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Effects of zinc and fluoride on the remineralisation of artificial carious lesions under simulated plaque-fluid conditions

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Declaration

There are no conflicts of interest of which we are aware

Abstract

The aim was to study the effects of zinc and fluoride on remineralisation at plaque-fluid (PF) concentrations. Artificial carious lesions were created in two acid-gel demineralising systems (initially infinitely under-saturated and partially-saturated with respect to enamel) giving lesions with different mineral distribution characteristics (high- and low-R respectively) but similar integrated mineral-loss values. Lesions of both types were assigned to one of four groups and remineralised for 5 d at 37°C. Zinc and fluoride were added, based on PF concentrations 1 h post-application, to give four treatments; 231 µmol/L zinc (Zn), 10.5 µmol/L fluoride (F), zinc/fluoride combined (Zn/F) and an unmodified control solution (non-F/non-Zn). Subsequently remineralisation was measured using microradiography. High-R lesions were analysed for calcium, phosphorus, fluoride and zinc using electron-probe microanalysis (EPMA). All lesions underwent statistically-significant remineralisation. For low-R, remineralisation was in the order non-F/non-Zn^a < F^a < Zn^{ab} < Zn/F^b, and for high-R, F^a < non-F/non-Zn^b < Zn^b < Zn/F^c (treatments with the same letter not significantly different ($p < 0.05$)). Qualitatively, remineralisation occurred throughout non-F/non-Zn and Zn, predominantly at the surface-zone (F) and within the lesion-body (Zn/F). EPMA revealed zinc in relatively large amounts in the outer regions (Zn, Zn/F). Fluoride was abundant not only at the surface (F) but also in the lesion-body (Zn/F). Calcium:phosphate ratios were similar to hydroxyapatite (all). To conclude, under static remineralising conditions simulating PF, Zn/F gave significantly greater remineralisation than did F, possibly because zinc in Zn/F maintained greater surface-zone porosity when compared with F, facilitating greater lesion-body remineralisation.

Introduction

Zinc is incorporated into many fluoride toothpaste formulations, to reduce calculus, as an anti-bacterial agent and to reduce oral malodour [Segreto et al., 1991; Saxton et al., 1986; Young et al., 2003]. However, the primary oral health benefit conferred by the regular use of fluoride toothpastes is a reduction in caries incidence. Fluoride effects this by retarding demineralisation and promoting remineralisation. Therefore when additional agents are incorporated into fluoride toothpaste formulations, it is important to ensure that the anti-caries efficacy of fluoride is not compromised.

The effects of zinc on the de- and remineralisation dental mineral and hydroxyapatite have been studied extensively. It was established long ago that it can reduce enamel solubility [Brudevold et al., 1963], and mechanistic studies have shown that it can reduce crystal-growth of Brushite (Br), octacalcium phosphate (OCP) and hydroxyapatite (HA), even at relatively low concentrations [reviewed by Legeros et al., 1999]. Thus it has the potential to influence both demineralisation and remineralisation. However, data from in vitro pH-cycling studies incorporating both de- and remineralisation have reported no such effects for zinc when delivered from fluoride toothpastes [ten Cate, 1993; Laucello et al., 2007]. A reduction in enamel demineralisation in situ was reported by ten Cate [1993], but it was concluded that this could not be attributed to direct interaction with the enamel substrate, and may have been the result of anti-bacterial effects to some extent. In a rat-caries study, zinc had no effect on the anti-caries effect of fluoride [Ingram et al., 1984] and subsequently, in a three-year caries clinical trial (CCT), the addition of zinc to fluoride toothpastes containing 1000, 1500 and 2500 ppm fluoride (as sodium monofluorophosphate (SMFP)) the same finding was reported, with zinc having no effect on caries, either positive or detrimental, at any of the three fluoride concentrations [Stephen et al., 1988]. Ripa et al. [1990] reported that during a further CCT, there was no significant difference in anti-caries effectiveness between two anti-calculus toothpastes, both of which contained zinc, one containing fluoride as SMFP and the other as sodium fluoride (NaF), and an SMFP control toothpaste.

