

# *Gerodontology*

*Volume 20 Number 2  
December, 2003*



Published by The Gerodontology Association

# Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial

J Holmes

UKSmiles Dental Practice, Wokingham, Berkshire, UK

## Abstract

**Objective:** To assess the effect of an ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery primary root carious lesions (PRCL's), in an older population group. **Design:** A total of 89 subjects, (age range 60-82, mean  $\pm$  SD,  $70.8 \pm 6$  years), each with two leathery PRCL's, were recruited. The two lesions in each subject were randomly assigned for treatment with ozone or air, in a double-blind design, in a general dental practice. Subjects were recalled at three, six, 12 and 18 months. Lesions were clinically recorded at each visit as soft, leathery or hard, scored with a validated root caries severity index. **Results:** There were no observed adverse events. After three months, in the ozone-treated group, 61 PRCL's (69%) had become hard and none had deteriorated, whilst in the control group, four PRCL's (4%) had become worse ( $p < 0.01$ ). At the six-month recall, in the ozone group, seven PRCL's (8%) remained leathery, the remaining 82 (92%) PRCL's had become hard, whilst in the control group, 10 PRCL's 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, two PRCL's remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCL's had progressed from leathery to soft, i.e. became worse, 65 PRCL's (75%) were still leathery, and one remained hard ( $p < 0.01$ ). At 18 months, 87 (100%) of ozone-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 and only one of the control PRCL's had reversed ( $p < 0.01$ ). **Conclusions:** Leathery non-cavitated primary root caries can be arrested non-operatively with ozone and remineralising products. This treatment regime is an effective alternative to conventional "drilling and filling".

*Key words:* Root caries, reversal, arrest, ozone, toothpaste, mouth-rinse, spray

## Introduction

### *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<sup>1</sup>. 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 (PRCL'S) was thought likely to increase<sup>2</sup>.

Ageing individuals tend to have gum tissue recession, and the associated exposed root surfaces are more susceptible to caries<sup>3,4</sup>. A survey conducted in the United Kingdom in 1988 by Downer<sup>5</sup> 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 PRCL's was reported to be over 80% in elderly institutionalised people<sup>6,7</sup>. Hand *et al.*<sup>8</sup> 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<sup>9-11</sup>. 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 PRCL's has been shown to contain large numbers of acidogenic and aciduric microorganisms, which correlate with the severity of root caries<sup>12-19</sup>.

#### *The detection of root caries*

More accurate detection systems need to be developed for the detection of PRCL's<sup>19-21</sup>. Root carious lesions are classified as soft, leathery or hard, based on differences in the degree and pattern of mineralisation. Nyvad and Fejerskov<sup>22</sup> 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<sup>22</sup> 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<sup>12,23</sup>. Research suggests that soft and leathery lesions can remineralise and may become hard<sup>3,12,23,24</sup>. 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.*<sup>25</sup> and the term primary root carious lesion (PRCL) was proposed by Lynch in 1986<sup>20,23</sup>. 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<sup>26</sup> and marginal adaptation<sup>27</sup> necessitating frequent restorative replacement<sup>28</sup>.

Previous studies have shown that root caries prevalence increases with age<sup>6,29,30</sup> from about one in nine root surfaces at risk for under-30's, to approximately two in three for the over 60's<sup>4</sup>. Attachment loss and exposure of root surfaces to the oral environment are accepted to be nearly universal prerequisites to the development of root caries<sup>31-33</sup>.

Papas *et al.*<sup>34</sup> emphasised the high involvement of posterior teeth with root caries. The microbial colonisation of PRCL's has been extensively investigated. Attention has focused on the causative micro-organisms in the aetiology of root caries<sup>12,23,35-37</sup>. These studies showed the importance of the acid-producing group of bacteria in PRCL's. Further studies linked salivary and plaque conditions<sup>38,39</sup>. It is now recognised that the most advantageous treatment for root caries is remineralisation<sup>40</sup>. The use of pharmaceutical agents<sup>41,42</sup> and fluoride-containing dentifrices may provide some protection for high caries risk patients. Various pharmaceutical agents have been used, such as fluoride<sup>43-46</sup>, and chlorhexidine or chlorhexidine in combination with thymol<sup>47</sup>.

Keltjens *et al.*<sup>48</sup> 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<sup>49</sup>.

