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Effects of fluoride concentration on enamel demineralization kinetics *in vitro*

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ABSTRACT

Objectives: The aim of the present study was to measure the effects of fluoride concentration on the real-time *in vitro* demineralization of enamel during exposure to caries-simulating conditions using Scanning Microradiography (SMR).

Methods: Enamel blocks obtained from non-carious human molars were fixed in SMR environmental cells, through which acidic solutions (0.1 M acetic acid, pH 4.0) were circulated for periods of 48 h. SMR was used to quantitatively measure continuous mineral mass loss. Subsequently, the effects of sequentially increasing fluoride concentration (0.1–4500 mg/L [F^-]) in the acidic solutions were measured on the rate of enamel demineralization.

Results: The data shows a log-linear relationship between [F^-] and reduction in demineralization up to 135 mg/L [F^-]. Above 135 mg/L, no further significant decrease in demineralization occurred.

Conclusion: The optimum range of local fluoride concentration for reducing enamel demineralization was in the range 0.1–135 mg/L [F^-] under the conditions studied.

Clinical significance: Relatively low [F^-] can exhibit near-optimum protection. Increasing the fluoride concentrations above 135 mg/L may not necessarily give an increased cariostatic benefit. Improving the means of delivery of relatively low fluoride concentrations to the oral fluids through slow releasing mechanisms, such as the oral fluoride reservoirs, is the more appropriate way forward for sustaining long-term clinical efficacy.

1. Introduction

Although the incidence of dental caries has declined in the past forty years, it remains the most common global disease in both adults and children.[1] The increasing preference for more 'western' diets high in carbohydrates and refined sugars is a leading cause for this. Fluoride (F^-), the most effective preventive measure, can be found in various forms and concentrations ranging from ~0.5 to 1 mg/L in drinking water, ~1000–1500 mg/L in dentifrices, 250–500 mg/L in mouthwashes, and >5000 mg/L in gels and varnishes.[2,3] There is continued interest in fluoride's therapeutic potential particularly its optimum concentration for anti-caries efficacy.

The predominant caries-preventive mechanisms of action of fluoride are post-eruptive through 'topical' effects, including (1) inhibition of demineralization and (2) enhancement of remineralization at the crystal surfaces. Fluoride present in

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solution at sub-ppm concentrations surrounding the enamel crystals can markedly protect dissolution of tooth mineral by acid.[4] Furthermore, the incorporation of fluoride into the apatite crystal surface during remineralization can enhance the resistance of the tooth to acidic challenges.[5,6]

In hydroxyapatite ($Ca_{10}(PO_4)_6OH$, HAp), the complete substitution of F^- for OH^- on the *c*-axis forms fluorapatite ($Ca_{10}(PO_4)_6F_2$, FAp). The F–OH exchange at the apatite–solution interface is thermodynamically favourable at the apatite surface and occurs readily even at low fluoride concentrations.[7] This interaction of fluoride within the apatite lattice structure increases crystal stability and results in a decrease in its solubility.[8] Fluoride-substituted apatite ($Ca_{10}(PO_4)_6(OH)_{2-x}F_x$) phases form when the degree of fluoride substitution in the apatitic mineral is less than that of FAp. It was reported that the solubility behaviour of HAp with various levels of fluoride substitution showed maximum stability at 50% substitution of OH^- groups with F^-, this apatite phase being less soluble than pure FAp.[8] At around 50% substitution of fluoride in the hydroxyl column, hydrogen bonding between neighbouring F^- and OH^- ions are maximum, thus stabilizing the lattice structure.[9]

The anti-caries effect of topically applied fluoride has also been attributed to calcium-fluoride (CaF_2)-like deposits formed on enamel, which are thought to act as a protective barrier on the surface, as well as serving a fluoride reservoir.[10,11]

Whilst most studies have focused on the more general cariostatic action of fluoride, there are very few which measure the direct physical effects of fluoride on real-time enamel demineralization. Most *in vitro* systems involve indirect discontinuous analysis methods, *e.g.* solution Ca^{2+} are quantified.[12] Additionally, it is difficult to fully replicate the complex nature of the oral environment, or allow for the strict chemical control of individual variables of a multi-factorial disease, this includes the salivary pellicle. Furthermore, significant variations exist naturally in human enamel tissue composition, and therefore demineralization rates can vary significantly between different teeth specimens, and, at different locations in the same tooth specimen.

