes in the hardness and resistance to acid attack.

It is believed during the process of ozone treatment, a number of important changes occur within the carious lesion. The specific ecological niche infection by selective micro-organisms is eliminated. Furthermore, acidity is reduced in these lesions as pyruvic acid produced by micro-organisms is converted to acetate and carbon dioxide. Some proteins which are natural inhibitors for remineralisation are also removed. It is also possible that dentine channels are opened which might assist remineralisation. These factors allow the bio-available minerals from the patient's saliva, naturally supersaturated with calcium and phosphate, as well as the use of remineralising mouth rinses, sprays and dentifrices to flood into the lesion; and penetrating the lesion. In the research studies, remineralisation occurs throughout the entire depth of the lesion, not just in the superficial layers. This could be contrasted with resin dentine-bonding agents that are very superficial in their depth of penetration. The new mineral deposition in the dentinal tubules obliterates the tubular structure, so rendering it impenetrable to bacteria. This morphological change, as well as the inability of the pathogenic ecological niche to re-establish in a short time span, means that the lesion will remineralise, rather than be prone to further decay. Lastly, the high fluoride and calcium mineral content of the new mineralised tissue means it is very resistant to acid attack.

There is a good evidence to assess the prophylactic uses of ozone treatment modality. Holmes has treated 348 newly erupting occlusal pits and fissures in teeth of caries prone children for 26 months using 10 seconds of ozone treatment every 4 months. Not a single tooth has required any drilling or filling in this population group. There are firm cost effective arguments for this type of use of the ozone technologies. In comparison to the cost of placing fissure sealants, their potential failure and the cost of providing restorative repair at a later time, ozone-treated teeth should, in theory, be very resistant to carious development. Research has shown that virtually all stained fissures have some enamel decay present. If newly erupted teeth in caries prone individuals were ozone treated, coupled with regular and improved preventive care, there should be a dramatic reduction in the cost of provision of dental care. Research is required to show what frequency of ozone treatment is required to achieve the best results.

Once remineralisation has taken place, and a firm foundation for any restorative care developed, the amount of tissue that has to be amputated is minimal or zero. This has a number of very clear benefits. The amount of tooth tissue that is usually destroyed during historic amputation therapy is huge, compared to the volume of original tooth tissue. In most cases where exposure of the pulpal tissue would be expected, the remineralised tissue avoids the need for expensive root canal treatment. And of course, if the tissue no longer has to be removed, the material used to restore the tooth is reduced, so driving down the cost of restorative care.

Finally, as the carious lesions can be treated at a very early stage, time taken off work and lost productivity to employers is reduced. Prevention has always been the most cost effective treatment modality for any centrally funded health scheme or managed care system. Whilst prevention has always been the goal of the dental profession, until now the tools for predictable and effective prevention have not always been available. With improved oral health care advice and prophylactic ozone treatment, there are huge benefits and compelling rea-

sons for this technology to be embraced into socialised dental care programs.

From the aspect of a modern private dental care program, the benefits of such a system outweigh the cost of the initial investment into the equipment required. If the cost of a 10 to 40 second treatment time, the disposable treatment items and wear & tear of the unit, are compared to the cost of preparing and placing a filling, its replacement some years later with further loss of tooth tissue, there are huge cost saving implications for any centralised managed and funded dental care schemes.

For example, the recent figures for England and Wales show that 19.7 million permanent fillings, at a cost of £ 223 million, were placed in 2001/2 in the NHS, and 3.7 million of these fillings were placed in under 18-year-olds. Research has shown that drilling and filling can weaken tooth structure, and sometimes more tooth material is removed than was strictly required to remove the area of disease, or decay, and a crown may be required at some stage in the future life cycle of this tooth and associated dental treatment. The patient will also have taken time off work to attend the dental appointment.

If the cost of just the drilling and filling is looked at, assuming that just 20% of these teeth with caries could have been prevented, the cost saving is about £44.6 million. If it is assumed that 45% of these cases could have been reversed, a cost saving of about £100.35 million may be achievable with the application of ozone technologies.

From the figures, it is plain that dental care with ozone may offer huge cost savings in any social dental health care scheme, and at a time when most centrally funded dental and medical care systems are looking for cost savings, and an increased and predictable improvement in dental health, ozone technologies would seem to offer both objectives. It is clear that the traditional amputation therapy is expensive, leads to further dental treatment being required, and does not offer a strategy that always leads to improved dental health. Ozone treatment is easier and quicker. However drilling and filling will still be required in the deep hidden occlusal carious lesions, often combined with Ozone treatment.

Management Strategies of Caries

Ozone can now be considered as a clinical alternative management strategy for early caries, and this statement is well supported in the increasing volume of published research. Research has shown that ozone kills the cariogenic micro-organisms, essential for the progression of the carious lesion. Research has shown that ozone application for either 10 or 20 seconds is effective in killing 99% or more (99.9% after 20 seconds) of micro-organisms in primary root carious lesions *in vitro,* and an application for a period of 10 seconds is capable of reducing the numbers *of Streptococcus mutans* and S. *sobrinus in vitro* (Baysan et al, 2000). Baysan also confirmed the killing of 99% or more of micro-organisms in primary root carious lesions *in vivo* (Baysan et al, 2000). Further research has shown that occlusal caries in deciduous teeth can be effectively controlled with ozone treatment.

Caries reversal is associated with several factors including the level of microbial reduction and the oxidant effects of ozone. The dramatic reduction effect of ozone on the microbial flora will have eradicated the ecological niche of the acidogenic and aciduric micro-organisms. This shifting of microbial flora to normal oral commensals would predominantly allow remineralisation to occur within the carious process. Previous studies have shown that ozone reduces microbial by-products and metabolites. The by-products such as pyruvic acid, cannot cause further demineralisation of the tooth. Also the removal of some metabolites might deny other bacterial types in the lesion their important nutrients. In this way, ozone has several mechanisms of action when used to control caries.

Other studies have shown an oxidant (sodium hypochlorite) can improve the remineralisation potential of demineralised dentine. Inaba et al, found that the use of an oxidant (10% sodium hypochlorite) on demineralised root dentine lesions improved their potential to remineralise since sodium hypochlorite is a non-specific proteolytic agent and was effective in removing organic components in the lesions (Inaba et al, 1995). Further published research in this field by Inaba et al, has shown that when root dentine samples were treated with this oxidant for 2 minutes, the permeability of the lesion to fluoride ions increased (Inaba et al, 1996). The conclusion of this study was that removal of organic materials from dentine lesions was an acceptable approach to enhance remineralisation.

In this light, part of the dramatic remineralisation results shown after ozone application in this and other studies can be accounted for, as it is known that ozone

is one of the most powerful oxidants available. It may also indicate that ozone has the ability to remove proteins in carious lesions, and to enable calcium, phosphate and fluoride ions to diffuse through the lesions, a phenomenon resulting in remineralisation of the majority of carious lesions after ozone application.

Follow-up

Research teams have recalled their patients at 3 and 6-month intervals to re-measure treated lesions. Our group of patients showed around 90% remineralisation, with no further progression of treated lesions. The control group lesions, which were not treated with ozone, showed no improvement, and sometimes the decay process progressed. Numerous pathfinder dental practices in the UK have reported their 12-month recalls results. The majority of lesions treated have shown signs of complete reversal. A recent study at UKSmiles, a dental practice in Berkshire, England, showed that with the use of the HealOzone toothpaste, mouthrinse and spray, the treatment could be made even more predictable. In this study by Holmes 99% of all the lesions treated showed signs of remineralisation (Holmes, 2003). In the control group, no reversal occurred, and there was a general trend for the control lesions to get worse in the study period.

To date, the results in the UKSmiles study mirror those achieved by the research team in Belfast. Patients in this study are as astounded as the researchers have been at the success of this painless technology. Recent research has looked at the acceptability of this treatment modality by patients (Domingo et al, 2001). This study emphasised that patients are prepared to pay a greater fee than that of traditional amputation therapy provided the modality offers the following criteria.

the treatment must:

- be painless
- be quick and durable
- avoid the use of drills and injections
- be effective

This study showed that ozone treatment fulfilled all of these criteria, and that the cost of the treatment was not an issue. Indeed, this aspect surprised the researchers, as the overwhelming majority of patients questioned indicated they would pay more for the ozone treatment than the fee for a traditional filling. The implications for a private dental clinic offering this treatment modality are plain; here is a system that can halt and completely reverse the decay process, in a simple short treatment time. There is no need for injections of anaesthetic and the resultant potential postoperative pain and dysfunctional tissues. The need to drill and place materials that are prone to failure is avoided, and most importantly, the dental profession does not have to remove huge volumes of diseased tissue just to gain a firm foundation. This process can, in some of the larger carious lesions, result in the exposure of the pulpal tissue, the need to remove the nerve tissue, place a root filling, with the possible removal of this tooth some years later. Large tissue removal results in weaker tooth structure, so the avoidance of this can save the cost of crowning a tooth for structural stability.

To summarise, ozone dental treatment has the potential to have a huge impact on the provision of dental treatment, at a national level, and at a local dental practice level. At a national level, there is the potential to reduce the cost of the provision of dental treatment and care. At the local level, there is an opportunity for dental practices to increase their profitability. At last, the dental profession has a technology to minimise the loss of tooth tissue prior to restorative care, and reduce the quantity of materials used to restore a tooth to full function and form. The real winners are the patients.

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CHAPTER 5.3

Ozone: A New Treatment Modality For Dentally Anxious Patients

Ruth Freeman, Julian Holmes & Edward Lynch

Introduction

Sixty-four percent of people in the Adult Dental Health Survey for the United Kingdom stated that some forms of dental treatment frightened them (Kelly et al 2000). Furthermore the Survey showed convincingly that fears concerning dental treatment were not related to dental attendance pattern. Over 40% of adults were fearful of some aspects of treatment irrespective of their dental attendance pattern. For adults who are dentally anxious it is the pain associated with the local anaesthetic injection and the drill that is the most feared aspect of dental treatment (Moore et al 1996; Kleinknecht et al 1973).

Similar patterns of dental fear are found in children (Carson and Freeman, 2000). Children admit to being fearful of some forms of dental treatment whether or not they have regular patterns of dental attendance. In general these fears are related to the drill and injection. Interestingly, the least feared item for all children was the polishing cup used in prophylaxis.

Dentists must be in the position to provide objective and empathetic care for all their patients. For the most part patients will accept the treatment that is being provided. However, problems arise when patients are too fearful, or if their fears are fixed upon the local anaesthetic injection and/or the drill and/or if they present in pain. In such situations the dentist must be able to provide care quickly and in a manner that will not unduly distress the patient. Hence there is a need for the dentist to be able to readily identify dentally anxious patients who may be amenable to dental treatment in the practice setting. Furthermore the availability of a rapid, non-invasive treatment modality that avoids the need for the injection or the drill would assist in the clinical management of dentally anxious patients, or those who present in pain.

The aim of this chapter is to provide the dentist with a schema with regard to the identification of the dentally anxious patient. The schema is based upon detailed history taking and the use of dental anxiety questionnaires to assess anxiety status. In addition the chapter aims to demonstrate the usefulness of ozone as a new treatment modality in the care of the dentally anxious patient.

Dental Anxiety and Dental Phobia: Some Theoretical Constructs

The term dental anxiety was first coined by Coriat to describe the anxiety associated with dental treatment (Coriat, 1948). The term dental anxiety is both descriptive and explanatory as it provides the dentist with the means of recognizing and understanding fear associated with dental treatment. In descriptive terms dental anxiety describes the fearful patient who attends for treatment. The anxious patient is usually, pale, quiet or withdrawn and unable to maintain eye contact. In explanatory terms all energies are taken up with the feared situation the patient is unable to connect, speak or form any type of interaction with the dentist – their energies are elsewhere – as Florence Nightingale stated:

'Remember [*the patient*] is face to face with his enemy all the time, internally wrestling with him, having long imaginary conversations with him'.

Entering the dental surgery, seeing the dental chair and instruments revives the occasion that initially gave rise to the anxiety. This present day experience diverts the patient's energies and forms a nexus of fear from which the patient is unable to flee. Hence Coriat's (1946) view that the patient experiences an 'anticipatory anxiety' and a 'fear of the unknown' provides an

explanation as to why the anxious patient fears dental treatment in the 'here and now'. It is as if the past has caught up with the present and the patient fears experiencing the original terrifying dental treatment all over again. This is what the patient internally and eternally wrestles with, and about which (s)he has the long imaginary conversations. The amount of anxiety experienced in quantitative terms will affect the ability of the patient to form a treatment alliance and accept the treatment the dentist is offering and providing.

Dental anxiety in Coriat's (1946) explanatory framework is associated with the past, and is relived in the 'here and now' of dentistry which results in the anticipatory anxiety and the fear of the unknown. For many patients with dental anxiety identifying the anxiety-provoking experience and ventilating fears, worries and concerns will allow the formation of the treatment alliance and accept the care the dentist is offering and providing.