Given that zinc can clearly affect both demineralisation and remineralisation, an apparent contradiction exists. The aim of the present study was to study the effects of zinc and fluoride on remineralisation of demineralised enamel at concentrations based on those found in plaque-fluid [Saxton et al., 1986; Newby et al., unpublished] one-hour after application. Previous studies have shown that the R value (i.e. total lesion mineral-loss/depth) of artificially-created lesions can affect the outcome of remineralisation studies [Lynch et al., 2007]. Therefore two types of lesion, of differing R-value, were employed.

Materials and methods

Preparation of enamel blocks

Sound enamel blocks (120) were prepared from bovine permanent incisors. The central portions of the labial surfaces were abraded to a depth of about 0.5mm using wet 600-grit carborundum paper on a rotary grinder (Silfradent model 801, Silfradent, Sofia, Italy). The abraded areas were then polished using 9 µm silica (Logitech, Glasgow, Scotland). Blocks of approximate dimension 6 x 3 mm were cut laterally from this polished area with a water-cooled rotary disc cutter (Microslice 2, Malvern Instruments, Malvern, UK) and painted with nail varnish (Number 7, Boots, Nottingham, UK), to leave only polished enamel windows ca. 5 x 2mm exposed. After drying at room temperature the blocks were mounted in dental wax (Beading Wax, Kemdent, Swindon, UK) in the bases of two crystallising dishes.

Lesion formation

Two types of lesion were created in two different acid-gel demineralising systems, based on the initially infinitely-undersaturated (US) and partially-saturated (PS) systems described by Lynch and ten Cate [2006a], to create lesions with similar mineral-loss (ΔZ) values but different R-values.

In both cases, the blocks were demineralised at pH 4.6 and 37°C in a methyl cellulose/lactic acid system (50 mmol/L lactic acid/8% methyl cellulose (aqueous, 1500cPs, 63 kDa, Sigma Chemicals, UK), pH adjusted with KOH). For PS, calcium chloride dihydrate / potassium dihydrogen orthophosphate were added to both the acid and gel, at 4.1 / 8.0 mmol/L respectively. US and PS were incubated for 14 and 18 d respectively.

Microradiography

A thin slice was cut from each tooth block polished on an etched glass plate using 9 μm silica (Logitech, Glasgow, Scotland), to a final thickness, measured accurately, of ca. 120 μm and mounted on a plastic template along with an aluminium step-wedge. Microradiographs of the templates were taken on Kodak Type 1A high-resolution plates (Kodak, Rochester, USA) exposed to a $\text{CuK}\alpha$ X-ray source operating at 10 mA and 20 kV. Exposure time was 35 min and the distance from source to template was 300 mm. Microradiographs were examined under an optical microscope (Leica, Wetzlar, Germany). An image of the central, homogeneous portion of each lesion, centred optically, was taken, typically capturing 300 μm of the lesion. The integrated mineral loss (ΔZ) was measured using a computerised image-analysis system (TMR2006, Inspektor Research Systems, Amsterdam, the Netherlands). ΔZ was the product of the lesion depth (LD) in μm , and the mean mineral loss, or R value, over that depth, relative to sound enamel, which was assumed to be 78% v/v mineral. Hence units were $\text{vol}\% \cdot \mu\text{m}$. LD and maximum mineral density (vol%) in the surface zone of each lesion (SZmax) were also calculated and at baseline, the R value (i.e. average mineral-loss, where $R = \Delta Z/\text{LD}$ [Arends et al., 1997]) was calculated to confirm that US and PS were different with respect to this parameter. R-values for high- and low-R lesions (standard deviations in brackets) were 29.0 vol% (4.07) and 22.7 vol% (3.17) respectively.

Remineralisation

Lesions were stratified and assigned to four treatment groups, in both the high- and low-R groups (i.e. a total of 8 groups, 9 lesions per group), so that average ΔZ values were not significantly different between groups. Lesions were re-mounted on individual glass microscope slides in dental wax (as above), one slide per treatment group and each slide placed into 150 mL of remineralising solution and incubated for 5 d at 37°C. The remineralising solution, described by Lynch et al. [2007], was intended to simulate plaque-fluid, based on the data reported by Gao et al. [2001] and Carey et al. [1986]. It comprised 1.0 mmol/L calcium chloride dihydrate, 12.7 mmol/L potassium dihydrogen orthophosphate, 20 mmol/L HEPES, 130 mmol/L potassium chloride, pH 6.58. For both the high- and low-R lesions there were four treatment solutions, created by the addition of zinc (as zinc acetate) and / or fluoride (as NaF) to the remineralising solutions. These were a non-fluoride/non-zinc control (non-F/non-Zn), 231 $\mu\text{mol/L}$ zinc (Zn), 10.5 $\mu\text{mol/L}$ fluoride (F) and 231 $\mu\text{mol/L}$ zinc/10.5 $\mu\text{mol/L}$ fluoride combined (Zn/F). The fluoride and zinc concentrations were