Recent publications conclude that ozone should be considered as an alternative pharmaceutical management strategy<sup>19,50-54</sup> rather than the traditional drill and fill approach. Ozone (a pale blue-coloured gas, chemical formula O<sub>3</sub>) 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, known as the HealOzone (CurOzone, USA and KaVo GmbH & Co, Germany) has been available commercially in Europe for more than two years. Ozone is a very powerful antimicrobial agent. Recently, Baysan *et al.*<sup>51,52</sup> reported that ozone application either for 10 or 20 seconds was effective to kill the great

majority of microorganisms in PRCL's (>99% microbial killing after 10 seconds ozone application).

O<sub>3</sub> is naturally produced by the photodissociation of molecular O<sub>2</sub> into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating HO<sub>3</sub>, which, in turn, decomposes to hydroxyl radical. Further reactions convert O<sub>3</sub> to an even more powerful oxidant, the hydroxyl radical (OH<sup>-</sup>)

In view of its powerful oxidising properties, O<sub>3</sub> can attack many biomolecules such as the cysteine, methionine and the histidine residues of proteins. The effects of ozone on cell structures, metabolism and microorganisms is well-documented in published papers<sup>19,55-59</sup> in both dentistry and medicine. Research has shown that ozone disrupts the cell walls of microorganisms 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 *et al.*<sup>19,51</sup> have published reductions from log<sub>10</sub> 6.0 to log<sub>10</sub> 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 PRCL's<sup>19,50,54</sup>.

### Aim

The aim of this study was to assess the effect of an ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCL's, in an older population group.

### Method

After ethics committee approval, all participants were recruited from consecutive subjects presenting with two leathery non-cavitated PRCL's at UKSmiles Dental Practice, 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 two leathery lesions each (178 PRCL's 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<sup>12,23</sup>. Lesions were randomly assigned into two groups; Group 1; treated with ozone, or Group 2; no ozone for the control PRCL's.

All subjects were prescribed a course of oral hygiene instruction by a member of the practice hygiene team followed by scaling and polishing

with non-fluoride-containing paste at baseline only. Professional instruction was given on brushing, the use of floss and interdental brushes. 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<sup>12,23</sup>. 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 PRCL's 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 PRCL's 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 volume 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 PRCL's 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 re-mineralising 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 four 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 three, six, 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. One week after assessment by the first dentist, 15 subjects (30 PRCL's) were recalled and examined by a third dentist. There was a good agreement in the classifications of hardness and severity of PRCL's ( $\kappa = 0.80$ ). Twenty subjects with 40 PRCL's 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 18 months, 87 subjects had completed the study. Two subjects had moved out of the practice area, and were not available for the 12 or 18 month re-assessment visits. There were no observed or reported adverse events at any of the treatment sessions or afterwards (Table 1).

After three months, in the ozone-treated group, 61 PRCL's (69%) had become hard and none had become worse, whilst in the control group, four PRCL's (4%) had become worse ( $p < 0.01$ ). At the six month recall, in the ozone group, seven PRCL's (8%) remained leathery, the remaining 82 (92%) PRCL's had become hard, whilst in the control group, 10 PRCL's 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, two PRCL's remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCL's had progressed from leathery to soft, i.e. became worse, 65 PRCL's (75%) were still leathery, and one remained hard ( $p < 0.01$ ). At 18 months, 87 (100%) of ozone-treated PRCL's had reversed, 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 and only one of the control PRCL's had reversed ( $p < 0.01$ ).

## 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

**Table 1.** Data sets (numbers of lesions in the first column and percentages in the second column) at baseline, 3, 6, 12 and 18 months

Group 1	Ozone-treated		2 subjects had dropped out at 12 months							
	Baseline		3 months		6 months		12 months		18 months	
Soft	0	0	0	0	0	0	0	0	0	0
Leathery	89	100	28	31	7	8	2	2	0	0
Hard	0	0	61	69	82	92	85	98	87	100
Group 2	Control Group		2 subjects had dropped out at 12 months							
	Baseline		3 months		6 months		12 months		18 months	
Soft	0	0	4	4	10	11	21	24	32	37
Leathery	89	100	85	96	78	88	65	75	54	62
Hard	0	0	0	0	1	1	1	1	1	1

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 elderly subjects have medical conditions that make dental treatment a challenge, such as sudden muscle spasms and movement. This study aimed to assess the effect of a novel ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCL's, 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<sup>60</sup> used to restore PRCL's have required frequent replacement. Preventive treatment regimes for PRCL's may be considered to have a better long-term prognosis than restorative treatment options<sup>61</sup>.

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<sup>62</sup>. In this respect, Bradshaw *et al.*<sup>63</sup> 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<sup>62</sup>. Papas *et al.*<sup>64</sup> reported the efficacy of a dentifrice containing 1,150 ppm sodium fluoride with soluble calcium and phosphate salts. Keltjens *et al.*<sup>48</sup> 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.

