ORIGINAL ARTICLE

International Dental Journal 2011; 61 (Suppl. 3): 46–54

doi: 10.1111/j.1875-595X.2011.00049.x

Zinc in the mouth, its interactions with dental enamel and possible effects on caries; a review of the literature

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Zinc is an essential trace element. In the mouth, it is present naturally in plaque, saliva and enamel. Zinc is formulated into oral health products to control plaque, reduce malodour and inhibit calculus formation. It has good oral substantivity, and elevated concentrations can persist for many hours in plaque and saliva following delivery from mouthrinses and toothpastes. Although low concentrations of zinc can both reduce enamel demineralisation and modify remineralisation, during caries clinical trials, the addition of zinc to fluoride toothpastes has not affected their ability to reduce caries. Mechanistic studies may help explain this apparent contradiction. Zinc is readily desorbed from hydroxyapatite by calcium, which is plentiful in plaque and saliva. Where crystal-growth sites remain occupied by zinc despite this, they may simply be 'over-grown' by remineralisation initiated at unoccupied sites. Further, under certain conditions, low concentrations of zinc can enhance remineralisation of enamel lesions, by retarding lesion arrestment. Although this may help to explain the apparent lack of an overall zinc effect on caries, it seems unlikely that any negative effects would be countered exactly by positive effects. Further mechanistic studies, complementing well-designed *in vitro* and *in situ* caries studies, should lead to further understanding of the zinc-enamel interactions relevant to demineralisation and remineralisation.

Key words: *Oral health*, *zinc*, *enamel*, *hydroxyapatite*, *caries*

Zinc is an essential trace element and is found in tissues throughout the body, approaching iron in its relative abundance[1]. The human body contains about 2g of zinc, of which approximately 60% is found in muscle tissue, 30% in bone and 5% in skin[2]. It is essential for growth and development in humans[3] and has diverse roles, being a critical component of several hundred enzymes and proteins[4]. Uptake and release of zinc are mediated by the bone reservoir[5]. It is found in foods including meat, cereals, milk and milk products[6]. Zinc is implicated in biomineralisation, where it stimulates both bone growth and mineralisation[7,8] and influences osteoclast activity[9,10]. Zinc-doped hydroxyapatite (HA) may improve bone formation around implant materials[11–13]. Retardation of bone growth in animals is commonly associated with conditions linked to zinc deficiency[14–18] and reduced bone density has been linked to a zinc-deficient diet[19,20].

While the effects of zinc on calculus and plaque-growth have been reviewed extensively, its interaction with the dental hard-tissues and possible role in de- and remineralisation have received less attention. The aim of this review is to summarise data regarding the oral disposition of zinc, before and after the use of zinc-containing toothpastes and rinses, the interactions of zinc with enamel and its analogues, and hence discuss the possible effects of zinc on caries.

ZINC IN THE MOUTH

Zinc is ubiquitous in the body and it is naturally present in saliva[21–33] and the teeth[34–38]. *Table 1* summarises reported background zinc concentrations for saliva. (Although the SI units are moles/m^3, values for zinc and other species, for example fluoride, are often reported in parts *per* million (ppm); hence ppm is used to facilitate comparison.) Relatively large amounts of zinc are incorporated into enamel prior to eruption, but after eruption, zinc concentration at the surface of the teeth apparently increases further (*Figure 1*)[34], suggesting that some incorporation does occur during post-eruptive exposure to the oral fluids. It seems probable that this is facilitated by many sub-clinical caries events, in a similar way to the post-eruptive uptake of fluoride, where repeated de- and remineralising challenges remodel the outermost layer of enamel[39]. This extra zinc is apparently lost over the following two to three decades (*Figure 1*), again in a similar fashion to fluoride[40].