based on 1 h post-application values reported by Newby et al. [unpublished] and Saxton et al. [1986] respectively. To confirm that the chemicals used to prepare the remineralising solutions was not contaminated with either fluoride or zinc, non-F/non-Zn was analysed in triplicate for fluoride, using an ion-selective electrode (model 9609BN, Orion Instruments, Beverly, USA), and zinc, using an inductively-coupled plasma spectrometer (model 7300 Dual View ICP-OES, Perkin Elmer, USA). The concentration of zinc was below limits of detection and the fluoride activity was 461 (SD = 98.0) nmol/L.

Subsequent to remineralisation, a further section was taken from each lesion, adjacent to the slice taken at baseline, for microradiography, and $\Delta\Delta Z$, ΔLD and ΔSZ_{max} values calculated.

Calculation of saturation with respect to calcium phosphates

The remineralising solutions' respective degrees of saturation with respect to HA (DS_{HA}), octacalcium phosphate (DS_{OCP}), Brushite (DS_{BR}), fluorapatite (FAp) and β -tricalcium phosphate (TCP) were calculated using a computer program [Larsen, 2001] as follows. Respective solubility-product constants of $2.51 \times 10^{-59} \text{ mol}^9\text{L}^{-9}$, $1.58 \times 10^{-49} \text{ mol}^8\text{L}^{-8}$, $2.51 \times 10^{-7} \text{ mol}^2\text{L}^{-2}$, $7.94 \times 10^{-61} \text{ mol}^9\text{L}^{-9}$ and $3.16 \times 10^{-30} \text{ mol}^5\text{L}^{-5}$ were used and respective DS values calculated were 9.14, 2.18, 1.31, 32.5 (where F was added) and 2.54 respectively indicating super-saturation with respect to all of these calcium phosphates.

Electron-probe microanalysis

Quantitative chemical analysis of the lesions was performed by electron probe microanalysis (EPMA). Four lesions from each of the high-R treatment groups were analysed. A Cameca SX100 fitted with 5 wavelength dispersive x-ray spectrometers was used to analyse calcium, phosphorus, fluorine and zinc. Operating conditions were 20kV and 10nA. Tooth mineral instability under the electron beam, a result of the heating effect which can adversely affect the quantitative analysis, was mitigated against by defocusing the beam to a few microns diameter, running at a lower beam current and reducing count times to a level that still yielded acceptable statistical errors. Samples were embedded in vacuum-compatible epoxy resin in 6mm brass tubes, then polished to a 1 μm finish and carbon-coated to neutralise electron charge build-up on the sample. Discrete readings were taken at various LD values. All elements were analysed using their respective $K\alpha$ radiation. Peak count times were 10s for calcium, 20s for phosphorus, 240s for fluorine and zinc. A Durango apatite

primary calibration standard was used for calcium, phosphorus and fluorine and for zinc, zinc metal was used.

Statistical analysis

Data were analysed using SAS v8.2 data-analysis software (SAS Institute Inc., Cary, USA). Variables analysed:-

- Change from baseline in mineral loss ($\Delta\Delta Z$)
- Change from baseline in lesion depth (ΔLD)
- Change from baseline in peak density (ΔSZ_{max})

Comparisons between different treatment groups were compared using an analysis of covariance (ANCOVA), as both lesion type [Lynch et al., 2007] and mineral-loss at baseline [Lynch and ten Cate, 2006b] in pre-formed lesions can have a marked effect on subsequent remineralisation behaviour. Therefore the ANCOVA model included factors for treatment, lesion type (high and low R value) and baseline as covariates. An interaction term was also included for treatment*lesion type. This interaction gave rise to the comparisons between the treatments at each lesion type level (low and high R value). A check was also performed on the treatment*baseline interaction and in all cases, this term was not statistically significant ($p > 0.10$) and was excluded from the final model.