If the construct of dental anxiety is to be fully understood it is necessary to revisit the notion that it is the amount of anxiety experienced in a quantitative sense which holds the key. Working with some patients who refuse to accept dental treatment suggests that the intensity of anxiety experienced is so great it results in avoidance of dental treatment – descriptively they are 'phobic' of dental treatment. It seems that in terms of the quantity of dental anxiety there must be an additional, cumulative factor, which provides the increased intensity of anxiety which results in the avoidance of treatment. It has been suggested that such patients have made a 'false connection' between traumatic experiences that have occurred outside the dental surgery with a frightening dental event that has occurred inside. In order for a false connection to occur the two situations must have some element(s) in common and two psychological processes must be operative. The first process is the displacement and substitution of anxiety from one situation (outside the dental surgery) to another (inside the dental surgery) and the second, the concentration of anxiety onto dental treatment. The anxieties from experiences outside the dental surgery are transferred onto dental treatment. The intensity of the accumulated anxiety becomes so great that it is both psychologically and physiologically unbearable for the patient. The result is the avoidance of dental care. For the purposes of this chapter the meaning of false connections and displacement (Freeman, 1998) are given as:

- false connections, are misunderstandings. They happen in childhood as a result of confusion of what has been seen, heard or experienced in one situation with what has seen, heard or experienced in another. The misunderstanding or confusion arises because the two situations have one or more elements in common.
- displacement describes the transfer or shift of emphasis from one situation, person or idea to another. With the transfer or shift in emphasis there is the formation of substitutes for the situation, person or idea.

The following vignette is illustrative:

Mrs. K is a 28-year-old married woman. She is happily married and has a son aged eight. She attended the dentist because she had an impacted wisdom tooth that was partially erupted and had been repeatedly infected and was now causing great pain. She described herself as 'phobic of the needle' and could not bear the thought of any type of injection. She spoke freely and divulged that although she enjoyed hospital programmes she could not look when a patient would be given an injection as it felt as if she was having it herself. Although not concerned about the pain of an injection she was always worried that the needle would break. Mrs. K talked about her son's illness and how she had become distressed when she saw him 'having the needle'. She feared that the needle would break in his body.

In the example of Mrs. K a false connection had been made between her son's injection and her 'needle fears'. The element in common was the injection and there had been a shift of emphasis from her son's injection to her own injection and her own injection fears were a substitute for the fears about her son's injection.

Within the context of false connections, displacement, substitution and concentration of affect, the differences between patients who are dentally anxious compared with those who experience phobic reactions may now be considered. The dentally anxious patient makes connections between two dental treatment experiences. The anxiety is associated with the past, disagreeable treatment experience and is displaced and substituted by present day anticipated dental care. The concentration of the anxiety is bearable and the patient attends for dental care.

Dental phobia is different. The phobic patient has to contend with a more profound intensity of anxiety. In this heightened state of arousal he can only repeat rather than remember the true reason for his dental fear. Nevertheless the dentist can help patients such as Mrs. K by encouraging them to speak freely, thereby helping them to identify the false connection. Mrs. K readily accepted the link between her son's illness and her own 'needle fears' and consequently was able to have IV sedation for the local anaesthetic extraction of her impacted tooth.

Occasionally adults and adolescents present for whom there appears to be no experience of a frightening dental experience and it is hard to discover where there might be a false connection with regard to linking something that happened outside with something that happened inside the dental surgery. These individuals experience such an intensity of anxiety that they refuse dental treatment despite being in considerable pain. It seems that for this group of patients their dental phobia is a symptom of a wider psychological problem associated with a disturbance in the individual's emotional development. The following clinical vignettes from the case histories of two adolescents are illustrative:

Case 1: John is 14 years old. He has over the years developed a 'needle phobia'. At the time of his referral despite experiencing considerable pain refused to have his painful tooth extracted either with conscious sedation or dental general anaesthesia. Close questioning of John's mother revealed John's food fads and avoidances and a considerable separation anxiety. John found it impossible to be on his own; he would not let his mother out of his sight and insisted that he slept in the same bed as mother. This suggested that all was not well with John.

Case 2: Jane is 16 years old. She also refused to have dental treatment and at the time of referral was in pain. Jane was fearful of the injection and the drill. She had never had an intra-oral injection nor had her teeth drilled. Jane's mother was most concerned about her daughter. Although being continent for about 3 years, from the age of 5 Jane had suffered from enuresis. She still wet the bed at night and continued to be wet during the day. Despite urological investigations nothing physical could be found to explain Jane's enuresis. As with John it seemed that there had been a disturbance in her psychological development.

It may be proposed that the emotional development of the two adolescents in the above vignettes had not proceeded smoothly. They both experienced dental phobia but this seemed to be a symptom of a wider psychological disturbance rather than an entity in its own right. While it is possible to identify these patients it is necessary to refer them for secondary level psychological care. The questions of how to deal with their dental treatment need remains.

There are yet another two groups of patients who may present with extreme dental anxiety – they are those with learning disability.

For the first set of such patients, it may be a situation that the patient is unable to understand what is happening and so feels distressed and anxious. To provide a treatment which can be used simply and easily may remove the need for referral for secondary level specialist dental treatment.

In the second set, there are a group of patients who have severe illnesses. They are anxious about any potential treat to their well-being. A good example of this is Charlotte, a 12-year-old, who attended at UKSmiles dental practice in Wokingham, Berkshire. Charlotte had a platelet count of 12. By all reasonable expectations, she has been very lucky to survive as long as she has. Charlotte knows that any penetrating injury, and that includes dental needles, can cause severe and uncontrolled bleeding into the injury site. Past dental care resulted in such an injury, that require hospital treatment to reduce the resultant haematoma, and that was just for a simple single surface filling in an upper tooth. Imagine the scenario of an inferior dental block for traditional dental treatment to a lower molar tooth. The result could have been life threatening. Yet with a very simple and short treatment with ozone, she has had the areas of decay reversed, and long lasting fillings placed, recently seen at recall after over two years. She and her mother do not have the anxiety they once shared about dental care.

Clinical experiences such as those above suggest that dental anxiety must be considered as a presenting symptom and the underlying causation differs as reflected by the intensity of affect. For patients presenting with dental anxiety who have had a frightening dental experience it is the connection from past to present in dental treatment terms that consolidates the fear. For patients with phobic reactions it is the false connection and displacement from traumatic episodes outside to inside the surgery that forms the nexus of the dental phobia.

A third category of dentally anxious patients exists in which details of their history suggest that their dental phobia is a symptom of a disturbance in their psychological development.

Finally there are those with learning disability for whom it a lack of understanding of what is happening that gives rise to their fears of dental treatment. In each of the above categories the need for dentists to be able to identify and use appropriate treatment techniques to forge the treatment alliance is the key to provide care for this patient group.

Dental Anxiety And Dental Phobia: Methods Of Psychological Assessment

The need to identify patients who are dentally anxious as opposed to those who are dentally phobic allows the dentist to envisage an appropriate treatment plan. It has been proposed that a continuum of dental anxiety phobia exists which will dictate the form and type of dental treatment that may be offered to the patient. For patients who present with varying intensities of dental anxiety the use of an alternative treatment modality that would be quick, painless and avoid the need for injections and/or the drill would provide means of acceptable and appropriate treatment of carious lesions.

There is, however, an additional requirement that is a reliable and valid means of assessing dental anxiety. Various questionnaires have been devised for the general practitioner and these include the Dental Anxiety Scale (Corah 1969), the Modified Dental Anxiety Scale (Humphris et al, 1995) for adult patients and the Child Fear Survey Schedule (Belfast version: Carson and Freeman, 2000) and the Modified Child Dental Anxiety Scale (Wong et al, 1998) for children. These scales are simple and easy to use and provide the practitioner with a means of confirming his diagnosis and developing a treatment alliance with them.

The Dental Anxiety Scale (DAS)

The DAS was developed by Corah (1969) to assess adult dental anxiety. It is a four-item inventory. The questions ask about the intensity of dental anxiety when waiting for, first the day of the appointment, secondly in the waiting room, thirdly for drilling and finally for scaling. Examples of questions to assess anxiety when visiting the dentist tomorrow and waiting in the waiting room for treatment are:

[1] If you had to go to the dentist tomorrow, how would you feel?

Would look forward to it as a reasonably enjoyable experience	☐ [1]
Wouldn't care on way or the other	☐ [2]
Would be uneasy about it	☐ [3]
Would be afraid	☐ [4]
Would be very frightened	☐ [5]

[2] While you are waiting in the waiting room for your turn in the dentist's chair, how do you feel?

Relaxed	☐ [1]
Uneasy	☐ [2]
Tense	☐ [3]
Anxious	☐ [4]
So anxious, I feel sick and break out in a sweat	☐ [5]

Each question has 5 possible responses from feeling relaxed (scoring 1) to feeling anxious (scoring 5). This gives a possible range of total scores from 4 to 20 with the total score of 8.89 representing the population average score. Scores between 17 and 20 correspond to dental phobia.

The MDAS was developed in 1995 by Humphris et al. This is a modification of Corah's scale and includes a question about local anaesthesia. The questions assess the intensity of dental anxiety when waiting for, first the day of the appointment, secondly in the waiting room, thirdly for drilling and for scaling and finally for local anaesthesia. Examples of questions to assess dental anxiety when waiting for a dental appointment and treatment are:

*If you went to your dentist for **TREATMENT TOMORROW**, how would you feel?*

Not anxious	☐ [1]
Slightly anxious	☐ [2]
Fairly anxious	☐ [3]
Very anxious	☐ [4]
Extremely anxious	☐ [5]

*If you were sitting in the **WAITING ROOM** (waiting for treatment), how would you feel?*

Not anxious	☐ [1]
Slightly anxious	☐ [2]

Fairly anxious ☐ [3]
Very anxious ☐ [4]
Extremely anxious ☐ [5]

The scoring system is the same as for the DAS with total scores ranging from 5 to 25. Scores above 19 indicate dental phobia with 10.97 being the population average, for people attending general dental practitioners.

Child Fear Survey Schedule (Belfast version)

The children's dental anxiety may be assessed using the Children's Fear Survey Schedule(CFSS) which they were asked to complete. The CFSS was adapted and validated to assess child dental anxiety by Carson and Freeman (2000). The child was asked to rate how anxious they felt with regard to 10 dental items on a 5-point scale; a score of 1 corresponding to 'no fear' and 5 corresponding to 'very afraid'. This gives a range of total scores between 10 and 50.

The Modified Child Dental Anxiety Scale

The MCDAS was developed by Wong et al, in 1998 to assess children's dental anxiety when having dental general anaesthesia (DGA) or relative analgesia (RA). The questionnaire consisted of 8 items. A score of 1 corresponded to 'relaxed/not worried' and a score of 5 corresponded to 'very worried'. It has been modified to include faces (Figure 1). The scores range from 8 to 40.

Figure 1: The Modified Child Dental Anxiety Scale

How Do You Feel About:					
going to the dentist	1	2	3	4	5
having your teeth looked at	1	2	3	4	5
having your teeth scraped and polished	1	2	3	4	5
having an injection in the gum	1	2	3	4	5
having a filling	1	2	3	4	5
having a tooth out	1	2	3	4	5
being put to sleep for treatment	1	2	3	4	5
having a mixture of gas and air' to help you feel comfortable but not asleep for treatment	1	2	3	4	5

Figure 2: Assessment of dental anxiety inventory.

Assessment of Dental Anxiety Inventory

The child has to identify which of the pairs of drawings explains how they feel about dental treatment (Figure 2). There are 8 pairs of drawings and the possible range of scores is from 0 to 8 (Venham, 1979).

By combining the patient's history and scores from the psychological questionnaires to assess dental anxiety, the dentist is now in a position to identify the patient with dental anxiety, phobic reactions and for whom their dental phobia is merely a symptom of a greater psychological disturbance. Working in this way the dentist is forging the treatment alliance in preparation for the next stage of care that is negotiating the treatment plan.

Dental anxiety and dental phobia: what treatment modalities are possible?

The use of conventional dental treatment modalities such as the local anaesthetic injection, the fast and slow handpieces have been related to patients' fears of dental treatment. Writing in the 1970s, for instance, Kleinknecht et al (1973) stated that the most anxiety provoking items of dental treatment cited by patients were the drill and the injection. Later research agreed with these earlier findings and demonstrated that the drill, the injection and pain were the most commonly reported fears of dental treatment (Freeman, 1991). Furthermore it was fear and use of the local anaesthetic injection that was predictive of patient dental anxiety status (Moore et al, 1996).

Examining the links between reported fears and dental anxiety status Freeman (1991) suggested that memory acted to distort the original frightening dental treatment experience. The difficulty with the distortion was that the dentally anxious patient would perceive each new dental treatment experience as potentially harmful. In this heightened state of anxiety patients would be more likely to recall, re-experience and learn more about events which corresponded to their mood. Essentially a vicious circle of anxiety would be developed and with it the incubation and maintenance of the patient's dental anxiety.

This work suggested that if patients were given a 'corrective dental experience' within a framework of behavioural management it would be possible to cut the vicious circle of dental fear and assist patients to contain their dental fears and accept treatment. However, until recently, the requirement for alternative treatment modalities that avoid the need for the injection or the drill and allow the patient to accept the treatment offered and provided by the dentist, remained unfulfilled.

With the advent of ozone as an alternative to conventional restorative techniques, such as local anaesthesia, drilling and filling, it seemed that ozone would be a useful treatment modality for the management of dental anxious patients requiring conservative treatment. Ozone is currently being used in a variety of clinical settings. Ozone treatment (HealOzone Unit, CurOzone USA and KaVo) has been shown to reverse carious process in just a single application. The HealOzone Unit (Figure 1) delivers Ozone for 10 seconds through a tightly fitting cup that is applied over the tooth. In terms of its non-invasive mode of application and the short treatment time it seems an ideal, treatment modality for the dentally anxious patient.

Recent research on 377 patients in London who required conservative treatment for two carious lesions assessed patients' attitudes, satisfaction and dental anxiety status with regard to conventional conservative treatment and treatment with ozone (Domingo et al, 2002). The patients were asked to complete a questionnaire before and after treatment of their carious lesions with either ozone, or with conventional restorative treatment. The questionnaire assessed their satisfaction with treatment, their dental anxiety before and after treatment, and contrasted dental anxiety status with regard to conventional dental treatment and ozone. The Modified Dental Anxiety Scale (Humphris et al 1995) was used to assess dental anxiety.