Scanning Microradiography (SMR) is a technique which uses X-ray absorption to continuously measure mineral mass loss at fixed local positions on an enamel sample.[13–17] SMR can be used to measure the demineralization rates of an enamel block during the course of acid reaction, always at the same location, thus eliminating the need for separate controls. The advantages of SMR include; (1) it allows continuous mineral mass measurements at fixed local positions on the same specimen over periods of several days[18,19]; (2) separate controls are not required as each position measured acts as its own control; (3) it is highly sensitive to small changes in mineral mass of the order of 0.1%, with statistical accuracy determined for X-ray photon counting, therefore accurate demineralization kinetics can be measured over periods of $\leq$48 h[17,20]; (4) considerably thicker samples can be used which provides a better representation of enamel tissue *i.e.* blocks of enamel (>3 mm) rather than thin sections ($\leq$350 μm) that is required for conventional microradiography.[21]

The aim of this *in vitro* study was to use SMR to measure mineral mass loss in enamel during exposure to acidic solutions simulating caries (pH 4.0) in real-time. Subsequently, the fluoride concentration [F^-] of the acidic challenge was successively increased and SMR measurements continued for periods of 40 h. Thus, real-time measurements of the effects of [F^-] (in the range 0.1–4500 mg/L) on the rate of enamel demineralization were obtained, on the same sample and at the same location.

2. Methods

2.1. Preparation of human enamel blocks

Anonymized caries-free permanent molars (tooth A, B and C) extracted for orthodontic purposes were randomly selected. Ethical approval was obtained from Queen Mary Research Ethics Committee (QMREC 2011/99). Enamel blocks (~5 mm × 5 mm) with a thickness of ~2 mm were cut from each tooth using an annular diamond blade (Microslice 2, Malvern Instruments, UK) and dentine was polished off using a P600 grit silicon carbide paper. One enamel block was obtained from tooth A (sample A), one from tooth B (sample B), and two enamel blocks were cut from a third tooth specimen C (sample C1 and sample C2) at different sites on the same molar. Each sample was located in a separate SMR environmental cell. The cut internal surfaces of the enamel blocks were coated with acid-resistant varnish, leaving only the natural surface perpendicular to the X-ray beam. Fig. 1 shows an enamel block enclosed in an SMR cell (volume ~2 cm^3) through which acidic solutions were circulated at 0.788 mL min^{-1}, whilst the narrow X-ray beam is targeted at a fixed position on the sample.

2.2. Preparation of [F^-]-containing acidic solutions

A series of acidic solutions were made from analytical grade reagents. A 10.0 L stock of 0.1 mol/L acetic acid (AnalaR

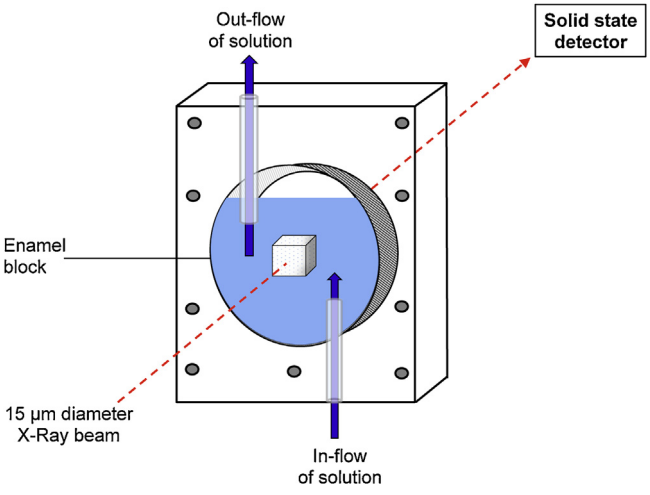

Fig. 1 – Schematic diagram of polymethyl methacrylate SMR environmental cell containing an enamel sample. The narrow X-ray beam repeatedly measures 3 fixed scan positions on a sample during the experiment, and the attenuated beam is detected by a solid-state detector.