The main comparison was the zinc-fluoride combination (Zn/F) versus fluoride alone (F). However, all comparisons were investigated and a Tukey adjustment for multiple comparisons was used on the treatment comparisons.

Results

Table 1 gives the results of microradiography and the statistical analysis. Figures 1 and 2 show average mineral-density profiles before and after remineralisation. One lesion each from the low-R/Zn and low-R/F groups, were insufficiently robust for a post-treatment section to be taken and polished for microradiography. Statistically-significant remineralisation was observed for both lesion types and in all treatment groups. For F (high- and low-R), remineralisation occurred predominantly at the surface-zone and a small but statistically-significant amount of demineralisation

occurred in the deeper parts of the lesion body. For Zn/F (high- and low-R), while the exaggerated remineralisation of the surface-zone seen in F was absent, large amounts of mineral were deposited in the lesion body, giving rise to laminated lesions in all cases, although this was much less pronounced in low-R, where it was more a broadening of the surface-zone in two of the lesions. For non-F/non-Zn (high- and low-R), mineral was deposited throughout the lesion with no obvious preferential sites for deposition. For Zn (high- and low-R), remineralisation took place throughout lesions, but the deeper parts were remineralised preferentially as indicated by the significantly larger reduction in LD when compared with non-F/non-Zn. Some lamination was seen in low-R. For F (high- and low-R), remineralisation occurred predominantly at the surface-zone and a small but statistically-significant amount of demineralisation occurred in the deeper parts of the lesion body. All of these effects were more pronounced in the high-R lesions.

Figure 3 shows EPMA-generated elemental distribution profiles overlaid with net remineralisation profiles for high-R lesions. For Zn, % (w/w) zinc was highest at the lesion surface, diminishing as amount of remineralisation increased. For F, whilst fluoride was clearly concentrated in relatively large amounts at the surface, dropping rapidly with increasing lesion depth, in Zn/F it was present in similar amounts at the surface but even higher amounts deeper into the lesions, at a slightly lesser depth than that of maximum remineralisation. Ca:P ratios for the non-F/non-Zn, Zn, F and Zn/F treatment groups (SD in brackets) were 2.16 (0.04), 2.16 (0.03), 2.17 (0.03) and 2.14 (0.09) respectively, consistent with a theoretical value for HA of 2.15. When the Ca:Pi ratio was compared with $\Delta\Delta Z$ as a function of depth, there were no apparent trends for any of the treatment groups, as would be expected given the small standard deviations.

Discussion

The concept of crystal-growth inhibitors not necessarily being incompatible with, and potentially enhancing, remineralisation is not new. Featherstone et al. [1981] reported that zinc and strontium, in combination with fluoride, had a synergistic effect on enhancement of remineralisation. Subsequently, ten Cate et al. [1985] reported enhanced lesion-body remineralisation with dipping solutions containing zinc. However, the aims of these studies were not to study the effect of zinc alone and the presence strontium, capable of affecting enamel de- and remineralisation in its own

right [Featherstone et al., 1983], was a confounding factor, and so it cannot be said with any certainty precisely what role zinc played. More recently, Fujikawa et al. [2008] reported that salivary macromolecules associated with crystal-growth inhibition enhanced remineralisation in a similar fashion to that reported here. There is a striking similarity between the lesions which had been remineralised in the presence of salivary macromolecules and fluoride depicted by those authors and the high-R, Zn/F lesions from the present study.