Zinc is also found naturally in dental plaque, but comparison between reported values is not

Table 1 Some reported values for background concentrations of zinc in saliva and plaque, with standard deviations in brackets (except * where ranges are quoted). Values for 'dry' plaque have been reduced sevenfold to facilitate comparison[41–43]

Authors	Salivary Zn conc/ppm	Authors	Plaque Zn conc/ppm
Kim *et al.*	0.0135 (0.0122)	Schafer *et al.*	15.2 (13.0)
Burguera-Pascu *et al.*	0.055 (0.017)	Hall *et al.*	31.6 (4.70)
Menegário *et al.*	0.127 (0.0744)	Günbay *et al.*	6.41 (0.687)
Watanabe *et al.*	0.080 (0.043)	Creeth *et al.*	19.5 (2.70)
Özdemir *et al.*	0.17 (0.176)	Dobl *et al.*	18.6 (11.2)
			17.9 (10.6)
Kuraner *et al.*	0.112 (0.048)	Gilbert and Ingram	< 40
Gilbert *et al.*, Gilbert and Ingram	< 0.200	Saxton *et al.*	15.9 (pooled sample)
Sighinolfi *et al.*	0.0465 (0.0152)	Harrap *et al.*	17.4 (5.86)
Tan-Walker and Gilbert	0.150 (0.06)	Afseth *et al.*	7.86 (2.29)
	0.190 (0.06)		
Harrap *et al.*	0.244 (0.0883)	Afseth J.	< 10.7
Klinger *et al.*	0.0654 (0.0418-0.0100)*	Schamschula *et al.*	17.1 (12.3)
	0.079 (0.0347-0.178)*		
Henkin *et al.*	0.051 (0.014)	Schamschula *et al*	13.4 (7.46) – 15.5 (6.71)

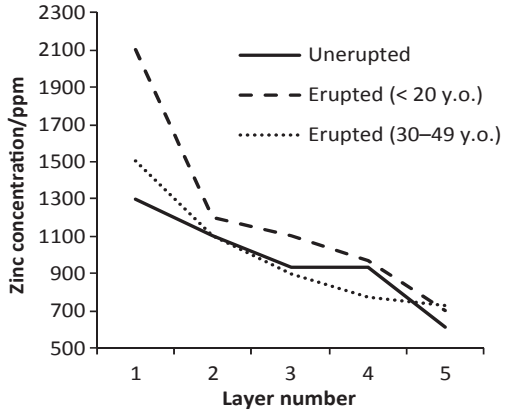

Figure 1. Zinc concentration in outer enamel. Layers relate to successive biopsies, each deeper than the previous[34]. The apparent increase in layer 1 relatively soon after eruption, in subjects less than 20 years old, was absent in subjects aged 30-49 years old.

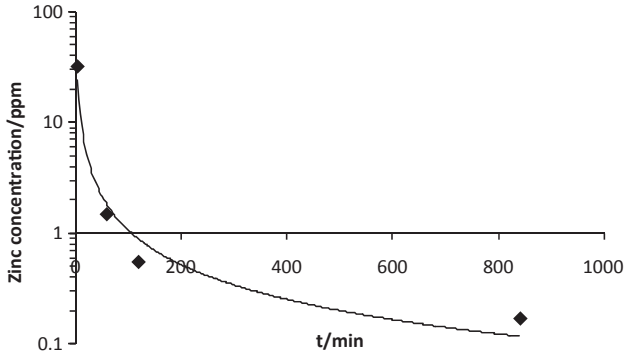

Figure 2. Clearance of zinc from saliva.

straightforward, as concentrations for 'wet' or 'dry' plaque are reported. However, assuming that drying increases the apparent concentration sevenfold[41–43], values are broadly similar[29,31,44–53] (*Table 1*). Zinc is taken up by the salivary pellicle[29,54] and it seems likely that the oral mucosa is the most important oral reservoir[46,55,56], though insufficient data exist to support this proposition conclusively.

ZINC APPLIED FROM TOOTHPASTES AND RINSES

Zinc is added to toothpastes and mouth rinses, as an anti-bacterial agent to help to control plaque, to reduce oral malodour and to reduce calculus formation through crystal-growth modification/inhibition (discussed below)[57–59]. To exert these effects, it must be present at the site of action at an effective concentration for sufficient time. Accordingly, zinc pharmacokinetic data for both plaque and saliva, following zinc delivery from these vehicles, have been well reported[22,25,28–31,44,45,50,56]. Despite differences in experimental protocols, for example different zinc salts, doses and rinsing regimes, all of which may affect zinc delivery and release, some general trends can be discerned.