This study has shown that dentistry has the ability to reverse lesions with just 40 seconds of ozone treatment. At 18 months, 100% reversal and remineralisation had been achieved. The studies by Baysan<sup>19</sup>, Baysan *et al.*<sup>50-52</sup> 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 PRCL's, only a single lesion (1%) showed reversal, despite improved oral hygiene instruction and care, regular brushing and the use of a remineralising dentifrice, spray and mouth-rinse. These measures alone cannot produce predictable caries reversal. This study has shown that over time, leathery PRCL's can gradually become worse, becoming soft. 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 two 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<sup>12,17-19,23,50-52</sup> for PRCL's and hard root caries lesions are arrested<sup>12,19,23</sup>.

O<sub>3</sub> has the unique feature of decomposing to a harmless, non-toxic and environmentally safe material (oxygen). O<sub>3</sub> has been used in medicine for many years. The first O<sub>3</sub> 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 O<sub>3</sub>. To date, O<sub>3</sub> therapy has been a recognised treatment modality in 16 nations. Research by Baysan showed that the HealOzone system has no potential to leak into the oral cavity<sup>19</sup> due to the unique ozone delivery system. No side effects have ever been documented in either the dental research centres or the numerous 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<sup>65</sup>. 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<sup>66</sup>. It

is important to remember that in 1968, only 21% of people aged 65-74 had any teeth compared to 66% in 1998. Such data illustrate 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<sup>19,51,52</sup>, 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. 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 US population over the age of 65 shows evidence of root caries<sup>66</sup>. 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 PRCL's 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.

### Conclusion

The restorative management of PRCL's 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.*<sup>67</sup> 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.

### References

1. **Steele J G, Walls A W, Ayatollahi S M, et al.** Major clinical findings from a dental survey of elderly people in three different English communities. *Br Dent J* 1996; **180**:17-23.
2. **O'Mullane D M, Whelton H.** Oral health in Irish adults 1899-90. Government Publications Stationery Office, Dublin, 1992.
3. **Hellyer P H, Beighton D, Heath M R, et al.** Root caries in older people attending a general dental practice in East Sussex. *Br Dent J* 1990; **169**: 201-206.