In another study in Ireland, patient's attitudes to managing caries with Ozone was assessed (Domingo et al, 2002). Attitudes and dental anxiety were assessed on a 5-point Likert scale. Fifty patients, who had a carious lesion treated with conventional drilling and filling within the previous 3 months, and who had a similar carious lesion requiring treatment by the same dentist took part. All 50 patients participated and 100% completed the questionnaire.

The results showed that all patients were happy or satisfied with the ozone treatment they received, and were happy or satisfied with the amount of time the ozone treatment required. 96% were satisfied to choose this treatment even if the ozone treatment cost more than regular treatment, 100% would recommend this treatment to a friend or close relative, and 100% would like to receive this treatment again. 100% of subjects were not anxious after the ozone treatment and reported less anxiety after, compared with before, the ozone treatment. 80% of patients reported a reduction in anxiety ($p<0.05$).

The results of this survey suggest that patients attending a general dental practice were happy or satisfied with ozone treatment to manage their dental caries and would be happy to even pay more for this treatment than conventional drilling and filling. The ozone treatment was associated with a reduction in anxiety.

Patients' attitudes toward and satisfaction with managing caries with ozone as a routine treatment in a Dental Private Practice was investigated in Italy by Megigh-

ian and Dal Vera (2003). Patients (n=250) who had a carious lesion treated with conventional minimally invasive drilling and/or air abrasion and filling within the previous 6 months and who also had a similar lesion requiring treatment by the same dentist took part in the study. Attitudes, dental anxiety and satisfaction, one month after treatment, were assessed on a 5-point Likert scale questionnaire. All 250 patients participated and 94% completed the questionnaire within three months of treatment. All patients were happy or satisfied with the ozone treatment they received and were happy or satisfied with the amount of time the ozone treatment required. 85% considered the check up appointments to monitor the progression of clinical reversal of lesions a minor draw back. 55% were satisfied to choose this treatment even if the ozone treatment cost more than regular treatment, 100% would recommend this treatment to a friend or close relative, and 100% would like to receive this treatment again. 80% of patients reported a reduction in anxiety ($p<0.05$).

A similar questionnaire was administered to 45 patients who had undergone conventional minimally invasive treatment within the last 6 months, but who did not require ozone treatment. 100% of these patients were happy or satisfied with the treatment received, but 65% reported anxiety before and after the treatment.

Patients attending our private dental practice were happy or satisfied with ozone treatment to manage their dental caries. The ozone treatment was associated with a strong reduction in anxiety even in patients used to minimally invasive, and not traumatic, dental approach.

The findings from this preliminary work are positive and encouraging. Nearly all of the patients stated that they were happy and satisfied with the ozone treatment. The reduced time in the dental chair was perceived as a highly desirable characteristic of the new treatment. All of the patients stated that ozone was their preferred treatment option – irrespective of the financial costs. In terms of their dental anxiety status patients were less anxious before and after ozone treatment compared with conventional treatments for their carious lesions.

It is known that a large proportion of patients attending for dental treatment show varying degrees of apprehension, worries and anxieties that may result in avoidance of dental treatment (Kelly et al 2000). As the majority of people perceive the injection and the drill to be the most fearful aspects of dental treatment it would seem that non-invasive methods of treating the carious lesion must have a role to play in the clinical management of the dentally anxious patient. Ozone treatment, which requires no local anaesthesia, drilling or filling and is completed in seconds, is an ideal solution for those patients who are dentally anxious. The reduction of dental fear experienced by those receiving ozone compared with conventional methods of caries management suggested that ozone provided a new treatment modality for dental anxiety management.

Conclusions

It is not only patients who present with dental anxiety who provide the busy general dental practitioner with management difficulties. Knowing that time and patience are at the centre of the objective and empathetic treatment of this patient group, the dentist also appreciates that it may sometimes be impossible to spend the necessary time to help the patient accept dental treatment. The patient's close relatives may need more reassurance than the patients themselves. In a general dental practice setting, the GDP may have the anxiety of mothers and fathers to cope with, quite apart from the young child. GDPs spend as much time calming the patients' relatives in this case, as they do in the provision of effective dental care for the patient. In this scenario, ozone has had a major impact in the group of dental practices who have adopted the ozone technologies. As the treatment is fast, painless, and does not involve the use of anaesthesia or any form of drilling, the anxiety of the relatives is reduced. The guilt that this group of carers has is reduced too, a point often overlooked by researchers, and GDPs. Professor Edward Lynch and Dr Julian Holmes both have young daughters of similar age. Both had each a large hidden carious lesion found not by themselves, but by their child's orthodontists. Both expressed guilt at this lack of care, despite regular and routine screening sessions. With the ozone technologies, both children avoided the need for traditional drilling and filling. Their dental care was simple, and predictable. The end result was complete remineralisation of the lesions, and the guilt factor for both parents was reduced by the knowledge that their children's teeth were saved by a modern technology. Both children are happy youngsters, who still have no traditional cavities, and have no dental anxiety associated with modern dental care. It remains to be seen if

this technology has the potential to encourage a group of dental patients who will take up a could-not-care-less attitude, in the knowledge that modern technology may be able to reverse the effects of decay!

The practitioner must identify patients who can accept surgery-based treatment and those for whom it would be impossible. Engaging the patient and discovering the cause of their dental anxiety together with the use of questionnaires to assess the affect allows the dentist to quickly identify those who can and cannot accept dental treatment. Some patients will remain with the practice while others will be referred for secondary and specialist care.

For patients with carious lesions who are fearful of the injection and the drill this poses yet another management difficulty and the next stage is to decide how the dentally anxious patient's conservative treatment will be conducted. With the advent of ozone it seems that a reasonable alternative to conventional restorative treatment may exist for those who are dentally anxious. The reduced treatment time of seconds compared with 30 minutes, together with the use of snugly-fitting cup over the tooth, ozone would seem to have all the characteristics to reduce dental anxiety and allow the fearful patient to accept the treatment the dentist is providing. In addition it may have a part to play in the care of the dentally anxious child as children perceived the prophylaxis polishing cup as the least fearful aspect of dental treatment. Ozone is a new treatment modality which seems to have the potential to provide treatment of the carious lesions which is acceptable for the dentally anxious adult and child patient.

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CHAPTER 5.4

The Utility of Ozone Therapy for Dental Caries in the Elderly

Jonathan Ship & Kenneth Allen

Introduction

The aging population is the most rapidly growing segment of most civilized countries. In 1950, only ~10% of the USA population was aged 65 years or older. This value increased to 13% in 1997, and is expected to reach 20% by the year 2030. These demographic trends are even more dramatic for very old adults: individuals aged 85+ years will undergo nearly a 3-fold increase in the next 35 years. In comparison to statistics from 1900, the population has changed dramatically around the world. For example, the median age of death has reached 80 years in the USA (in 1900 the value was 58 years) and 1.5% of the population survives to age 100 (in 1900 the value was 0.03%).

While most of the world's elderly population lives at least partially independent lives, there is a significant number of older adults that are completely dependent for all aspects of life. For example, over 6% of USA adults over age 65 years are in nursing homes, currently exceeding 2 million persons. Nursing home residents have more than doubled since 1965, and USA nursing home beds exceed hospital beds. The lifetime risk for institutionalization is 52% for females and 30% for males. These trends in the aging population, both community-dwelling independent and institutionalized, have profound implications for oral health, particularly, the status of the natural dentition. The loss of teeth has long been associated with aging, yet major epidemiological changes have occurred over the past several decades regarding retention of the dentition. Only about 25% of adults aged 65+ years are completely edentulous, and the prevalence of edentulism decreased 10% between 1983–1993 in the USA in adults aged 65+ years. These dramatic changes have been attributed to advances in dental treatment, disease prevention, increased availability of dental care, and improved awareness of dental needs. Furthermore, these trends are expected to continue with improved oral health care, greater tooth preservation, and enhanced restorative techniques and materials.

In summary, there are two concurrent epidemiological trends that have broad implications for dental health: the elderly are the most rapidly growing segment of the population, and they are increasingly retaining their natural dentition. Accordingly, the dental profession must be prepared to provide diagnostic, preventive, and interceptive techniques for the healthy and medically compromised older dentate adult to preserve their teeth for functional and pain-free purposes.

Over a person's lifetime the structure and appearance of the tooth undergoes significant change. Occlusal attrition, pulpal recession and fibrosis, and decreased cellularity occur, and secondary and reparative dentin contribute progressively to acellular and dehydrated dentin. A lifetime of occlusal stresses induce microscopic and macroscopic fractures. A plethora of local (e.g., gingival recession, salivary dysfunction) and systemic (e.g., medication- and systemic disease-induced motor and sensory disturbances) factors contribute to a greater susceptibility to dental caries, which can lead to oral infections and may jeopardize essential functions such as mastication, phonation, and deglutition.

As a dentate individual, there is susceptibility to coronal caries as a result of new and recurrent dental decay. Increases in caries are influenced by two trends: a greater retention of teeth among the elderly, and a decline in caries among younger people. While it was previously believed coronal caries was restricted to paediatric and young adult populations, coronal caries is now considered to be a prevalent problem among older adults. Due to gingival recession, remaining teeth are

also at risk for developing cervical or root surface caries. For example, there is an 18-fold increase in the average number of tooth surfaces with root caries between persons age 20 and those age 64; this demonstrates that root caries often begins well before old age. With extended retention of the natural dentition into older ages, previously restored teeth are more prone to recurrent decay due to defective restorations, fractured fillings, poor oral hygiene, and inaccessible restoration margins.

Data from several USA national health surveys demonstrate a large number of decayed coronal as well as root surface carious surfaces in the older adult population. These data have been substantiated from population studies from around the world including China, Scandinavia, United Kingdom, Canada, Australia, Germany, and the USA demonstrating a significant impact of new and recurrent dental decay patterns among older cohorts. For example, there are estimates that greater than 30% of the population aged 65+ years has untreated coronal and root caries.

The problem of untreated dental caries in the elderly is magnified in populations that are dwelling in nursing homes. One Canadian study reported that there were high caries prevalence rates (50% coronal and 69% root surface) in elderly dentate residents of intermediate and extended long term care hospitals. Over a one year period of time, there was an incidence rate of 64% for coronal caries and an incidence rate of 49% for root surface caries in older Australians living in nursing homes.

Risk factors for dental caries in the elderly

There are multiple risk factors for dental caries in the elderly, the most important being retention of teeth, gingival recession, existing restorations, xerostomia and salivary dysfunction, manual dexterity problems, cognitive changes, and the effects of medications and medical disorders. The retention of the natural dentition among older adults has changed dramatically over the past century. For example, the proportion of adults with their natural dentition increased from 30% in 1957 to greater than 65% in a span of approximately forty years in the USA. Combined with reports that over half of older adults had a least one dental visit per year, it is predicted that there will be substantially more people requiring dental treatment needs as the population ages. In some regions there are anticipated shortages of dental care professionals, which will place greater burdens on oral health professionals to maintain and retain the natural dentition of all segments of the elderly population.

The elderly population is susceptible to periodontal diseases due to the increased retention of the natural dentition, and this phenomenon has implications for dental disease as well. The incidence and severity of gingival recession have been reported to be directly proportional to age. The prevalence of gingival recession in adults aged 18 to 64 years was recently reported to be 50%, and increased to 88% in adults older than age 65 years. The lack of enamel covering the root surface leaves only cementum to protect the tooth, and therefore gingival recession increases the risk of dental pathology: abrasion, abfraction, and caries. Cementum, a specialized calcified connective tissue, has only one eighth the Knoop hardness number of enamel and is more susceptible to erosion by acids produced by decay-causing bacteria. Root surface caries as a result of gingival recession also creates restorative challenges. The tooth is narrower in a mesial-distal and facial-lingual dimension as gingival recession exposes the root. Restorations placed in this narrower portion of the tooth will have greater proximity to the dental pulp increasing the likelihood of endodontic involvement.

A major component of dental treatment is replacement of existing restorations. Recurrent caries, defined as caries at the junction of an existing restoration and the tooth, is an indication of microleakage and must be addressed to prevent further destruction of the remaining natural tooth. This is the most common reason for replacement of both amalgam and composite restorations. Successful treatment outcomes are predicated upon multiple factors, including the properties of the restorative material, and the stresses from the forces of mastication and habits such as grinding and clenching. In the older population, with more restored teeth, replacement of defective restorations will result in a greater burden on dental professionals and on society to facilitate unimpaired access for oral health care. Other challenges remain for recurrent caries in the elderly: detection may be difficult since the margins of the previous restoration may be interproximal and/or subgingival. Contributing to the difficulty in detecting recurrent

caries are large old metallic restorations that reduce the diagnostic ability of intraoral radiographs.

Another risk for new and recurrent carious lesions is conditions which cause decreased salivary output. Xerostomia (the subjective complaint of a dry mouth) and salivary dysfunction are side effects of prescription and non prescription medications and can also be caused by multiple diseases. Such conditions and diseases include Sjögren's syndrome, Parkinson disease, head and radiation for cancer, and chemotherapy. Dry mouth is not a normal consequence of the aging process, but rather is related to an underlying aetiology. The most common cause of salivary disorders is prescription and non-prescription medications. For example, 80% of the most commonly prescribed medications have been reported to cause xerostomia, with over 400 medications associated with salivary gland dysfunction as an adverse side effect. The side effects of medication utilization are not limited to impaired salivary output, but also include changes in the viscosity of saliva causing plaque to adhere more readily to the teeth. With the growing elderly population, particularly those taking prescription medications, there is a large prevalence of medication-induced xerostomia amongst the elderly which will most likely worsen in the future.