Following application, relatively large amounts of the applied zinc dose are retained in the mouth, with reported values typically between about 15-40%[28–30,46,56]. Good oral substantivity was confirmed by Gilbert and Ingram[29], who reported that when zinc was applied from a toothpaste, of the remaining 30% retained in the mouth, only a further 5.7% was removed by rinsing three times.

Zinc is cleared from saliva bi-modally[57], with relatively high post-application concentrations falling rapidly over 30-60 min, after which low concentrations, significantly elevated when compared to baseline, may persist for many hours[e.g.28–31] (*Figure 2*). This trend reflects rapid clearance of loosely-bound zinc followed by slower clearance of more firmly-bound zinc[57], as is the case for fluoride. A similar trend is seen in plaque, but elevated concentrations can persist for at least 12 hours after application[44,45,50]. Repeated application of zinc has shown that a build-up effect occurs in plaque[45,50], as for fluoride[41].

Pharmacokinetic data relating to zinc concentrations in plaque-fluid following application are apparently absent from the dental literature. We were able to locate only a value measured 1 hour after application from a mouthwash[49]. It is reasonable to assume, however, that the clearance profile will be broadly similar to that seen in plaque and saliva. A statistically-significant correlation was reported between concentrations of another divalent metal cation, magnesium, in plaque-fluid and saliva, which lends some support to this assumption[60] although differences in dissociation constants[61] should also be considered. However, it has been reported that most of the zinc in plaque is associated with the particulate phase[49], with only 1% of the total amount present in the plaque-fluid, and the relative distribution would be very likely to change during a cariogenic challenge.

Zinc competes with calcium for bacterial binding-sites in model biofilms and it has been proposed that half of the bound zinc would be released under cariogenic conditions, through, for example, protonation of carboxylate and phosphate groups in bacterial lipoteichoic acid[61]. Circadian variations in concentration exist[62], further complicating the matter.

DIETARY ZINC, ENAMEL ZINC CONTENT AND CARIES

Increased caries incidence was reported when rats were fed a zinc-deficient diet[63,64], although in the latter study the co-administration of vitamin B_6 with the zinc makes estimation of any zinc effect difficult. However, while transient sub-clinical zinc deficiency is relatively common, prolonged chronic deficiency, as was the case in the rat studies, is rare in humans[65,66]. Further, caution should be exercised when extrapolating the findings from studies of caries in rodents, as salivary pH tends to be higher than in human saliva[67], concentrations of calcium and phosphate may differ, and differences have been noted in some other factors which can influence caries[68]. Nonetheless, zinc distribution in rat enamel does at least seem to be similar, with higher concentrations reported near the enamel surface[69]. Studies into the effect of zinc concentrations in human enamel and caries incidence have failed to show a consistent, significant correlation[70,71]. One factor which has been proposed as a confounding influence is that zinc is a common component of dental restorations, leading to contamination in subjects who are, or have been, by definition caries-prone[71].

EFFECTS OF ZINC ON FLUORIDE EFFICACY AND EFFECTIVENESS IN USE

Zinc, along with other metal cations, has long been associated with reductions in enamel solubility[34,72,73] and can also modify crystal-growth of the calcium phosphates implicated in remineralisation (discussed below). Therefore, it has the potential to influence the dynamic de/remineralisation balance in the mouth.

However, data from pH-cycling studies where zinc and fluoride were delivered from a toothpaste and both de- and remineralisation were alternated do not suggest an overall zinc effect[74–76]. Increased remineralisation after application of dipping solutions containing zinc has been reported[77,78] but the presence of strontium, also capable of affecting enamel de- and remineralisation[79] was a confounding factor and the role of zinc cannot easily be deduced. A reduction in enamel demineralisation with the use of a zinc-containing fluoride toothpaste was reported during an *in situ* study. Lesions in the zinc/fluoride group did not demineralise significantly when compared to baseline, whereas those in the fluoride control group did[74]. However, it was concluded that this could not be attributed wholly to direct interaction with the enamel substrate, and may have been the result of anti-bacterial effects to some extent. Churchley *et al.* report a similar anti-demineralisation effect in this issue[80].