In the present study, static remineralisation conditions with no acidic challenge were used in order to attribute any effects observed to the presence or absence of zinc; without an acidic challenge, capable of modifying surface-zone porosity, as a potentially confounding influence. While 5 days without an acidic challenge of sufficient severity to modify surface-zone porosity might be a relatively uncommon event in vivo, it serves to demonstrate the potential of crystal-growth inhibitors to modify remineralisation in a positive fashion. We used a zinc concentration representative of plaque-fluid, rather than whole plaque or saliva, because it is zinc in the aqueous phase of plaque, available to react with tooth mineral, which would influence remineralisation in vivo. Ideally, a range of zinc concentrations would be studied, as the effect of zinc on the various calcium phosphates implicated in remineralisation is concentration-dependent [LeGeros et al., 1999]. It is difficult to estimate immediate post-application zinc concentrations for plaque-fluid. Although pharmacokinetic data for zinc in saliva, following the topical application of zinc from toothpastes and mouthrinses, have been reported by several authors [e.g. Harrap et al., 1984; Saxton et al., 1986; Gilbert, 1987; Gilbert and Ingram, 1988; Günbay et al., 1992; Özdemir, 1996], with zinc concentrations reported at several post-application times, data for whole plaque tend to be reported only at one or two times [e.g. Afseth et al., 1983; Schäfer et al., 2007]. The concentration for plaque-fluid used in the present study was the only reported value of which the authors were aware. However, immediate post-application concentrations are likely to be much higher, as indicated by whole-plaque data [Gilbert and Ingram, 1988], and salivary pharmacokinetic data suggest that, many hours after application, the zinc concentration in plaque-fluid would fall below the 1 h value used here. A further consideration is that zinc is bound to plaque in a similar fashion to calcium [Rose, 1996] and will presumably be liberated in a similar way during acidogenesis. Further work is needed in vitro to determine the effects of zinc and fluoride over a range of concentrations and, ideally, also using intra-oral models to simulate more closely the clinical situation.

The anti-caries effectiveness of zinc-containing fluoride toothpastes has been confirmed during caries clinical trials, with both NaF and SMFP as fluoride salts [Stephen et al., 1988; Ripa et al., 1990]. At the mechanistic level it is unclear if the interaction between Zn, enamel and the two fluoride salts would differ. Here, we used NaF as a source of ionic fluoride, since it is currently considered to be the species responsible for the anti-caries efficacy of both NaF and SMFP, the latter after hydrolysis by oral phosphatases. Given that ionic fluoride concentrations in plaque-fluid tend to be higher following application of NaF mouthrinses and toothpastes when compared to SMFP- or mixed NaF/SMFP ones [Ekstrand, 1997; Vogel et al., 2000; Newby et al., unpublished], the effects seen in the present study may be more pronounced when NaF is the fluoride source, rather than SMFP.

Our findings demonstrate that zinc has the potential to enhance fluoride-induced remineralisation in early caries lesions. Clinically, this may be beneficial in the case where a lesion is arresting. In simple terms, a sub-surface lesion might be described as active, in which case it would be expected to progress towards cavitation, or arrested, in which case de- and remineralisation have effectively ceased. The rate at which a lesion tends towards either of these two extremes may be influenced by a number of factors, including fluoride [Featherstone, 2008]. It is accepted that exposure to fluoride may arrest lesions but that the sub-surface region will likely remain hypo-mineralised, as a highly mineralised surface-zone, a characteristic of arrested lesions, acts as a barrier to diffusion of ions into the lesion [Larsen and Fejerskov, 1989]. If fluoride-induced arrest were delayed by zinc, and surface-zone porosity maintained for longer, then this could allow more sub-surface remineralisation to take place than would have otherwise been the case, leading to a more complete consolidation of lesions. The use of crystal-growth inhibitors such as zinc in combination with fluoride may be useful in cases when fluoride is applied topically at relatively high concentrations, from toothpastes and gels, and where lesion arrest is likely or indeed the aim. Laminations, as seen in the lesions exposed to Zn/F, are a natural phenomenon, occurring in between 5 and 22% of ex vivo white-spot caries lesions, where values have been reported [Kostlan, 1962; Crabb, 1966; Silverstone, 1970; Palamara et al., 1986; Driessens et al., 1986; Theuns, 1987]. The lamination observed in our Zn/F lesions may be an exaggerated form of this naturally occurring structure. A further consideration is that the present study looked at only one aspect of the dynamic caries process, i.e. static remineralisation, whereas in reality both de- and remineralisation occur alternately. Arends and Christoffersen

[1986] concluded that fluoride stabilises the nascent surface-zone, by inhibiting demineralisation and also by promoting growth of enamel crystallites. As zinc can also reduce enamel demineralisation [Brudevold et al., 1963], it may enhance this stabilisation by increased inhibition of demineralisation during caries challenges.