4. **Galan D, Lynch E.** Epidemiology of root caries. *Gerodontology* 1993; **10**: 59-71.
5. **Downer M C.** The improving dental health of United Kingdom adults and prospects for the future. *Br Dent J* 1991; **170**: 154-158.
6. **Banting D W, Ellen R P, Fillery E D.** Prevalence of root surface caries among institutional older persons. *Community Dent Oral Epidemiol* 1980; **8**: 84-88.
7. **Beck J D.** The epidemiology of root surface caries: North American Studies. *Adv Dent Res* 1993; **7**: 42-51.
8. **Hand J S, Hunt R J, Beck J D.** Incidence of coronal and root caries in an older adult population. *J Public Health Dent* 1988; **48**: 14-19.
9. **Silwood C J, Lynch E J, Seddon S, et al.** <sup>1</sup>H-NMR analysis of microbial-derived organic acids in primary root carious lesions and saliva. *NMR Biomed.* 1999; **12**: 345-356.
10. **Silwood C J, Lynch E, Claxson A W, et al.** <sup>1</sup>H NMR investigations of the molecular nature of low-molecular-mass calcium ions in biofluids. *J Biol Inorg Chem.* 2002; **7**: 46-57.
11. **Silwood C L, Grootveld M, Lynch E.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic analysis of human saliva. *J Dent Res.* 2002; **81**: 422-427.
12. **Beighton D, Lynch E, Heath M R.** A microbiological study of primary root caries lesions with different treatment needs. *J Dent Res* 1993; **73**: 623-629.
13. **Lynch E, Beighton D.** Relationships between mutans streptococci and perceived treatment needs of primary root carious lesions. *Gerodontology* 1993; **10**: 98-104.
14. **Ship J A, Fox P C, Baum B J.** How much saliva flow is enough? "Normal" function defined. *J Am Dent Assoc* 1991; **122**: 63-69.
15. **Brailsford S R, Lynch E, Beighton D.** The isolation of *Actinomyces naeslundii* from sound root surfaces and root carious lesions. *Caries Res* 1998; **32**: 100-106.
16. **Lynch E, Beighton D.** Short term effects of Cervitec on the microflora of primary root carious lesions requiring restoration. *Caries Res* 1993; **27**: 106.
17. **Lynch E.** Relationships between clinical criteria and microflora of primary root caries. Proceedings of the First Annual Indiana Conference, Indianapolis. Indiana University School of Dentistry (ISBN 0-9655149), 1996; 195-242.
18. **Lynch E, Beighton D.** A comparison of primary root caries lesions classified according to colour. *Caries Res* 1994; **28**: 233-239.
19. **Baysan A.** Management of primary root caries using ozone therapies. PhD Thesis, University of London, 2002.
20. **Lynch E.** The measurement of root caries for research purposes. *J Dent Res* 1986; **65**: 510.
21. **ten Cate J M, van Amerongen J P.** Caries diagnosis, conventional methods. Proceedings of the First Annual Indiana Conference, Indianapolis. Indiana University School of Dentistry (ISBN 0-9655149), 1996, 27-37.
22. **Nyvad B, Fejerskov O.** Scanning electron microscopy of early microbial colonization of human enamel and root surfaces *in vivo*. *Scand J Dent Res* 1987; **95**: 287-296.
23. **Lynch E.** The diagnosis and management of primary root caries. PhD. thesis, University of London, 1994.
24. **Hellyer P, Lynch E.** Diagnosis of root caries - a critical review. *Gerodontology* 1991; **9**: 95-102.
25. **Hazen S P, Chilton N W, Mumma R D.** The problem of root caries; 1. Literature review and clinical description. *J Am Dent Assoc* 1973; **86**: 137-144.
26. **Taylor M J, Lynch E.** Microleakage. *J Dent* 1992; **20**: 3-10.
27. **Taylor M J, Lynch E.** Marginal adaptation. *J Dent.* 1993; **21**: 265-73.
28. **Lynch E, Tay W M.** Glass ionomer cements part III- clinical properties II. *J Irish Dent Assoc* 1989; **35**: 66-73.
29. **Vehkalahti M, Rajala M, Tuominen R, et al.** Prevalence of root caries in the adult Finnish population. *Community Dent Oral Epidemiol* 1983; **11**: 188-190.
30. **Manji F, Fejerskov O, Baelum V.** Pattern of dental caries in an adult rural population. *Caries Res* 1989; **23**: 55-62.
31. **Stamm J W, Banting D W, Imrey P B.** Adult root caries survey of two similar communities with contrasting natural water fluoride levels. *J Am Dent Assoc* 1990; **120**: 143-149.
32. **Katz R V.** The clinical diagnosis of root caries. Issues for the clinician and researcher. *Am J Dent* 1995; **8**: 335-341.
33. **Galan D, Lynch E.** Prevention of root caries in older adults. *J Can Dent Assoc.* 1994; **60**: 422-433.



