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Effect of Fluoride on Artificial Caries Lesion Progression and Repair in Human Enamel: Regulation of Mineral Deposition and Dissolution under *In Vivo*-Like Conditions

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Abstract

Objective—This study was carried out to determine *in vitro* the effect of fluoride on 1) the demineralization of sound human enamel and 2) the progression of artificial caries-like lesions, under relevant oral conditions.

Methods—Thin sections of sound human enamel were exposed to solutions undersaturated with respect to tooth enamel to a degree similar to that found in dental plaque fluid following sucrose exposure *in vivo*, containing fluoride concentrations (0 – 0.38 ppm) found in plaque fluid. Mineral changes were monitored for 98 days, using quantitative microradiography. The effect of fluoride (1.0 – 25.0 ppm) on the progression of artificial caries-like lesions was similarly studied.

Results—Fluoride concentrations of 0.19 ppm and greater were found to prevent the demineralization of sound enamel *in vitro*. However, significantly higher concentrations of fluoride (25.0 ppm) were required to prevent further demineralization of artificial caries-like lesions. Demineralizing solutions with intermediate fluoride concentrations (2.1 – 10.1 ppm) induced simultaneously remineralization in the outer portion of the lesion and demineralization in the inner portion. Simultaneous remineralization and demineralization were also observed in hydroxyapatite pellets.

Conclusions—Our results show that the observed effect of fluoride on enamel demineralization is not solely a function of bulk solution properties, but also depends on the caries-status of the enamel surface. A mechanistic model presented indicates that, in comparison to sound enamel surfaces, higher concentrations of fluoride are required to prevent the progression of artificial caries-like lesions under *in vivo*-like conditions since the diffusion of mineral ions that promote remineralization is rate-limiting.

Keywords

dental enamel; remineralization; demineralization; mineral distribution; fluoride

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Introduction

Previous studies have shown^{1–3} that low levels of fluoride in solution can prevent enamel demineralization *in vitro*, when an appropriate concentration is added to an undersaturated organic acid solution that, in the absence of fluoride, induces rapid and significant demineralization. For example, in a study carried out in our laboratory some years ago¹, demineralization of sound enamel was completely inhibited by the addition of 1.0 ppm of fluoride to a lactic acid solution that was undersaturated (*i.e.*, $DS < 1$) with respect to tooth enamel with a degree of saturation with respect to tooth enamel (DS_{En}) of 0.12. Here, DS_{En} is defined as the ratio of the mean ionic activity product for enamel mineral (assuming enamel to have the stoichiometry of hydroxyapatite) to an estimate of its thermodynamic solubility product constant, expressed in the same fashion. In the presence of 1 ppm fluoride, no mineral loss was observed for up to 10 days exposure to the demineralizing solution. In addition, it was found that the fluoride content of the enamel surface increased significantly, relative to controls. Since the solution was also supersaturated (*i.e.*, $DS > 1$) with respect to fluoroapatite ($DS_{FA} = 1.8$), a working hypothesis was developed that states that the net rate of enamel demineralization would be reduced in a demineralizing medium that is also supersaturated with respect to less soluble fluoridated phases (*e.g.*, $DS_{FA} > 1$), due to the enhancement of the rate of precipitation of fluoridated apatitic phases relative to the rate of dissolution of the original enamel surface. A model describing this mechanism is illustrated in Fig. 1. An important corollary to this hypothesis is that these two processes (*i.e.*, dissolution and reprecipitation) occur simultaneously and are in dynamic competition. Based on this model and results from other published studies on the effect of fluoride on enamel demineralization *in vitro*, we proposed that the amount of fluoride required to prevent enamel demineralization is inversely related to the relative driving force for enamel dissolution *in vitro*⁴.

Since the driving force for enamel demineralization *in vivo*, *i.e.* in dental plaque fluid, has been found to be significantly lower than those used in the noted laboratory studies⁵, with the lowest DS_{En} values obtained after sucrose exposure ranging from 0.2 to 0.45⁶, one would predict that even lower levels of fluoride would be sufficient to inhibit enamel demineralization under *in vivo*-like conditions. Thus, the present study was carried out to test this hypothesis *in vitro*, by determining the effect of fluoride on the demineralization of sound human enamel under relevant oral conditions. In addition, to further explore the effectiveness of fluoride in caries prevention and repair, the influence of fluoride on the progression of pre-formed artificial incipient caries lesions in human enamel was explored under similar *in vivo*-like conditions.

Material and methods

The overall experimental design is outlined in Fig. 2. Details of the methods and materials used are described in the following sections.