Medical conditions that reduce manual dexterity will decrease the effectiveness of oral health maintenance and can subsequently result in an increase in primary and recurrent caries. These medical conditions (e.g., Parkinson's disease, stroke, arthritis) are more prevalent in the elderly. For example, arthritis symptoms, which often directly affect dexterity, affect 12% of adults younger than 44 years of age, but more than triple to 40% of adults over the age of 75. While an estimated 40 million USA adults had some form of arthritis in 1995, by the year 2020, an estimated 18.2% (59.4 million) will be affected.

Finally, there are significant numbers of older adults living with cognitive, behavioural, and psychiatric disorders who are susceptible to the ill-effects of impaired oral hygiene. These individuals are frequently administered anti-psychotic drugs with xerostomic side effects, further increasing their risk of developing new and recurrent dental caries. In summary, the elderly present multiple risk factors for increased dental disease, and the prevalence of tooth-related disorders is expected to increase in the future with the increased proportion of the growing elderly population retaining their natural dentition.

Treatment of dental caries in the elderly

The treatment options for restoring natural dentition to biologic form and function in the elderly are varied and include amalgam, composite resins, compomers and glass ionomers. The decision as to which material will be best can only be made after the practitioner evaluates the medical and dental history of the patient, aetiology of the carious lesion, properties of the restorative material, and availability for periodic recall examination and assessment.

Dental amalgam is an alloy primarily composed of mercury, silver, copper, zinc and tin with a history that traces back to China in 600 AD and France in the 1830s. Among the many advantages of amalgam are its strength, durability and relative easy of application and placement. As a low cost, durable material that mimics the properties of a natural tooth, amalgam is often the material of choice for use in posterior teeth. It is relatively easy and quick to use, has excellent longevity and compressive strength, and can be repaired readily in the presence of recurrent caries rather than be completely replaced which reduces the expense of follow-up treatment. There are, however, concerns about mercury hygiene and safety. Furthermore, there are a great number of available tooth-coloured alternatives that provide greater aesthetics and have fluoride-containing cariostatic activity, which have significantly reduced the use of amalgam.

As an alternative to amalgam, composite resins were introduced into dentistry in 1968, and are particularly helpful where retention is limited and aesthetics is of primary concern. A distinct advantage of composite resins, especially in the elderly, is its use in repairing defective restorations and/or carious tooth surfaces. An existing resin restoration with recurrent caries is repaired by removing the decay or defect, etching, bonding and placing the resin. Composites are also indicated for repairing carious defects adjacent to amalgam restorations as well as for repairs of defective amalgam restorations. In such cases, bonded resins can avoid the necessity for a crown and thus reduce patient visits and expense. Resins do have limitations: they are more likely to have open contact areas and poorly contoured proxi-

mal surfaces. In the elderly population, these problems are a concern for the longevity of posterior resins and the gingival health of the surrounding tissue.

Enhancing the desirability of posterior resins are new glass ionomer liners that can protect pulpal and axial walls under the resin restoration. When such liners were placed under Class II resins, there was a statistically significant reduction in microleakage. The new generation of glass ionomer liners provides sustained fluoride release that reduces the incidence of recurrent caries. The fluoride is considered 'rechargeable' when the patient uses a fluoridated rinse or toothpaste.

As an alternative to composite resin restorations, glass ionomers were introduced in 1972. They possessed several desirable characteristics, including the ability to chemically bond to enamel and dentin, fluoride release and pulpal compatibility. However, their poor physical properties and sensitivity to water contamination made them a poor choice for restorative use. Glass ionomer resins were then modified with the addition of methacrylate derivatives which increased fluoride release, but their weak physical properties persisted and they are primarily indicated for non stress bearing areas. In the older person at high risk to new and recurrent caries, glass ionomer resins may be desirable due to their fluoride release and their 'rechargeability'. However, even in non stress bearing areas, glass ionomer restorations experience severe surface degradation over time.

In an attempt to reduce the limitations of glass ionomer and resin modified glass ionomer restoratives, a compomer material emerged in the late 1980s and early 1990. The compomer, a polyacid-modified resin composite, also releases fluoride but at a reduced rate when compared to traditional glass ionomers. In addition, the compomer material is not fluoride 'rechargeable', leaving out an important feature for restorative materials in the elderly. The high bond strength of compomer restorations make them desirable for use in Class V lesions. Since the fluoride releasing capabilities are less than that of glass ionomers, they are best suited for areas which are more easily kept free from plaque.

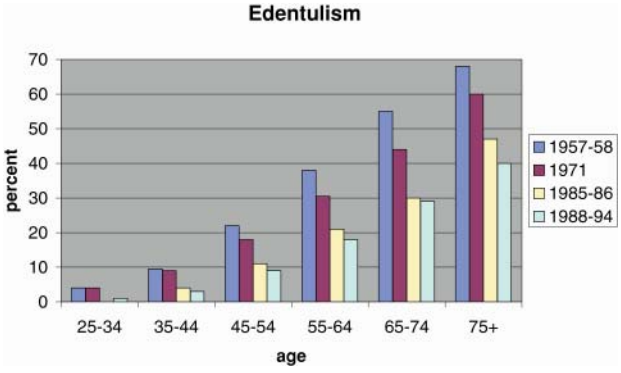
Proper home care can postpone the need for replacement restorations. This is especially important for older adults who may have limited access to dental care. However, the elderly may have inadequate home care due to limited manual dexterity, xerostomia, or cognitive deficiencies. Therefore efforts must be made to enhance oral hygiene despite oral, systemic, physical, behavioural, and psychological problems. Some aids available to the elderly are electric toothbrushes, fluoride rinses, and prescribed home fluoride treatment.

There is still no perfect restorative material for use in the elderly. Each of the available alternatives has its own advantages and disadvantages and the selection of which material is best for the elderly patient must be based on the practitioner's knowledge of the properties of each material and the needs of the patient.

Utility of Ozone therapy for dental caries in the elderly

The earlier chapters in this book have clearly shown the dramatic benefits of using the HealOzone to manage root caries. The benefits of Ozone treatment for these lesions are clearly described with excellent clinical results achieved in the clinical trials carried out by Baysan as well as Holmes.

Table 1: Epidemiology of Dental Caries with Aging. (graph courtesy Ship and Allen)



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CHAPTER 5.5

HealOzone and Total Quality Management in Dental Practice in Germany

Volker Scholz

Introduction

To successfully implement the HealOzone (KaVo GmbH, Germany) in a general dental practice requires other management structures and skill. These encourage entrepreneurial skills, rather than have the dental practice focused on traditional dentistry. In Germany and other countries, the university undergraduate courses tend to focus on the legal regulations and in Germany, payment structures and systems for dental healthcare. For this reason it has been very difficult to introduce and adapt methodical changes in dental health care. Dentists have little professional training in business and management. The dental profession tends to be poor at communication with patients, and conversing with and advancing the media's understanding of dentistry and innovations in dental health care. For example, a cavity in a tooth is still looked upon as a carious disease, which is influenced in part by errors in treatment methods, and in part by the way health insurers pay the dental profession. Insurers tend to be focused on the symptoms of disease, rather than trying to encourage and reward those who treat the cause of the disease with a preventative approach. This encourages the 'drill-and-fill' mentality and condemns teeth to a cycle of treatment and re-treatment that could be avoided. There are potential savings to be made in adopting a new approach to dental care in these countries, and the HealOzone may provide the essential key to this new modality.

The use of the HealOzone emphasises a treatment methodology that puts the cause and prevention of caries back in the limelight, with a non-invasive treatment protocol. The success in the dental practice and with patients depends not only on the technical mastery of HealOzone but also to a great extent on how well the entire dental team succeeds in transferring their knowledge of the method and the results of the treatment to patients. Team members need to do this in such a way that patients understand the treatment and what their involvement in this treatment is, as well as being able to manage the patient's expectations.

The aim in this chapter is not to illustrate the application of the HealOzone methodology, as this has been done in other chapters, but to show which management structures, skills and methodological aids can be used for successful implementation on ozone dental care in daily practice.

The "European Foundation for Quality Management" (EFQM) model.

The official statement of the EFQM reads:

"Regardless of sector, size, structure or maturity, to be successful, an organisation needs to establish an appropriate management system. The EFQM Excellence Model is a practical tool to help organisations do this by measuring where they are on the path to Excellence; helping them understand the gaps; and then stimulating solutions". (EFQM for further information visit: www.efqm.org)

The EFQM model is demanding. The evaluation of the degree of achievement of the 9 criteria is accomplished by continuous self-assessment. This self-assessment can be compared with the internal audits of EN ISO 9001, and it is supported by a structural question catalogue. This model has become more and more a guideline in healthcare, and forms the basis of the applied KTM in hospitals. In Switzerland there is already a dental surgery, which, based on the principles of the model, has been awarded the highly acclaimed yearly 'European Quality Award' by the EFQM. The "Dental Excellence Standards" are meanwhile part of the EFQM "Commit-

ted to Excellence" validation for dental clinics (for more information – German language – only visit: *www.dentalexcellence.de*)

The model differentiates between so-called 'enablers', i.e. the criteria that make quality actually possible (in the widest sense) and the 'results', which, by planning ahead, should be or are reached. The philosophy of the model is that permanent effort to achieve quality will bring about measurable and excellent results. The lower level 'committed to excellence' shows that there is a closed circuit of 'continuous improvement'. The most important fact is that by permanent measuring and analysis of the results, a learning process is evolved which provides a continuous improvement – learning culture.

The 5 "enabler criteria" are summarised here:

'Leadership'. This section defines how the leading team in a surgery and other executives promote, support and stimulate a culture of comprehensive quality management. The (surgery) management has to set an example in every aspect to achieve excellent results. The main guideline has to be communicated by suitable means and efforts made by the employees have to be recognised and rewarded.

'Policy and strategy' determine how the company or organisation formulates 'surgery' policy and strategy, breaks it down step-by-step, checks these steps, and converts these steps into plans and how they can be measured. The surgery management has to develop acceptable targets and principles that are understandable. These targets and principles need to be followed up and re-assessed by strategic measures. All these need to be documented in the surgery model.

Employees define how their capability, skills and their placement should be developed, thus realising their entire potential of the employees. This concept can be promoted to employees by suitable measures, like target agreements, personnel planning, profit sharing for example. All these single measures can be united into an integrated personnel development concept.

'Partnerships and resources' describe how resources are used effectively to lower costs and waste. Procedures for the effective and efficient use of the main surgical resources (financial, technical, and information) have to be set up. With reference to the principal cooperation partners (cost bearer, supplier etc.) the fundamental rules of cooperation are based on mutual trust and acceptance of the partnership targets and rules.

'Processes' entail setting up of workplace descriptions and describing each process carried out in a surgery. These should guide employees how to achieve the set targets and to create values. The principal net product processes (diagnostic, therapeutic and infrastructural) must be identified and actively assessed via these targets, re-checked, and continuously improved.

'Customer results' are one of the most important criteria and are therefore highly assessed in the self-assessment. Patients are requested to fill in questionnaires. They are asked for information on satisfaction and what factors make them loyal to one practice in preference to another. Patients should be asked to evaluate their practice performance regularly. Measuring scales are to set up (taken from a basic nature of complaints) that illustrate the reached value. Besides this 'subjective' quality assessment by the patients themselves, quality statistics can also be taken from treatment provided for maintaining oral health by the Oral Health Manager (OH Manager).

'Results of employees' describes what level has been reached in respect of motivated and satisfied employees. Employee satisfaction must improve as a result of the personnel-related measures in particular, as well as the entire concept generally. This must be reflected in measurable results (i.e. rate of mistakes, rate of fluctuation, working climate index).

'Social responsibility' judges what the organisation achieves by fulfilling the wishes and expectations of the local, national and international society as a whole. Equally important to this effect is the assessment of society, in terms of the attitude of the organisation towards the quality of life, the environment and preservation of global resources. It also measures the levels of the effectiveness of the organisation itself. Related to these criteria would be the 'image' of the surgery and the surgical environment, with respect to this commitment. In particular, this criteria looks at the practice owner and their commitment to these social aspects. With this criteria it is assumed that each 'business' is

subject to a particular ethic and moral code. Here again, there's a key to success with a new methodology like the HealOzone, because on a long-term basis this method will have no economic success for the surgery if it is overused for superficial marketing purposes only, i.e. no records of follow-up success.

'Results' comprise the key achievements and show what has been reached in terms turnover, profit, number of patients and quality of life. Last but not least, TQM must also be financially feasible. The corresponding economic measure can be derived from TOWER, which gives these essential figures. TOWER is specifically designed control software for single profit centres such as dental practice. In common with other result criteria, 'benchmarking' is a comparison of results achieved by other dental practices. The principle 'Learning from the best' is common to all Dental Excellence Partners (DEP *www.dentalexcellence.de*).

Results

The EFQM model is applicable to the requirements of a dental practice. It provides an overall 'vision', underlined by striving towards excellence, amongst other criteria. DEP profit from a framework contract with EFQM in Brussels and can they can participate in group validation. At the end of this validation they are awarded a certificate by EFQM, known as the "committed to excellence" award.