However, zinc had no effect on the anti-caries effect of fluoride during a rat-caries study[81] and in a three-year caries clinical trial (CCT), the addition of zinc to fluoride toothpastes, containing 1,000, 1,500 and 2,500 ppm fluoride (as sodium monofluorophosphate (SMFP)), had no effect on caries, either positive or detrimental, at any of the fluoride concentrations[82] even though use of the zinc-containing toothpastes reduced calculus during the same trial[83]. During a subsequent CCT, no significant difference in anti-caries effectiveness was observed' between two zinc-containing fluoride toothpastes and a non-zinc fluoride control formulation[84].

While zinc has anti-microbial properties, no consistent link has yet been established between anti-microbial efficacy and caries reductions[85], except in the rather extreme case of, for example, use of chlorhexidine[86], or in the complete absence of the microflora in gnotobiotic rats[87].

Given zinc's potential to affect both de- and remineralisation, this apparent lack of a consistent effect of zinc on caries would seem to be contradictory.

ZINC, FLUORIDE DELIVERY AND DE/REMINERALISATION; MECHANISTIC STUDIES

CCTs are considered to be the ultimate predictor of anti-caries effectiveness, and *in vitro* and *in situ* studies can be useful pre-clinical screening tools. In most cases, however, net changes are only quantified after many applications of active agents and many de- and remineralisation events[88]. Thus is it difficult to assess the effect of an agent, in this case zinc, on fluoride delivery or efficacy, and here, mechanistic studies can be useful.

Fluoride-uptake (from SMFP) to enamel lesions was reduced by 60% in the presence of zinc citrate[89]. It was suggested that zinc may have reacted with phosphates in the enamel lesions and that subsequent precipitation blocked pores at the surface of the lesion, or that zinc-MFP complexes may have inhibited uptake. Subsequently, however, fluoride-uptake to enamel lesions from a zinc/SMFP toothpaste was shown to be substantially greater than from its control, although no difference was seen when a sound enamel substrate was used[74], and zinc citrate in a sodium fluoride toothpaste had no effect on fluoride-uptake to lesions[75]. Precipitation seems an unlikely explanation *prima facie*, as the experimental solutions were, in both cases, highly under-saturated with respect to both HA and zinc phosphate, based on respective –log10(solubility-product constants) of 58.4[90] and 35.2[91], but it is possible that localised supersaturation, and therefore thermodynamically favourable conditions for precipitation, may have existed in the unstirred solutions. In contrast, in solutions supersaturated with respect to HA, a combination of zinc and fluoride enhanced remineralisation of enamel lesions[92] when compared to fluoride alone, probably by retarding lesion arrestment, i.e. the opposite of the surface-blocking effect mentioned above. A very similar effect has been reported for salivary macromolecules[93].

Data from a pilot study into the effect of physiologically-relevant zinc concentrations[30], added to acid-gel demineralising systems, showed that zinc reduced demineralisation markedly (*Figure 3*). Significant reductions in both integrated mineral-loss and R-values[94] were seen in all groups where zinc was added, and in both cases, 25ppm Zn = 15ppm Zn = 5ppm Zn < 1 ppm Zn < 0ppm Zn ($p < 0.05$, Fisher's LSD multiple range test). Enamel is not stoichiometric HA but contains impurities associated with its solubility and which are lost during early demineralisation[95–97]. One possibility is that zinc retarded the dissolution of the more soluble mineral phases, leading to a reduction in R-value. Related to this