34. **Papas A, Joshi A, Giunta J.** Prevalence and intraoral distribution of coronal and root caries in middle-aged and older adults. *Caries Res* 1992; **26**: 459-465.
35. **Beighton D, Lynch E.** Relationships between yeasts and primary root-caries lesions. *Gerodontology*. 1993; **10**:105-108.
36. **Collier F I, Heath M R, Lynch E, et al.** Assessment of the clinical status of primary root carious lesions using an enzymic assay. *Caries Res*. 1993; **27**: 60-64.
37. **Beighton D, Hellyer P H, Lynch E J, et al.** Salivary levels of mutans streptococci, lactobacilli, yeasts, and root caries prevalence in non-institutionalized elderly dental patients. *Community Dent Oral Epidemiol* 1991; **19**: 302-307
38. **Fure S.** Five-year incidence of caries, salivary and microbial conditions in 60-, 70- and 80-year-old Swedish individuals. *Caries Res* 1998; **32**: 166-174.
39. **Beighton D, Lynch E.** Comparison of selected microflora of plaque and underlying carious dentine associated with primary root caries lesions. *Caries Res* 1995; **29**:154-158
40. **Allen E P, Bayne S, Becker I, et al.** Annual review of selected dental literature: Report of the Committee on scientific investigation of the American Academy of Restorative Dentistry. *J Prosthet Dent* 1999; **83**: 27-66.
41. **Lynch E.** Antimicrobial management of primary root carious lesions: a review. *Gerodontology*. 1996; **13**: 118-129.
42. **Baysan A, Lynch E.** Management of primary root caries with a high fluoride dentifrice. *Tissue Preservation and Caries Treatment*. Quintessence Book 2001, Chapter 2, 37-48.
43. **Lynch E, Baysan A, Ellwood R et al.** Effectiveness of two fluoride dentifrices to arrest root carious lesions. *Am J Dent* 2000; **13**: 218-220.
44. **Lynch E, Baysan A.** Reversal of primary root caries using a dentifrice with a high fluoride content. *Caries Res* 2001; **35**: 60-64.
45. **Baysan A, Lynch E, Ellwood R et al.** Reversal of primary root caries using dentifrices containing 5,000 and 1,100 ppm fluoride. *Caries Res* 2001; **35**: 41-46.
46. **Duckworth R.** The science behind caries prevention. *Int Dent J* 1993; **43**: 529-539.
47. **Lynch E, Brailsford S R, Morris-Clapp C, et al.** Effect on Cervitec on the treatment needs of primary root-caries. *J Dent Res* 1995; **73**: 535
48. **Keltjens H M A M, Schaeken M J M, van der Hoeven H.** Preventive aspects of root caries. *Int Dent J* 1993; **43**: 143-148.
49. **Wright P S, Hellyer P H, Beighton D, et al.** Relationship of removable partial denture use to root caries in an older population. *Int J Prosthodont* 1992; **5**: 39-46
50. **Baysan A, Lynch E, Grootveld M.** The use of ozone for the management of primary root carious lesions. *Tissue Preservation and Caries Treatment*. Quintessence Book 2001, Chapter 3, 49-67.
51. **Baysan A, Whiley R, Lynch E.** Anti-microbial effects of a novel ozone generating device on microorganisms associated with primary root carious lesions in vitro. *Caries Res* 2000; **34**: 498-501.
52. **Baysan A, Lynch E** Effect of ozone on the oral microbiota and clinical severity of primary root caries *Am J Dent*, 2004, Accepted for publication.
53. **Lynch E.** Kariesbehandlung mit Ozon. *Die Quintessenz* 2003; **54**: 608-610.
54. **Lynch E.** Leczenie prochnicy za pomoca ozonu. *Quintessence dla lekarzy stomatologow* 2003; **11**:198-200.
55. **Bocci V.** Ozonization of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis. *Med Hypothesis* 1992; **39**: 30-34.
56. **Bocci V, Luzzi E, Corradeschi F, et al.** Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. *J Biol Regul Homeost Agents* 1993; **7**: 133-138.
57. **Bocci V.** Does ozone therapy normalize the cellular redox balance? Implications for therapy of human immunodeficiency virus infection and several other diseases. *Med Hypotheses* 1996; **46**: 150-154.
58. **Bocci V.** Ozone as a bioregulator. Pharmacology and toxicology of ozonotherapy today. *J Biol Regul Homeost Agents* 1996; **10**: 31-53
59. **Bocci V.** Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *Br J Biomed Sci* 1999; **56**: 270-279.
60. **Lynch E, Tay W M.** Glass ionomer cements part III- clinical properties II. *J Irish Dent Assoc* 1989; **35**: 66-73.
61. **Arneberg P.** Dental caries in the elderly. 2. Root caries. Symptoms and treatment guidelines. *Nor Tannlaegeforen Tid* 1989; **99**: 676-679.
62. **Nyvad B, Fejerskov O.** Active root surface caries converted into inactive caries as a response to oral hygiene. *Scand J Dent Res* 1986; **94**: 281-284.

63. **Bradshaw D J, McKee A S, Marsh P D.** Prevention of population shifts in oral microbial communities *in vitro* by low fluoride concentrations. *J Dent Res* 1990; **69**: 436-441.

64. **Papas A, Russell D, Singh M, et al.** Double blind clinical trial of a remineralizing dentifrice in the prevention of caries in a radiation therapy population. *Gerodontology* 1999; **16**: 2-10.

65. **Nunn J, Morris J, Pine C, et al.** The condition of teeth in the UK in 1998 and implication for the future. *Br Dent J* 2000; **23**: 613-644.

66. **Anusavice K J.** Need for early detection of caries lesions: A United States Perspective. Proceedings of the 4th Annual Indiana Conference, Indianapolis. Indiana University School of Dentistry (ISBN 0-9655 149-2-7), 2000, 13-29.

67. **Emilson C G, Ravald N, Birkhed D.** Effects of a 12-month prophylactic programme on selected oral bacterial populations on root surfaces with active and inactive carious lesions. *Caries Res* 1993; **27**: 195-200.

Address for correspondence:

Dr Julian Holmes, BDS  
UKSmiles Dental Practice,  
3 Old Row Court, Rose Street,  
Wokingham, Berkshire,  
RG40 1XZ, United Kingdom

Tel: +44 1189795559

Fax: +44 To add

e-mail: julian@o3dc.co.uk