Aims of the EFQM model

- Strengthening competitiveness by improving profitability, at the same time increasing the effectiveness of treatment.
- Working within the management plan.
- Encouraging creativity and self-responsibility of all employees.
- Preventing mistakes takes precedence to crisis management.
- Constant improvement of the entire organisation through flexibility, creativity and willingness for innovation.
- Making sure all treatment is ethical and is carried out to patients'requirements.
- Reduction in running costs and material wastage.
- The accomplishment of these requirements and aims is measured and recorded.

The dental excellence standard
This EFQM model is consequently applied within the DEG. The following are regularly performed, evaluated and benchmarked:

- Self-assessment of the dental practices.
- Employee and patient questionnaires by DEG members are taken, both targeted and anonymous.
- Data relating to patients oral health and compliance using the OHManager.
- Data and codes for economic performance with TOWER.

The goal is to use dental excellence as a brand which gives a clear impression to the patients that in this dental practice, their teeth and dental health will be cared for and improved, and that the dental practice achieves positive financial growth.

The OHManager and software
Documenting the success of the HealOzone therapy is of vital importance. Multi-factorial diseases like caries and periodontitis cannot be recognised properly by traditional methods. Therefore, based on the IHCF Foundation Oral Health Manager concept for promoting health, appropriate software has been developed which was presented in 2003. This is available in a number of surgeries. The key lies in the result *sheet*. The OHManager software is much easier to work with and offers many more features.

Basic principles

The OHManager comprises 18 criteria but it is not necessary to register all 18 factors of the OHManager for every patient. Each patient is registered according to the presenting clinical criteria. Each dental practice can determine in the program setup, the factors for the different age groups that should be examined and which criteria should be preset to an average value. The graphic shows all the results and the risk line is common to all participating dental practices. This has been found to be excellent for patient motivation!

Processing instructions can be freely defined for every risk-grade of caries, and the program determines automatically which periodontal factors are important from the results of the prophylaxis program. The treatment program can be then tailored for each patient and determines the recall intervals. These basic settings have to be entered only at the initial program set-up. However the software identifies patterns so minor adjustments can be made.

Some main functions of the OHManager software include:

Registering a patient and their detail

The selection comprises 18 points of the RiscProfileGrafic program defined as follows:

- Point 1: fluoride exposure.
- Point 2: nutrition habits.
- Point 3: nutrition quality.
- Point 4: Mutans streptococci colonisation.
- Point 5: Plaque index.
- Point 6: Probing depth.
- Point 7: Tartar.
- Point 8: BOP bleeding index.
- Point 9: PSI degree.
- Point 10: Smoker.
- Point 11: DMF-T rate.
- Point 12: DIAGNOdent value.
- Point 13: Compliance.
- Point 14: General diseases.
- Point 15: Cleaning damages, open root surfaces.
- Point 16: Erosion, abrasions.
- Point 17: Secretion rate.
- Point 18: Buffer capacity.

The selection comprises degrees of seriousness from "0 to 3".

The patients 'complete report' consists of the following points, each with their own sub-data screen:

- Anamnesis check.
- Plaque index.
- PSI degree.
- Risk profile of health keeping.
- Contract and recall planning.
- Success chart.
- Process instructions.
- Recommendations for home.

Both the plaque index and the BOP index, as well as the number of DIAGNOdent measuring points are taken over to the screen "RPG risk profile health keeping". Registration and the follow-up of teeth with critical DIAGNOdent values is a valuable tool with this system, especially with the HealOzone therapy. A welcome future development would be the integration of a data port into the DIAGNOdent to automatically dump data directly into the screen entry points, so the DIAGNOdent can be read electronically and clinical pictures (intraoral digital camera images, for example) can be stored in individual patient's notes. Thus areas of success and areas where better oral care and treatment compliance is necessary for a positive outcome could be shown and demonstrated to the patient.

Example query:

All female smokers over 35 and BOP factor 2 plus

Aim: Offer smoke counselling, advice on how to stop the habit, and perio-intensive prophylaxis

Here, the recommendations and corresponding instructions are administered. This is required so that the recommendations for home care can be administered and determined. You can add, alter and delete your own recommendations, with or without instructions.

This function can be individualized by each practice. The OHManager software is ideal for the administration and recording the HealOzone therapy because the at-home therapy can be organised with the HealOzone patient kits.

The program determines the treatment steps, their sequence, the time period to the next treatment and the clinician responsible for carrying out the treatment. The program provides presettings for each health profile, which can be complemented or altered individually. The settings for each health profile are made separately. There are 5 caries and 5 periodontitis profiles available.

Recommendations for home care.

Here the recommendations for home care for each of the 18 points of the RPG diagram and for each degree of seriousness (0 to 3) are determined.

The program will generate addressed and personalised letters from the patient's database.

OHManagement – The instrument for the serious preventative dental practice.

Prevention has become the prime treatment offered by dental practices, and this can be confirmed by visiting the web sites of dental practices on the Internet, and discussion with dental colleagues. Prevention is the cornerstone of modern dental treatment and care. However there are few systems that can access and test how successful the practice is in delivering prevention. Every dental practice has a number of patients who do not attend for routine oral care and assessment, and others whose compliance with at-home oral hygiene is poor.

Every dental practitioner invests a great deal of financial resources, time, professional skill and effort to deliver quality care to their patients. The majority of practice administration programs are poorly suited to the management of a preventative practice. This is one of the problems associated with the more popular administration software systems, as these do not allow the entry of caries risk factors, periodontal screening index, defects of hard tooth substance, and DIAGNOdent data, for example, in such a way that this data can be accessed and assimilated into a coherent and educational report for the patient.

Statistics

The anamneses reports and the 18 risk factors can be selected freely in cross evaluations. That means that a query can be run, for example, to show how successful prophylaxis was on patients with a high BOP, male or female patients. The query will even divide the report into smokers and non-smokers. Equally, so-called 'problem patients' could be targeted with the latest information if, for example, a new treatment method has been introduced in the surgery that could be of specific value to these patients. These evaluations can be used as proof of successful healthcare, which, in future, will be essential for each surgery within the framework of quality requirements. The positive aspect of this is that these statistics are built up by the software in the background without any extra cost – no extra time is needed to establish them.

Patient relationship

For each registered patient, a pre-treatment report of the examination can be printed that illustrate the current risk profile, the planned professional care, the suggested recall intervals and recommendations for the patient's at-home oral prophylaxis. Recall letters and reminders of pre-set appointments can be printed automatically. The content of this information can be chosen freely by the dental practice and personalised to that practice.

(Information: *www.ohmanager.org*).

Monitoring HealOzone patients

Laser fluorescence makes it possible for the first time to diagnose early carious lesions that are reversible and to follow up the changes of the measured values over a period of time. If an intraoral camera is available, the lesion in question can be documented in a clinical picture for the patient as a differential diagnosis. A critical DIAGNOdent value then requires a specific therapy, which does not immediately have to be invasive but to support the natural self-healing process at first. This means first the caries has to be eradicated to stop the demineralisation caused by bacteria and their by-products. The HealOzone is used to disinfect the lesion and to promote the remineralisation of this lesion; the patient must be educated and informed of their role in the management of their disease, by suitable management and educational tools in the dental practice and by the patient's compliance in the use of an 'at-home' kit.

The HealOzone therapy is verifiable with the DIAGNOdent values as a check throughout their course of treatment. There is no common format at this moment in time on how the data should be acquired and recorded, especially when using an electronic patient record system. However, the OHManager software can acquire critical DIAGNOdent values as well as all the 18 factors discussed above.

A patient query can be sought selectively according to these criteria, which will allow improvements in the patient management as their treatment progresses. This also applies to the administration of intra-oral clinical pictures or x-rays of teeth treated with the HealOzone. The software provider has considered the requirements of the DIAGNOdent manufacturer by including the possibility to capture the data directly from the DIAGNOdent into the record screen. A data capture port

would be a very welcome improvement in future DIAGNOdent models.

Results and Conclusion

Since the beginning of the *IHCF- Stiftung zur Förderung der Gesundheit* (*www.ihcf.org*), discussions were centred on the premise that securing quality in dentistry would be best achieved by securing a healthy status. However up to now the technology has not been available. The author has been involved in the development of these systems for over 10 years. He has spent a great deal of time in his dental practice in Lindau to develop a targeted approach to prevention and prophylaxis that is acceptable to patients. Since the introduction of this software, patients are inducted into an annual contract, and HealOzone therapy is now included in these care contracts.

This means that the dental practice and the team do not have to 'sell' a vast number of single services any longer. They can now concentrate on product "professional health keeping of teeth by gentle and patient friendly dentistry". The diagnosis and therapy methods applied are clearly explained to the patient but the patient does not finally decide on the fact whether the dental practitioner applies these treatment options or not. After one year a new evaluation takes place, normally showing a positive result for the patient that their risk profile has improved and that they need less professional time, thus reducing their fees. The OHManager greatly facilitates such a concept and its management.

Marketing with the OHManager and HealOzone

The trend throughout Europe is towards patient-funded health care, or private care. Centrally funded systems have become too expensive. The trend towards private care has seen a necessity of acquiring new skills, and a growth in successful marketing. Marketing has to be carried out in an ethical manner to acquire new patients and retain them in the dental practice. Technologies like the HealOzone and the DIAGNOdent have the potential to build a loyal patient base, as they offer patient-oriented dentistry. This can be organised with the OHManager and embedded in a marketing strategy within a concept like dental excellence.

CHAPTER 6.1

The Use of Ozone in the Treatment of Dental Unit Waterlines

Hisham Al Shorman, Wilson A Coulter & Edward Lynch

Introduction

Contamination of dental unit water lines (DUWL) by micro-organisms is a real problem facing modern dentistry which has yet to be completely resolved even though the first report of contaminated water in the dental lines was as far back as 1963. Blake, working in the United Kingdom, recovered high concentrations of bacteria from dental waterlines and reported on the effectiveness of disinfectants as water decontaminants (Blake, 1963). Since then a lot of reports have addressed the problem and called for the solution, stating that microbial contamination could be up to 10^6 colony-forming units per millilitre (CFU/ mL) (Bagga et al, 1984; Coulter et al, 1993; Miller, 1996; Shearer, 1996; Walker et al, 2000).

Medical professionals, nowadays, see more immunocompromised patients than they used to see in the past as a result of the advances in medical care which have increased life expectancy of these patients. Advances in dental care should be no less perfect than other medical fields. Therefore, we are urged both medically and ethically to make the dental clinic as infection-free as possible.

Water is widely used in the dental clinics for various purposes including instrument cooling while performing the dental treatment and mouth rinsing. This makes it quite necessary that we use water of high microbiological quality.

An international goal was set in 1996 that by the year 2000 the water used in the dental clinics should harbour no bacteria more than 500 CFU/ mL (ADA 1999). In Europe, the drinking water standard is 100 CFU/ mL. Obviously, the water used for treatment of patients should be microbiologically similar or even better than the drinking water (Council directive, 1998).

The problem

Studies have shown that contaminated dental water not only poses risk to the patients but also poses a risk to the dental team because most dental procedures generate large amount of aerosols that may be inhaled. A proportion of dentists experience occupational exposure to *Legionella pneumophilia*, a waterborne pathogen that can cause Legionnaire's disease and Pontiac fever. Antibody titres to Legionella are significantly higher among dental workers than the general population (Atlas et al, 1995). Fortunately, in the vast majority of cases there is no evidence of it leading to pulmonary infection. However, another study has shown that the antibody titre is similar to that of the general population (Pankhurst et al, 2003). The health and Safety Commission in the UK have issued a new θApproved Code of Practiceö regarding the control of Legionella in water systems. It stated that water temperature can be used as a method of thermal control. The guidelines state that hot water should be stored at 60 °C and the cold water should be kept below 20 °C. The temperature of hot and cold tap should be checked monthly. Once a year a representative number of taps on a rotational basis should be checked. If taste or odour problems are noted then a microbiological investigation may be required as this could signal development of conditions that could promote growth of Legionella. The specific HSE guidelines on dental equipment state that they should be "drained down and cleaned at the end of each working day".

Dental literature refers to two other environmental bacteria as important micro-organisms in this context; Non-tuberculous mycobacteria and *Pseudomonas aeruginosa* which are of particular risk for patients with Cystic fibrosis.

While micro-organisms suspended in the water form

part of the problem, the real problem is the biofilm that forms inside the dental waterline tubing (Figs. 1–3) which constitutes a constant and continuous source of bacteria (Mayo et al, 1990). Biofilms are generally heterogeneous in microbial composition and morphology with a wide variety of species of bacteria, fungi and amoebae.

Biofilms are 30–50 μm thick and are enclosed in a polysaccharide layer known as the glycocalyx, which makes them resistant to many chemical agents. In addition growth within the confines of the biofilm enhances bacterial proliferation by binding and retaining a supply of nutrients (Costerton et al, 1987), which permits a higher level of metabolic activity whilst protecting bacterial population from biocides. Figure 1 illustrates the flow of water within the waterlines in a laminar flow that facilitates the adherence of micro-organisms to the walls.

Enormous effort has been done in research to achieve the international goal and wide range of material has been investigated. Table 1 summarises some of the biocides tested in various studies.