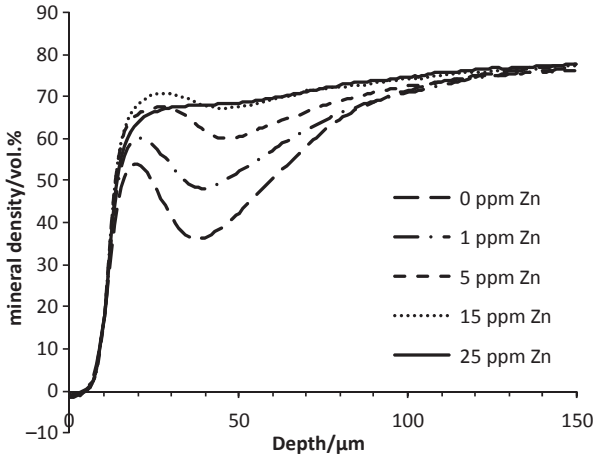

Figure 3. Mean mineral-density profiles for lesions formed in the presence and absence of zinc *in vitro*.

was the suggestion that an increase in R-value with increasing time in demineralising systems was caused by preferential dissolution of more soluble mineral[88]. Further, during a recent intra-oral de/remineralisation study, lesions with low-R values underwent net demineralisation, whereas high-R lesions with a similar integrated mineral-loss values underwent net remineralisation, and a similar preferential dissolution effect was proposed[98]. The observation that the acidified gels were supersaturated with respect to Hopeite is worthy of note (see 'interactions with HA' below).

REDUCTION OF HA SOLUBILITY

Enamel is a defective form of HA, and HA has often been used as an enamel analogue during mechanistic de- and remineralisation studies[99,100]. In simple terms, zinc can interact with HA by adsorption onto crystal surfaces[100–102] and/or incorporation into the crystal lattice[103,104].

Reductions in the solubility of apatites effected by metal ions are widely documented beyond the dental literature[105–107], but the mechanism is still unclear. Adsorption at high-energy 'kink' sites is one possibility[108]. Alternatively, the precipitation of Hopeite, observed during zinc-adsorption studies[109], may be implicated, and data from some studies suggest that above 1ppm, Hopeite dominates the surface-properties of HA under certain conditions[110]. Zinc uptake was reported to have continued beyond the point of complete monolayer coverage with increasing solution concentration, and the proposed explanation was the precipitation of Hopeite once surface adsorption was complete, again a concentration-dependent effect. Other reports tend to support an adsorption mechanism. Zinc was readily displaced from HA by calcium in an equimolar fashion[100]. More recently[111], HA specimens were exposed to a demineralising solution, whose zinc concentration was increased and then decreased. As zinc concentration increased, dissolution decreased but when the zinc concentrations was subsequently reduced, dissolution rates quickly increased again. This lack of a zinc 'memory' in the HA specimens used also suggests an adsorption mechanism. However, other factors[101], such as changes in pH during reactions[112], and use or otherwise of pre-equilibrated[113] systems, can modify zinc/HA interactions. The two mechanisms are apparently not mutually exclusive and it is likely that both are implicated in reduced solubility of HA and apatites, in the presence of zinc, to a greater or lesser extent.

MODIFICATION OF CALCIUM PHOSPHATE CRYSTAL-GROWTH

The ability of zinc to modify the crystal-growth of orally-relevant calcium phosphates has been exploited

to control dental calculus, and is reviewed extensively elsewhere[114]. Briefly, zinc can influence precipitation of the calcium phosphates relevant to remineralisation. It can affect the crystallinity of apatite precipitated *de novo* and can inhibit the crystal-growth not only of HA but also of two HA precursors, di-calcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP)[115–117]. Some effects are concentration-dependent. Inhibition of crystal-growth in HA, OCP and DCPD was reported below a pH-threshold, above which ACP or Zn-substituted TCP were formed[114]. In general, it has been concluded that zinc promotes the deposition of the more soluble calcium phosphate phase[114]. However, it has been shown that while PRP-3, a proline-rich phosphoprotein found in saliva, reduced HA crystal-growth to undetectable amounts in the absence of fluoride, with the addition of 1ppm fluoride, crystal-growth could be measured readily. It was proposed that even at maximum PRP-3 coverage, some crystal-growth sites remained uncovered[118] and that this would ultimately allow 'overgrowth' of occupied growth sites. A similar mechanism was proposed for zinc[74]. Calcium readily displaces zinc from HA[100] and reduces adsorption of zinc by some HA precursors[119], potentially enhancing this effect by increasing the number of growth-sites unoccupied by zinc.