Reduced or inhibited remineralisation through surface-zone blocking has been demonstrated during mechanistic studies in vitro [Silverstone et al., 1981; ten Cate and Duijsters, 1982]. Comparing F with Zn/F, the most likely explanation for the enhanced remineralisation in Zn/F is the smaller increase in SZ_{max} during remineralisation, with zinc retarding crystal growth at the surface of Zn/F lesions, facilitating ingress of mineral ions. The deeper penetration of fluoride into the Zn/F lesions is analogous to the findings of ten Cate and Duijsters [1982], who reported that a pH-cycling regime with a high cariogenic challenge effected fluoride deposition at a greater depth when compared with static remineralisation. In this case, the acidic challenge presumably maintained sufficient surface-zone porosity to allow continued ingress of fluoride ions. Similarly, Lynch et al. [2006] reported almost complete remineralisation at continuous low pH in the presence of much higher fluoride concentrations than those used by ten Cate and Duijsters [1982]. Once again, it seems likely that low pH facilitated remineralisation by maintaining surface-zone porosity. Silverstone et al. [1981] compared remineralising efficiency of solutions with different calcium concentrations and reported that when a more 'efficient' remineralising solution (i.e. high calcium) was used, remineralisation was limited to the surface-zone when compared with the low-calcium solution, which effected remineralisation throughout the artificial lesions used.

While it is likely that the presence of zinc affected surface-zone porosity, it is unclear why zinc failed to inhibit remineralisation in the lesion bodies of both Zn- and Zn/F-treated specimens. However, the preferential deposition of mineral in the deeper parts of the lesions suggests that Zn maintained porosity and hence mineral ingress. The composition of the PF solution and the temperature used were close to conditions which will favour deposition of dicalcium phosphate dihydrate (DCPD), an hydroxyapatite pre-cursor, and although the zinc concentration in the present study is roughly in the middle of the range of concentrations where inhibition of DCPD crystal growth has been demonstrated [LeGeros et al., 1999], remineralisation was still observed. Possible explanations have been reported by Ingram et al. [1992] and ten Cate [1993]. Both are very credible but neither fully explains our findings. Ingram et al. [1992] reported that concentrations of calcium similar to those in saliva displaced

adsorbed zinc from hydroxyapatite which had been pre-treated with zinc and went further to suggest that this might be how zinc reduces calculus formation without affecting fluoride-promoted remineralisation. Although the calcium concentration in the remineralising solution used in the present study was somewhat lower than that used by Ingram et al. [1992], it was applied at the same time as the zinc and at a much higher concentration (1 mmol/L vs. 231 $\mu\text{mol/L}$) so some competition for binding would most likely have occurred, mitigating against the potentially deleterious effect of zinc on remineralisation. Based on the earlier work of Margolis et al. [1982], ten Cate [1993] proposed that not all crystal-growth sites are affected by zinc and that in conditions of relatively high super-saturation, as in the present study, overgrowth of the inhibited sites can occur, presumably with zinc ultimately incorporated into the apatite lattice. If over-growth of inhibited sites had occurred then some zinc should have been detected in the remineralised lesion bodies of both Zn- and Zn/F-treated specimens, which it was to some extent, although not throughout the region of maximum remineralisation. So, while it is not possible to attribute the observed trends in remineralisation wholly to either of the mechanisms proposed above, it seems entirely plausible that both may have been partially implicated.

Previous studies have shown that the R-value of artificially-created lesions can have a marked effect on remineralisation, both in vitro [Lynch et al., 2007] and during intra-oral studies [Lippert et al., 2011]. Therefore, lesions with different R-values were used here. That the overall trends observed in both lesion types were broadly similar, but more pronounced in the high-R lesions, may be the result of the higher SZ_{max} value at baseline for the latter lesion type, resulting in retarded diffusion of ions into the low-R lesions [Silverstone et al., 1981; ten Cate and Duijsters., 1982]. In specimens exposed to Zn/F, the observation that lesion-body remineralisation was effected closer to the surface in low-R than in high-R ones may have been the result of more rapid depletion of mineral ions in solution in the pores of low-R lesions, with a higher specific area than the high-R lesions [Lynch et al., 2007]. Larsen and Fejerskov [1989] estimated that uptake of calcium and phosphate by enamel crystallites is so rapid that only marginal super-saturation may exist deeper in lesions, supporting this proposition. The same proposition may explain the deleterious effect of fluoride in high-R lesions, with a slower surface-blocking effect allowing remineralisation to continue for longer than in low-R ones. The small but significant amount of demineralisation observed in the lesion bodies of F, in both low- and high-R cases, may have been the result of so-called Ostwald-ripening of enamel in the surface-zone. Assuming that at some point remineralisation had slowed