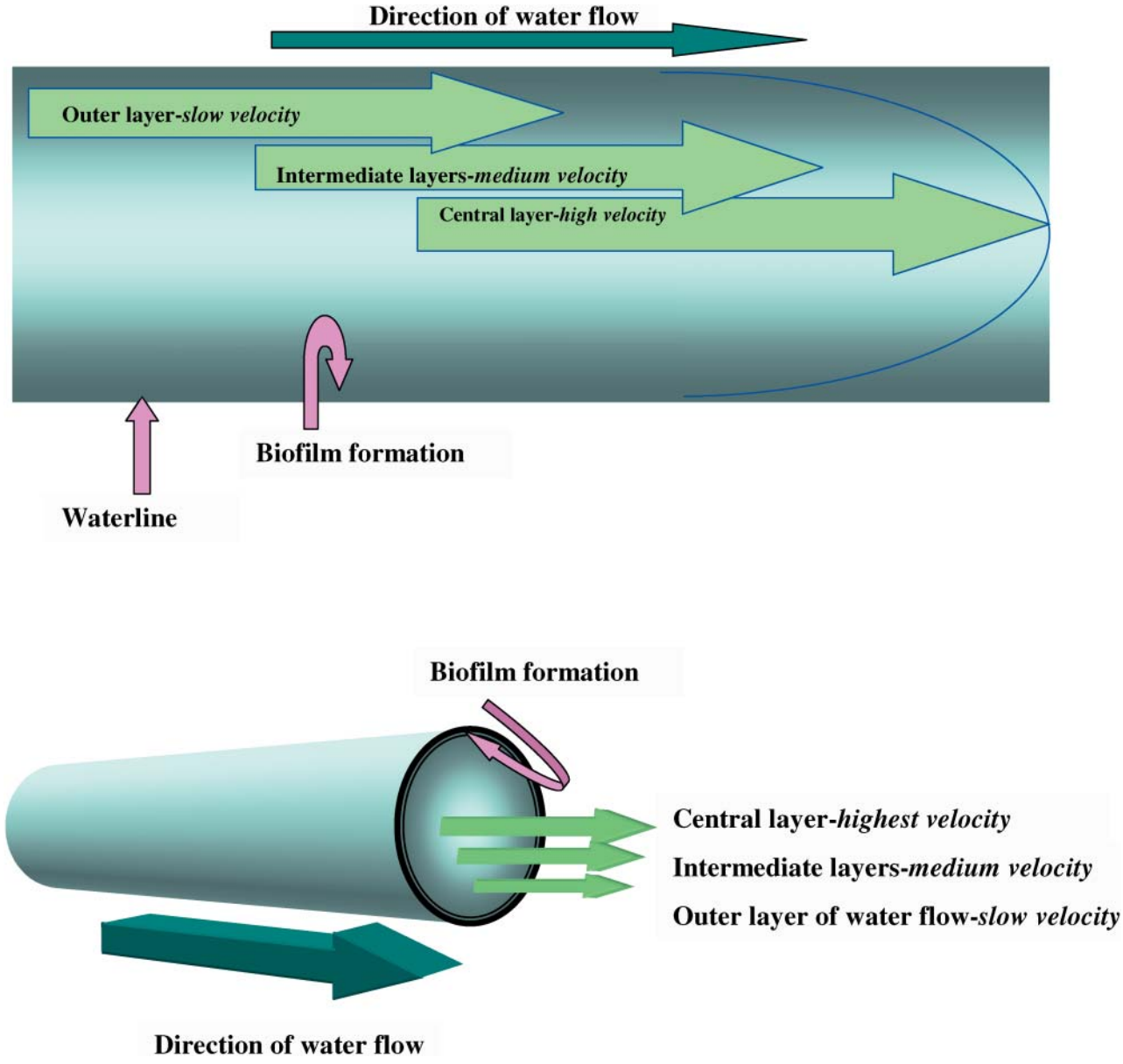

Figure 1: The laminar flow of water within the dental water lines in longitudinal and cross sections. The closer the water flow to the wall, the less the velocity becomes as a result of friction. At the wall the water becomes virtually stagnant where the biofilm develops.

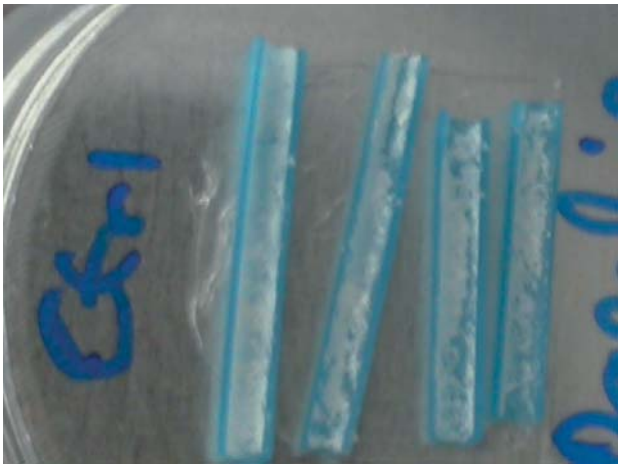

Figure 2: Photographic image of a waterline samples showing the gross appearance of the contamination of these lines when the source of water to the dental unit is normal tap water .

Ozone

The strong oxidative and antimicrobial activity of Ozone enabled it to be used and investigated in a wide range of medical applications (Bocci, 1996). In dentistry, it is getting more attention as clinical trails give more data of the potential especially in the treatment of dental caries (Baysan et al, 2000; Abu-Naba'a et al, 2003a, 2003b) and oral infections and wound healing (Filippi, 2001). A number of studies have investigated Ozone as an agent for control of the contamination of DUWL.

Ozone is a well-known effective means of wastewater (Ternes et al, 2003)and public water disinfection (Shin and Sobsey, 2003). Its biocidal efficiency and the lack of side effects of smell and taste when compared to the Chlorine made it the preferred choice.

Using the High-proton Nuclear Magnetic Resonance (^{1}H NMR), the authors (Shorman, 2001)examined the biochemical quality of ozonation of water in the DUWL. The analysis showed that the main biomolecules present were microbial-derived organic acid anions, notably acetate (A), formate (Form), lactate (L) and propionate (Prop).

Further biomolecules detectable included the amino acid glycine (Gly), a number of aromatic compounds (Ar) and occasionally ethanol (Eth) (Figs. 4 a,b). These results showed that Ozone changes the biochemical composition of the biofilm and microbial structure within the DUWL.

In a study over a period of 8 years, Filippi (1997) compared the efficacy of Hydrogen Peroxide (H_2O_2) and Ozone to control the DUWL contamination. He monitored the waterlines in 12 units treated with integrated H_2O_2 water disinfection units according to the manufacturer recommendations (Dentosep PL, Siemens, Germany) and compared them with two other units with similar work load and circumstances that were treated with Ozone from Cytozone water ozonation unit (Hansler, Germany). Dental units were monitored using microbiological culture for total viable count of water samples and for *Pseudomonas aeruginosa*. Units treated with H_2O_2 were tested 204 times in total with an average of 17 tests per unit. Units treated with Ozone were tested 36 times in total with an average of 18 tests per unit.

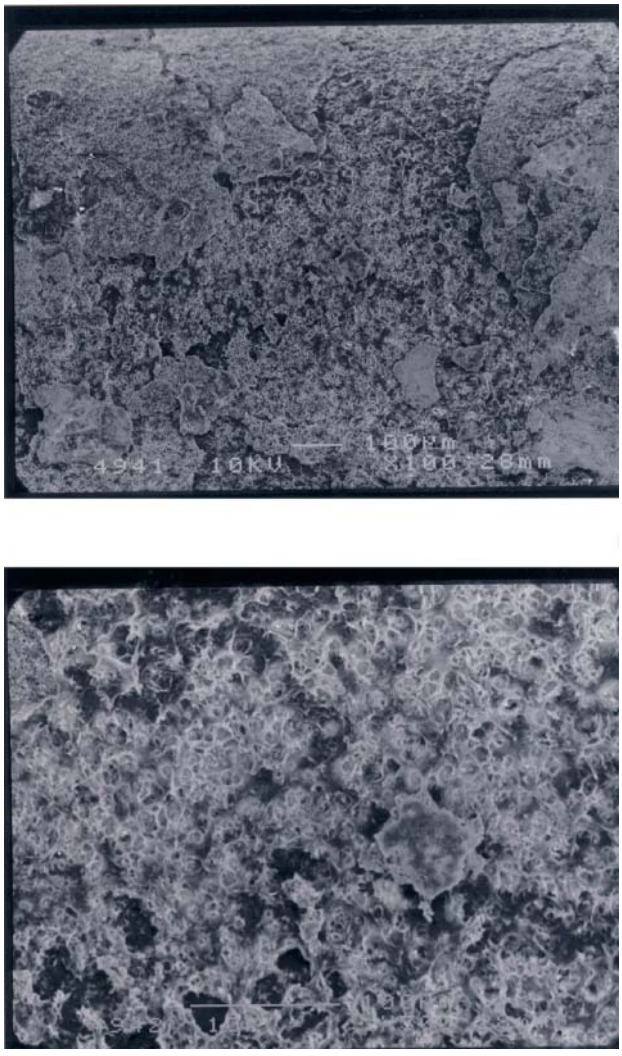

Figure 3: SEM image of a contaminated dental waterline showing the appearance of the biofilm at x100 and x300 magnification.

Table 1: Some of the biocides tested for the control of DUWL contamination. (Walker et al, 2003; Al Shorman et al, 2003)

- Alpron (Cl2 plus biofilm remover)
- Betadine (10%) – povidone iodine
- Bio2000 Ready-To Use
- Chlorhexidine (0.2%)
- Dialox Ready-To-Use (peracetic acid)
- Dioxiclear (chlorine dioxide)
- Enzyme based Biocatalyst (1.25%)
- Flushing (82 ml per min)
- Ozone
- Oxigenal (H_2O_2)
- Sanosil
- Sodium Hypochlorite (0.5%)
- Spor-klenz Ready-To-Use (peracetic acid)
- Sterilox (electrically activated water)
- Tegodor (1%) (aldehydes)
- Sterilex Ultra (alkaline peroxide)
- Calbenium

The results of that study showed that the water was of an acceptable microbiological quality in 99 tests out of 204 (<50% of tests) in the H_2O_2-treated units and *Pseudomonas aeruginosa* was found in 181 tests (90% of tests).

In the units treated with Ozone, none of the tests showed any recoverable micro-organisms including *Pseudomonas aeruginosa*. The author concluded that by using Ozone, it is possible to obtain a continuous freedom from micro-organisms in dental treatment units (Shorman, 2001).

It can be noted that the number of units in the two groups was not equal. Additionally, it would have been better to include bigger number of units in the Ozone-treated group. Involvement of a control group with no treatment could have been asset to perfect the study. The ethical issue, however, must have dictated the exclusion of this group. A valid criticism would be that the authors did not report the dosage of the Ozone used and the flow rate which are important parameters when dealing with such application of the gas.

In another study, Filippi (1998) measured the concentration of Ozone in the air of the dental clinic that uses the water treatment with Ozone. He found that the level of Ozone was far below the maximum concentration of Ozone in the air in the work place permitted in Germany. This led him to the conclusion that the use of Ozone for the control of DUWL contamination is a safe procedure.

Using a laboratory model designed in their institution, Walker et al (2003) investigated the efficacy of a range of material in maintaining acceptable level of bacterial load in the DUWL and on the biofilm inside them. The biofilm tested was created in the lab within the tubes over a period of 2 weeks. Ozone was one of the agents, which did not show a consistent high level of efficacy on planktonic bacteria and biofilm. The agents tested with the exception of Ozone were left within the lines overnight (contact time 16 hours) while Ozone was applied for 10 minutes. Moreover, the concentration used (200 mg/h) was relatively low for such a trial. As mentioned earlier, Ozone being highly reactive is rapidly neutralised so for a long contact time, a continuous supply of freshly ozonated water should be used if a long contact time is required.

In a laboratory trial, Cardon et al (2002) used 8 sections of tubing cut from older dental units. A recirculating level of 0.05 ppm Ozone consistently reduced the planktonic counts to 100 CFU/mL or less. However, samples taken after 30 minutes after Ozone treatment harboured more than 10^4 CFU/mL. The authors concluded that the ozonation system evaluated appears to have no long-term benefit on DUWL biofilm control. Unfortunately, the authors did not mention the flow rate of Ozone used in this experiment.

Another water ozonation system developed by Compact Membrane System, DE was evaluated by Puttaiah et al (2001a), which consisted of daily purging of the DUWL with ozonated water, and providing membrane mixed ozonated dental water. Daily purging was carried out for 10 and 20 minutes in two different groups of units. The study concluded that this Ozone system controlled the waterline biofilm and provided safe treatment water for dental care over a period of 4 weeks.

In another study over a period of 12 weeks, the municipal water to the dental units was irradiated with UV light. The waterlines were purged at a varying frequency (once weekly to daily) and duration (10 and 20 minutes) with ozonated air. The results in this study indicated that daily purging the lines with ozonated air using filtered and UV irradiated dental treatment water as an irrigant controls the waterlines biofilm, and provides safe water for dental care (Puttaiah, 2001b). Again, the authors did not report the dosage and the flow rate of the Ozone used in these experiments.

Using a new Ozone device (CurOzone unit, USA and KaVo, Germany) that delivers Ozone in a concentration of 2100 ppm and a flow rate of 615 ml/min, the authors conducted a series of studies to investigate the effect of Ozone on the biofilm inside the DUWLs and consequently in controlling the contamination.

In one of the studies, two different protocols were used. They were based on an assumption that Ozone may have the ability to reduce the bacterial count to the internationally acceptable level when used frequently in low concentrations. Ozone could reduce or remove the biofilm if administered in high concentration.

First protocol (multiple low doses of Ozone)

Three *KaVo* dental units, which were previously investigated and known to have an existing biofilm, were tested. The ADA and BDA recommend that the waterlines be flushed for 2 minutes every morning before treating the first patient and for 30 seconds between every two patients (ADA, 1999; Shorman, 2003). Therefore, all units were flushed daily for 2 minutes with water ozonated for different concentrations. This treatment was carried out for 7 days.