Thin Section Preparation

Natural surfaces of non-carious human molars and premolars were used in this study to test our hypothesis. Natural surfaces were used to better reflect the oral condition. Prior to thin section preparation, all surfaces of the teeth were cleaned with a rubber cup and alumina micro polisher (Alpha Micro Polish Alumina 1, Buehler, Lake Bluff, IL, USA). Thin longitudinal sections (130 to 160 μm) of the teeth cut perpendicular to the lingual or buccal surface were prepared as previously described.^{5, 7} All surfaces of each enamel section, except the natural surface edge (lingual or buccal surface), were then coated with UV light-curing resin (Scotchbond, 3M Dental Products, St. Paul, MN, USA) using a small brush. A gold finder grid for electron microscopy (200FI, Polysciences Inc., Warrington, PA, USA) was buried in the resin coating near the uncoated window described below.⁸ The grid was used to help align the microradiographic images on the computer before and after the specific treatments. Nail

varnish was finally applied over the entire surface of the section except for a window on the natural surface edge of approximately 1 mm in length. The sections were then exposed to experimental solutions that were both undersaturated with respect to tooth enamel and supersaturated with respect to fluoridated phases (Table 1).

Preparation of Compressed Hydroxyapatite Pellets

To further explore mechanistic aspects of the effects of fluoride on the demineralization of human enamel lesions, compressed pellets of hydroxyapatite were prepared for experimentation. Commercially available standard reference material hydroxyapatite powder (NIST, Gaithersburg, MD) was ground to a fine powder in a mortar by a pestle for 5 minutes. The hydroxyapatite powder (100 to 150 mg) was placed in an infrared spectroscopy pellet maker, and compressed (19,000 lb) in a hydraulic press for 10 minutes under vacuum. Compressed pellets were then sintered at 900°C for 5 h.⁹ Finally, the pellets were ground to a thickness of 130 to 160 µm and coated with resin using the same method described for enamel thin section preparation.

Artificial Lesion Preparation in Teeth and Hydroxyapatite Pellets

Initial lesions were prepared in thin sections of enamel and in the hydroxyapatite pellets in the following fashion. Individual coated sections were mounted on the end of a plastic rod and immersed into 20 mL of demineralizing solution, contained in a small plastic bottle. The bottles were maintained at 25°C in a constant-temperature incubator, without agitation. The demineralizing solution was prepared from reagent grade chemicals and contained nominally 100 mmol/L lactic acid, 18.0 mmol/L calcium chloride, 7.8 mmol/L monobasic potassium phosphate, and 3 mmol/L sodium azide as a bacteriostat. The pH was adjusted to pH 4.3 with potassium hydroxide. Similar solutions were shown previously to produce subsurface lesions in human enamel.⁷ Lesion progress was monitored using contact microradiography. In this fashion, subsurface enamel lesions were produced over a 1 to 2 week period.

Preparation of Experimental Demineralizing Solutions

Experimental demineralizing solutions were prepared in the same manner as the demineralizing solutions used to produce the artificial lesions. Solution pH was adjusted to the desired value using potassium hydroxide and all solutions contained 3 mmol/L sodium azide as a bacteriostat. Prior to use, solutions were analyzed for calcium and phosphate, as described below. Degrees of saturation, DS, of these solutions with respect to enamel, fluoroapatite, and calcium fluoride were calculated, as defined below. These solutions were stable and showed no evidence of precipitation prior to use. The compositions of the solutions used in this study are listed in Table 1, along with corresponding DS values. Solutions were specifically designed to have DS_{En} values in the range of those found in dental plaque fluid following sucrose exposure⁶. The experimental solutions, however, differ from plaque fluid in a number of respects. For example, plaque fluids⁶ contain other inorganic ions and organic acids, and generally have a higher ionic strength. However, the experimental conditions used in this study were chosen to primarily reflect *in vivo*-like DS_{En} values and fluoride concentrations (see below), since these factors have been shown to significantly influence the rate of enamel demineralization *in vitro*^{e.g., 1, 5}.

Demineralizing solutions S1-S4 were used to determine the effect of fluoride on the demineralization of sound (S) enamel surfaces. These solutions had very similar chemical compositions, but differed with respect to their fluoride concentration (0 – 0.38 ppm). Fluoride concentrations were selected to reflect levels found in dental plaque fluid before^{10–14} and after¹⁵ sucrose exposure. Prior to the preparation of these solutions, trace amounts of fluoride were removed from the calcium and phosphate stock solutions by equilibrating the solutions with hydroxyapatite powder for 48 hours⁸. Previously, we found this procedure to be quite

efficient and estimate that the final concentrations of fluoride in the control solution to be less than 0.002 ppm⁵. These solutions were undersaturated with respect to enamel ($DS_{En} \sim 0.24$) and increasingly supersaturated with respect to fluoroapatite ($DS_{FA} > 1$). These solutions were also undersaturated with respect to other calcium phosphate phases and calcium fluoride (Table 1).

Demineralizing solutions L1–L5 were used to determine the effect of fluoride (1.0 – 25.0 ppm) on the progression of artificial incipient lesions (L). In comparison to solutions S1–S4, these solutions had slightly lower DS_{En} values ($DS_{En} \sim 0.22$) and were increasingly more highly supersaturated with respect to fluoroapatite ($DS_{FA} > 1$) and calcium fluoride.

Studies on the Effect of Fluoride on the Demineralization of Sound Human Enamel

Thin sections of enamel were placed in individual bottles containing 15 ml of the demineralizing solution and maintained at 25°C in a constant-temperature incubator. Eight (8) thin sections were exposed to each of four different demineralizing solutions (S1–S4) described in Table 1. The experimental solutions were changed weekly at which time microradiographs were taken. Microradiographs were taken weekly over a 98-day period. Lesion development was assessed using quantitative microradiography, as described below.