INCORPORATION INTO HA

It has been predicted that zinc replaces calcium in the HA lattice[120], preferentially in the Ca(II) position[121–124], and that for fluorapatite, the 'columnar' Ca (I) position would be preferred[121], although recently it has been suggested that simple ionic exchange mechanisms might be too simplistic[125]. It adopts tetrahedral-coordination as might be expected[122,126]. Some 'relaxation' of the surrounding oxygens is reported, made possible by the smaller ionic radius of zinc, when compared to calcium[127,128]. Covalent zinc-oxygen bonds are predicted[124], whereas bonds in HA are ionic in nature[107]. When zinc is incorporated into both carbonated and non-carbonated HA then its solubility is generally reduced[106,129].

Reduced acid reactivity in zinc-substituted carbonated apatites[129], a closer analogue to human enamel than non-carbonated HA[130], may be linked to the reduction of paracrystalline disorder[131], the production of larger crystals during precipitation[132] and the formation of crystals with fewer structural defects[129] than in non zinc-substituted carbonated apatites.

An inverse relationship between enamel fluoride and enamel carbonate contents has long been suggested[133]. However, a combination of zinc and fluoride was even more effective at reducing carbonate-induced crystal-structural disorder in the HA lattice, when compared to fluoride alone, or to other combinations of fluoride and metal ions[132].

However, given concentrations found in the mouth, it is not clear if sufficient zinc would be incorporated to affect enamel solubility markedly. Carious enamel lesions remineralised under simulated plaque-fluid conditions, in the presence of zinc and fluoride, contained relatively little zinc[92], and in the region of maximum remineralisation, only background concentrations, suggesting displacement of zinc by calcium. Other authors have suggested that zinc was incorporated into enamel during remineralisation *in situ*, but again, the increase in zinc concentration was modest[134].

SUMMARY AND CONCLUSIONS

Zinc is ubiquitous in the body and is found naturally in plaque, saliva and the teeth. It has been suggested that the acquisition of zinc by enamel, following the eruption of the teeth, is a part of post-eruptive maturation[135]. Zinc has been formulated into oral-health products to give a range of benefits. It can reduce dental calculus formation, control plaque and reduce oral malodour. It has good oral substantivity and is retained in plaque and saliva for many hours after application, and a build-up effect occurs in plaque with repeated application.

While it can reduce the solubility of both enamel and HA, and retard/inhibit remineralisation, no net effect has been reported for caries during *in vitro* and *in situ* caries studies, or during CCTs. Mechanistic studies are useful where fundamental understanding is the aim. The findings from these studies, where zinc was of interest, suggest that while it can affect remineralisation, both displacement of adsorbed zinc by calcium, and over-growth of newly-deposited mineral from sites unoccupied by zinc, may counter potential negative effects. Further, in lesions, it may retard fluoride-induced lesion-arrest, allowing more complete lesion consolidation via remineralisation at greater depth.

During mechanistic studies, both chemical and physical physiological parameters, for example zinc, fluoride, calcium and phosphate concentrations, possible inhibition of diffusion by plaque and diffusion into, out of and within lesions should be considered before attempting to extrapolate findings to the clinical setting. Thus, a degree of caution should be exercised and these studies should complement, rather than be substituted for, properly-designed designed *in vitro* and *in situ* studies.

Regarding the clinical effects of zinc on de- and remineralisation, it seems unlikely that potentially beneficial effects, such as reductions in solubility and enhanced/prolonged lesion porosity to mineral ingress, will counter exactly any possible negative effects. Further work, with mechanistic studies complementing well-designed *in vitro* and *in situ* caries studies, should lead to

further understanding of the zinc-enamel interactions relevant to demineralisation and remineralisation.

CONFLICT OF INTEREST AND SOURCE OF FUNDING

The work described in this manuscript was funded by GlaxoSmithKline Consumer Healthcare. The author is employed by GSK but confirms no potential conflicts of interest.

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