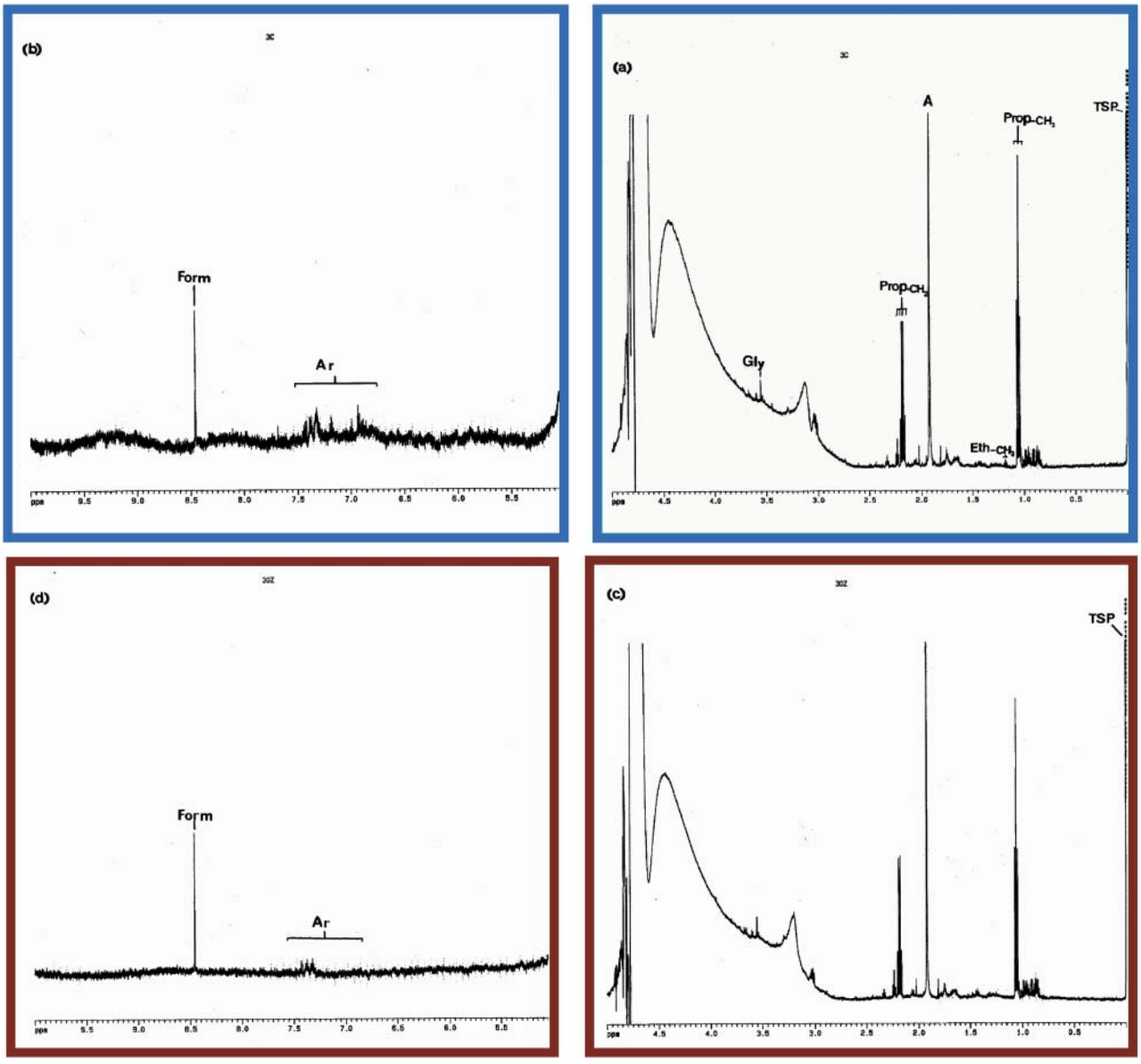

Figure 4(a): A 600 MHz single pulse ^{1}H NMR spectrum of a typical DUWL specimen.

Figure 4(b): The increase in formate (Form) concentration due to the oxidative consumption of carbohydrates.

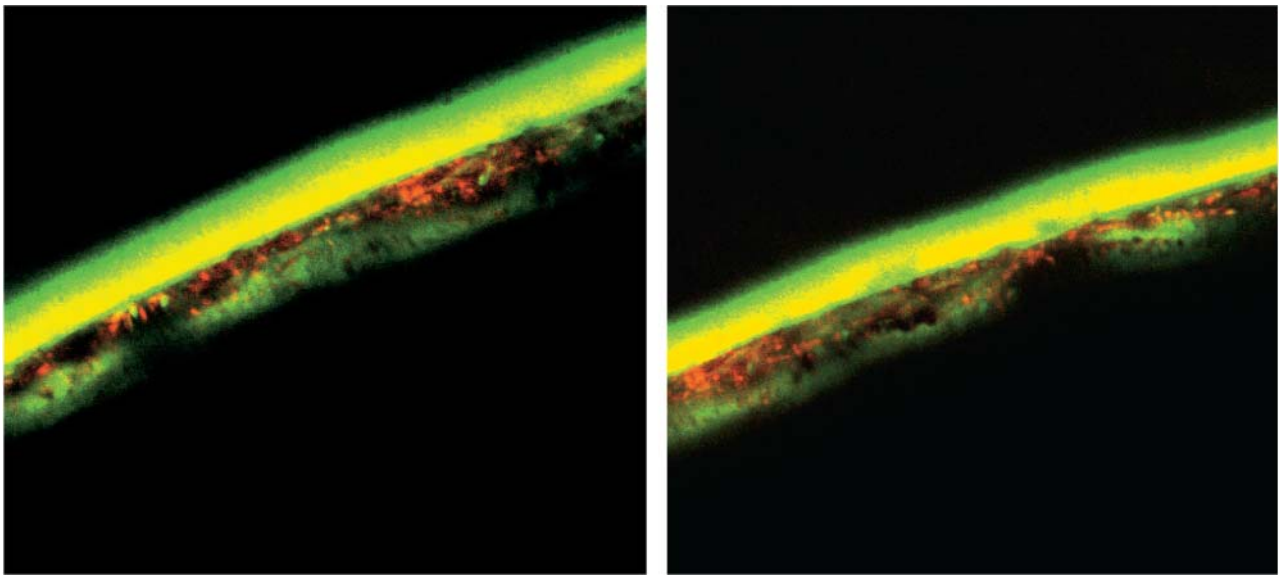

Figure 5: CLSM images of waterline baseline samples.

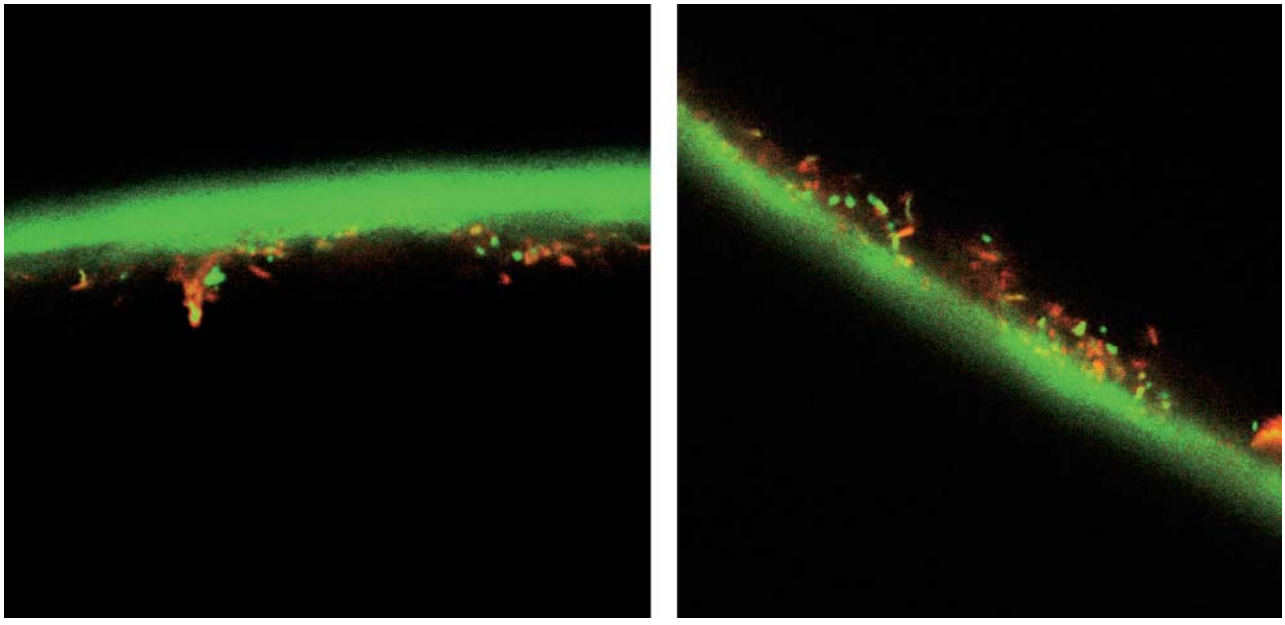

Figure 6: CLSM images of waterline samples after 5 minutes of flushing.

In the early morning, before the units were being used to treat patients, the water (3.5–4 litres) in the water bottle of unit 1 was ozonated for one minute after which the high-speed handpiece line was flushed for 2 minutes. Water samples were collected immediately before ozonation and immediately after flushing the line in a sterile container. This treatment was repeated daily for 7 seven days. The exact same treatment procedure was used with the other two units except that the water ozonation was done for 2 minutes in unit 2 and for 3 minutes in unit 3. The Ozone concentration was in the range of 0.02–0.03 mg/L.

Second protocol (single high dose of Ozone)

One dental unit (unit 4) was tested. A baseline water sample was collected via the high-speed handpiece and a 3-cm baseline sample of the waterline was collected

in two separate sterile containers. The water (4 litres) in the water bottle was then ozonated for 15 minutes resulting in Ozone concentration of 0.25 mg/L. The high-speed handpiece line was subsequently flushed for 15 minutes with this ozonated water. Water and 2–3 cm waterline samples were collected after 5, 10 and 15 minutes of flushing.

The results of this experiment showed that the baseline bacterial counts of water samples from units 1, 2 and 3 were respectively 2.9×10^4, 9×10^4 and 2.7×10^4 CFU/ml. After 7 days the bacterial counts became 2×10^5, 0 and 0 CFU/ml in the same order.

In unit 4 treated with higher concentration of Ozone, the baseline bacterial count was 2.1×10^4 CFU/mL. It became 0 CFU/mL after 5 minutes of flushing onwards.

The four units were monitored for any side effects of the Ozone treatment in terms of water leakage or any other faults reported by the operating dental staff.

Confocal Laser Scanning Microscopy (CLSM) was

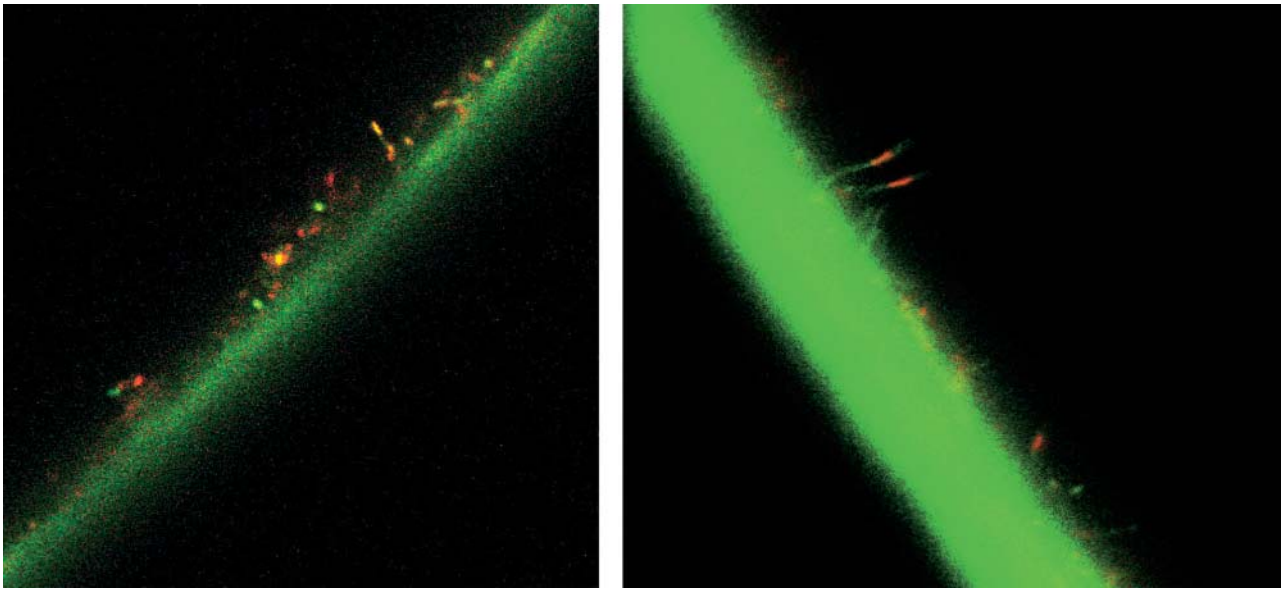

Figure 7: CLSM images of waterline samples after 10 minutes of flushing.