considerably or ceased, and stagnation conditions prevailed within the lesion, the larger, more thermodynamically-stable crystallites in the surface-zone [Silverstone, 1983] may have gained mineral at the expense of crystallites in the lesion body. It has also been proposed that in the presence of fluoride, mineral is drawn away from the lesion pores during surface-zone remineralisation [ten Cate and Loveren, 1999]. A reduction in pH concomitant with surface-zone remineralisation may have occurred leading to demineralisation of the lesion body and, potentially, all of these mechanisms may have played a part.

The finding that in high-R lesions fluoride concentrations towards the surface of Zn/F-treated specimens were around double those seen at the surface of those exposed to F alone is intriguing. It may reflect relative rates of remineralisation in Zn/F- and F-treated lesions, with a reduced rate in the latter following surface-blocking and hence less time for deposition of F onto and into the lesion. A similar trend was seen in zinc concentrations, but here a more likely explanation is that substantially more remineralisation occurred on exposure to Zn/F, leading to greater Zn incorporation.

In conclusion, under conditions optimised for net remineralisation, zinc and fluoride combined, at concentrations based on those found in plaque-fluid 1 h after application, gave significantly greater remineralisation than did fluoride alone. The most likely explanation is that zinc maintained surface-zone porosity to ingress of mineral ions and thus enhanced lesion-body remineralisation.

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Legends

Table 1: Results of microradiographic analysis. Baseline data are denoted by suffix (base) in the first three columns and means-adjusted changes from baseline are given in the following columns. Means with the same letter were not significantly different (ANCOVA, $p < 0.05$). Asterisk denotes a significant change from baseline. Standard errors are given in brackets.

Fig. 1: Mean mineral density profiles for high-R lesions. Dashed line = baseline, solid line = post-treatment. For clarity, standard deviations are given at 10 μm intervals.

Fig. 2: Mean mineral density profiles for low-R lesions. Dashed line = baseline, solid line = post-treatment. For clarity, standard deviations are given at 10 μm intervals.

Fig. 3: elemental amounts (solid lines) vs remineralisation (dashed lines) for high-R lesions.

Lesion type	treatment	$\Delta Z(\text{base})$ /vol%. μm	SZmax(base) /vol%	LD(base) / μm	$\Delta\Delta Z$ /vol%	ΔSZmax /vol%	ΔLD / μm
High-R	Non-F/non-Zn	2280 (83.8)	84.2 (4.70)	39.4 (1.90)	729 (63.5) ^{b*}	12.6 (5.00) ^{a*}	10.9 (2.0) ^{b*}
	Zn	2330 (95.5)	78.8 (3.10)	35.3 (1.10)	790 (63.5) ^{b*}	28.8 (5.30) ^{a*}	-0.630 (2.10) ^a
	F	2420 (101)	86.6 (5.10)	35.8 (2.50)	380.5 (64.1) ^{a*}	17.8 (5.00) ^{a*}	17.9 (2.10) ^{b*}
	Zn/F	2472.5 (92.0)	81.6 (2.60)	32.9 (2.30)	1320 (64.7) ^{c*}	26.7 (5.10) ^{a*}	4.70 (2.20) ^{ab*}
Low-R	Non-F/non-Zn	2170 (159)	98.1 (4.00)	52.2 (1.90)	471 (64.2) ^{a*}	-3.60 (5.10) ^{ab}	16.7 (2.30) ^{a*}
	Zn	2020 (103)	94.5 (3.20)	53.2 (2.00)	711 (70.4) ^{ab*}	-1.0 (5.30) ^{ab}	13.0 (2.40) ^{a*}
	F	2310 (88.0)	94.3 (6.30)	42.5 (2.70)	410 (67.4) ^{a*}	-12.9 (5.30) ^{a*}	22.4 (2.00) ^{a*}
	Zn/F	2380 (145)	105 (4.00)	48.8 (2.00)	833 (63.8) ^{b*}	10.5 (5.50) ^b	16.9 (2.10) ^{a*}

Label: change non-F to non-F/non-Zn (2x)