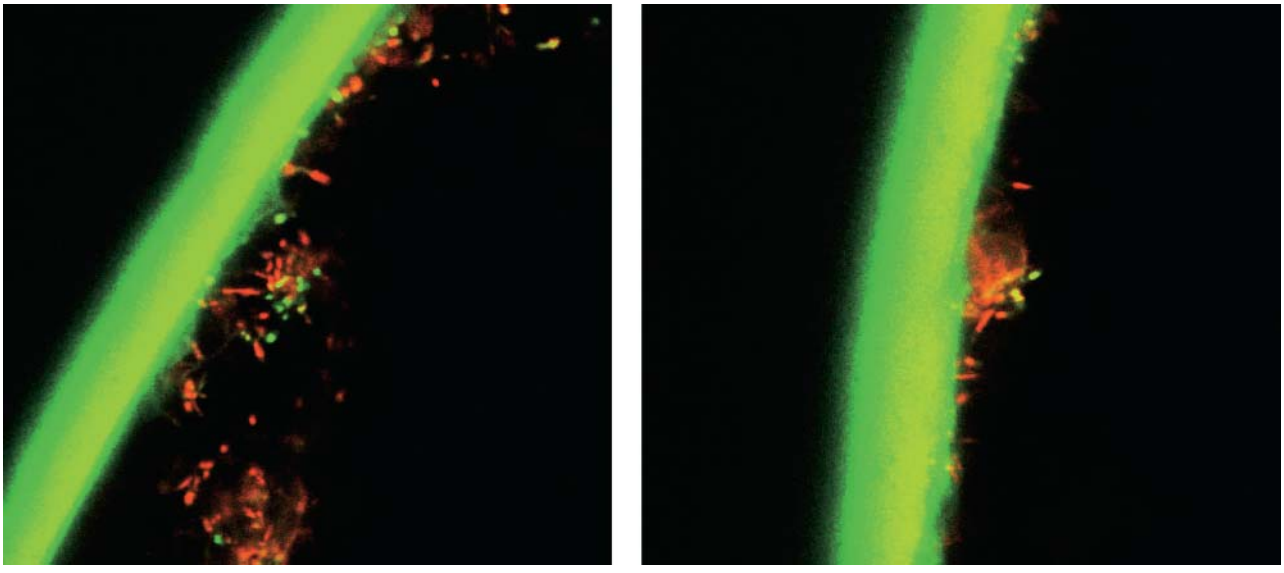

Figure 8: CLSM images of waterline samples after 15 minutes of flushing.

used to view the biofilm status in unit 4. The microscopic study of the baseline sample showed clearly the presence of the biofilm harbouring dead and live bacteria (Fig. 3). The biofilm appeared to have been reduced after 5 minutes of flushing with the ozonated water and contained a mixture of living and dead bacteria (Fig. 4). The biofilm reduction became more evident after 10 minutes of flushing with almost no living bacteria (Fig. 5). In most of the sections cut from the sample collected after 15 minutes of flushing, the biofilm was absent and there were only some remnants of sloughing biofilm that is shown at high magnification in Fig. 6.

There were no noticed or reported side effects or faults after the treatment in any of the units.

The authors conducted another work to treat DUWL after the emergence of *Pseudomonas aeruginosa* in a newly installed dental unit (Fig. 7) (Al Shorman et al, 2003). Ozone from the HealOzone unit was introduced into the water bottle in the unit (1 L) for 5 minutes and the line was flushed with the ozonated water for 10 minutes. Before the Ozone treatment the microbiological culture of water sample from this unit was pure *Pseudomonas aeruginosa* 3.4×104 CFU/mL. The sample that was taken after the treatment showed the *Pseudomonas aeruginosa* count to have reduced to 60 CFU/mL. Another similar treatment session was done that brought the line to sterility.

In conclusion, it is evident that Ozone has a very good position as an agent to treat or prevent the contamination of DUWL. However, as the idea is relatively new and the research done in this field is not sufficiently extensive, more investigations are needed. It is important that any published data include the dosage and the flow rate of the gas used to enable the dental professionals to compare products and protocols.

As Ozone destroys rubber, an important disadvantage when used in the dental unit is the possibility to destroy some rings and diaphragms made from rubber. Nonetheless, there are no reported such events in any of the studies published. Our work in this field is consistent with these observation in that we had not noticed and drawback of the use of Ozone from the HealOzone system in the treatment of DUWL contamination.

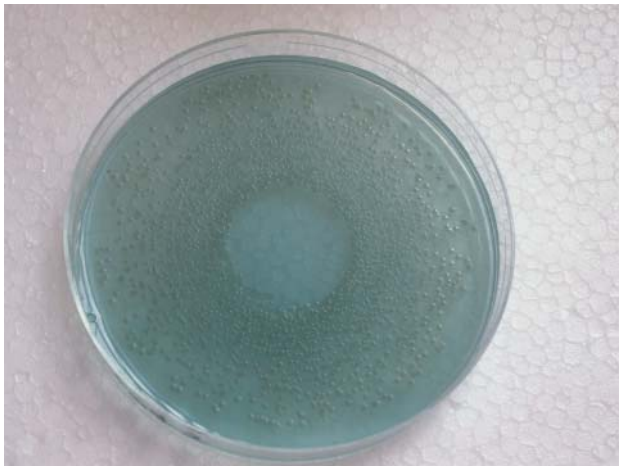

Figure 9: The appearance of Pseudomonas aeruginosa on culture medium.

Figure 10: The quick-release coupling fitted on the water bottle of the dental unit.

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CHAPTER 6.2

Synergistic Combination of HealOzone and Ozonated Oil Treatment

Julian Holmes

It has been stressed in various places in this book, that ozone treatment is not new to medicine or dental care. The Swiss dentist E.A. Fisch was using ozone in dentistry before 1932, bubbling ozone into saline. Dr Fisch used this ozonated saline to irrigate in oral surgery. He is credited with introducing a friend, the German surgeon Erwin Payr to the properties of ozone, who began to use this treatment modality in his operating rooms in the late 1930s. Ozone from the HealOzone unit has been researched in more than one hundred studies to date and every single study has proven the clinical reversal of caries. This chapter discusses ozonated oils and the best results have been obtained when they have been used in conjunction with the use of ozone from the HealOzone unit (KaVo).

In Eastern Europe and Cuba, where alternative medical care and treatment has been researched, ozonated oils are used in every day treatment of skin conditions and to combat infections. They have been used for the treatment of intra-oral and extra-oral infections and lesions.

There are many incidences where an alternative delivery system is beneficial. An example of this would be Amoxycillin. It is available as a liquid for children and those who cannot manage to swallow tablets or capsules, a capsule for adults, and as a cream for topical application.

Hence the development of the HealOzone unit, which releases ozone only if a seal is formed around the periphery of the delivery cup. This would be difficult in the case of a periodontal pocket, or where ozone was required over a larger surface. In contrast, when dissolved in an oil base, it forms long complex molecules called ozonoids. These ozonoids are effective anti-microbial agents, as well as stimulating the reparative and regenerative pathways at a cellular level.

Traditionally, ozone has been dissolved in olive oil. But the purity of olive oil is variable from one region to another, and depends on the soil type, the country of origin, and the method of oil extraction from the pressed olive fruit pulp. ^{1}H NMR studies by Professors Lynch and Grootveld have investigated different types of oils for ozonisation. Professor Lynch presented original research in this field at the annual meeting of the American Association for Dental Research in 2003, and this was published in the Journal of Dental Research in 2003. The work was entitled: ^{1}H NMR Analysis of Ozone-treated Grapeseed, Olive, and Sunflower Seed Oils and the authors were Professors Lynch and Grootveld as well as Dr's Holmes, Silwood, Claxson, Prinz and Toms.

The antimicrobial actions of ozonated oils represent a novel pharmaceutical approach to the management of a variety of medical and dental problems. The aim of this study was to determine which compounds might be responsible for the therapeutic benefits offered by these products.

Commercially-available grapeseed, olive and sunflower seed oils (GO, OO and SO respectively) were divided into two equivalent portions. The first was treated with Ozone (O_3) generated by the HealOzone unit (KaVo) for 10 minutes; the second group of portions served as untreated controls. ^{1}H NMR spectra of these samples were acquired on a Bruker AMX-600 spectrometer in the University of London.

Treatment of each vegetable oil with O_3 gave rise to

the consumption of polyunsaturated fatty acids present (i.e. significant reductions in their *mono-* and *bis*-allylic-CH_2 group resonances located at 2.06 and 2.76 ppm respectively, and also that of their vinylic protons at 5.38 ppm), consistent with their ozonation. Indeed, signals present in the 5.10–5.25 ppm regions of the ozonated GO and SO spectra are assignable to the ring protons of ozonides. Further O_3-induced modifications to the oils included the production of aldehydes, i.e. -CH_2CHO aldehydic group triplet resonances at 9.65 (ozonated GO and SO) and 9.74 ppm (all ozonated oils), terminal products arising from the decomposition of ozonides. This study concluded that ozone treatment of commercially-available vegetable oils gives rise to the production of ozonides and aldehydes, agents that are likely to account for the antimicrobial properties of ozonated oil products.

These oils are produced by Rainbow-Smiles, and are used in the UK, Europe and in the USA. The oils are presented as a thick gel, which should be cooled and refrigerated when received. They are supplied in 20-ml containers.

The Uses and Indications

The uses and indications for this type of product are numerous:

Periodontal pockets

Several dental practices in the UK and the USA have used these oils in periodontal cases for the last 12 months. The oil gel is loaded into a small 1.25 ml syringe, and with a fine delivery tip (Ultradent, USA supply the best delivery products for these oils) the oil-gel can be delivered into a periodontal pocket with ease. As there is stimulation of the reparative cellular mechanisms, the appearance of the tissue is pink and healthy.

Surgical areas

After surgery for removal of teeth, abscess drainage, third molar tooth removal, apicectomy, etc, patients are dispensed 20 ml of ozonated oil. They are instructed to use a clean 'q' tip or cotton wool to apply the oils to the surgical site 3–4 times each day, for a minimum of 5 days. However numerous dentists have used the large cup to deliver ozone from the HealOzone unit for this purpose, and report excellent results without a single adverse event despite many thousands of applications.

Herpes simplex

Herpes infections present a particular problem in dental care. Not only are they potentially debilitating for the patient, but also it is possible for members of the dental team to become infected if vesicles burst and cross-infect skin lesions or wounds. There are a number of commercial anti-viral agents available, such as aciclovir, famciclovir, valaciclovir and inosine pranobex. All depend on the application of these preparations at the prodromal stage; this is the stage when the skin area begins to prickle, just before the blisters or vesicles appear. If the ozone gas is applied using a large cup from the HealOzone unit at this stage, the vesicles do not appear in the majority of cases. Thousands of patients have prevented cold sores developing after Ozone application from the HealOzone. Patients have also commented that once treated in this way, herpes does not recur at the same site. The cycle of infection and dormancy of the virus depends on the nervous pathways and ganglion to reside in. At the moment this observation cannot be explained, and needs to be further researched. Oils can also be used.

Ulcers

Ozone from the HealOzone has been used on many patients with aphthous ulcers. All patients have reported a decrease in the time taken for the ulcers to heal and resolve, compared to previous treatment modalities. Patients have also commented that any pain disappears without further pain management after about 6 to 12 hours. Oils can be given to the patient for application at home.

Denture sore mouth

Denture sore mouth is a yeast infection. Patients are instructed to apply the oil to the fitting surface of the

denture, and then replace the denture in their mouth. This is a very simple protocol for the patient to use and follow. If applied at 4-hourly intervals, the average time to eradicate the yeast infection, and see soft tissue healing is about 2 days. Also, the denture-fitting surface appears to be much cleaner, due to the stain removal properties of the ozone compounds.

Nail infections

Several of the dentists who were the first to trial these ozone oils also reported success in clearing nail infections. Nail infections are chronic (yeasts and fungi) or acute (bacterial). Ozone gas is easy to apply if the practice has a Healozone unit. Ozone from the HealOzone and oils in these cases were effective in eradicating these infections within 5 to 10 days, and promoting normal nail growth by week 5–6.

Other uses

Denture sore mouth and athlete's foot are very similar yeast infections. Athlete's foot can be eliminated in about 48 hours using ozone from the HealOzone and oils. The inter-toe areas are cleaned and dried, and the oil applied every 4–6 hours.

In acne and other dermatological conditions, ozone and the oils have been used with remarkable effects, not only keeping the areas free of potential cross-contamination and opportunistic infection, but also stimulating tissue repair and healing.

The use of ozonated oils over the last 2 years in selected dental practices has seen accelerated healing and resolution of cases where traditional pharmacology has not offered resolution. There have been no adverse results reported by the group of users. Every patient has reported decreased pain, accelerated healing, and minimal scarring after use. The taste of these oils has been commented on, but all patients have been impressed with the accelerated healing of their lesions.

The huge advantage these ozone technologies have over traditional anti-microbial agents is that as the anti-microbial effect is so fast, there is no time for the microbial populations to establish a resistance to them. In effect, this is the perfect antibiotic! Essentially, if you consider any infective process as a bio-film problem, ozonated oil has a potential part to play in its eradication, and also in the healing process of the surrounding tissue. The uses outside dental care have huge potential in medicine and animal care.

The oil-gels are made by O3DC and distributed by Rainbow-Smiles. To order these oils, and for current costs, please visit contact Dr Julian Holmes on *www.julian@o3dc.co.uk* All the profits raised from the sales of these oils are being used for research and providing HealOzone treatment free to children from low income families.

References

1. Lynch E, Grootveld M, Holmes J, Silwood C, Claxson A, Prinz J, Toms H. ^{1}H NMR Analysis of Ozone-treated Grapeseed, Olive, and Sunflower Seed Oils. AADR 2001 Journal of Dental Research 182.