Studies on the Effect of Fluoride on the Demineralization of Artificial Carious Lesions in Human Enamel and Hydroxyapatite Pellets

Each thin section containing a subsurface lesion was mounted at the end of a plastic rod and immersed into 20 mL of a demineralizing solution (L1–L5), contained in a small plastic bottle. The bottles were maintained at 25°C in a constant-temperature incubator. Three (3) sections were used for each of the five experimental solutions. At selected times, the sections were removed from the demineralizing solution, rinsed with distilled deionized water, and patted dry. Microradiographs of each section were then taken, using the procedure described in the next section. The sections were then returned to the demineralizing solution. All solutions were changed weekly and experiments were allowed to continue until further changes in mineral content were not apparent (up to 760 h). To confirm findings observed in human enamel, two compressed pellets of hydroxyapatite containing artificial lesions were similarly exposed to solution L2 for up to 374 h.

Quantitative Assessment of Changes in Lesion Mineral Content

Changes in lesion mineral content were assessed as a function of time using quantitative microradiography, as we have previously described.^{7, 8} Microradiographs of the thin enamel sections, obtained using a Faxitron X-ray system (Hewlett Packard, McMinnville, OR, USA) and a high-speed holographic film (SO-253, Eastman Kodak, Rochester, NY, USA), were analyzed using a video image analysis system and software developed by Ed Marcus Laboratories (Newton, MA, USA) in our laboratory. The resolution of the system is limited by the magnification of the microscope and the dimension of a pixel, or less than 3 μm^2 . The gold grid attached to the cut surface of each section and the outer enamel edge were used to align the microradiographs obtained for each section prior to and at various times following exposure to experimental solutions. The inclusion of a calibrating aluminum step-wedge in the microradiograph facilitates the evaluation of the relative mineral contents in the enamel thin sections. The relationship between video brightness (gray level) and aluminum thickness (steps) was assessed using a polynomial function. Gray levels of the microradiographs were within the range of the video brightness of the step wedge. The mineral profiles were normalized with respect to the mineral content of the plateau region observed in each microradiograph, corresponding to the unaltered inner enamel. The plateau region is designated to have a mineral content of 100%, as we^{7, 8} and others^{e.g., 16, 17} have done previously. In this fashion, the change in the relative mineral content of each section is determined as a

function of time of exposure to the demineralizing solutions. To obtain quantitative comparisons between observed rates of demineralization (or remineralization) of the pre-formed artificial caries lesion, “% mineral change” was evaluated as follows. The area between the mineral content profile obtained after demineralization and the corresponding profile obtained after the exposure to the experimental solutions was integrated numerically. This area was then divided by the initial lesion area, which was similarly determined by numerically integrating the area between the mineral content profile of original sound section and the corresponding profile obtained after the initial demineralization. In this fashion, rates of demineralization and remineralization were normalized with respect to the size of the initial lesion. Given distinct changes observed in the inner (demineralization) and outer (remineralization) portions of the lesions (*e.g.* Fig. 5b), mean changes in the mineral content of these portions of the examined sections as a function of time for each solution were plotted separately (Fig. 6), although the processes of simultaneous remineralization and demineralization are clearly interrelated. The mean net change in mineral content of sections exposed to each experimental solution was also determined (Fig. 7).

Chemical Analyses

Prior to use, demineralization solutions were analyzed for calcium (atomic absorption) and phosphate (spectrophotometrically), as previously described.¹⁸ Fluoride concentrations were determined using a fluoride ion specific electrode (Model 9409, ThermoOrion, Beverly, MA, USA) and a micro reference electrode (Micoelectrodes, Londonderry, NH). Prior to analyses, fluoride-containing solutions and standards were diluted 1:1 with TISAB II solution (Orion Research, Cambridge, MA, USA).

Calculation of the Degrees of Saturation

Based on the analyses of the demineralization solutions, the degrees of saturation with respect to enamel (En), fluoroapatite (FA), and calcium fluoride (CaF₂) were calculated using previously reported procedures¹². The degree of saturation, DS, with respect to these phases is defined as the ratio of their mean ionic activity products in solution to their solubility product constants (K_X), defined in the same fashion. Thus,

$$DS_{En} = \left[\left(Ca^{2+} \right)^5 \left(OH^- \right) \left(PO_4^{3-} \right)^3 / K_{En} \right]^{1/9}$$

$$DS_{FA} = \left[\left(Ca^{2+} \right)^5 \left(F^- \right) \left(PO_4^{3-} \right)^3 / K_{FA} \right]^{1/9}$$

$$DS_{CaF_2} = \left[\left(Ca^{2+} \right) \left(F^- \right)^2 / K_{CaF_2} \right]^{1/3}$$

where ( ) represent the activity of the enclosed ions. Enamel was assumed to have the stoichiometry of hydroxyapatite (Ca₅(PO₄)₃OH) with an estimated thermodynamic solubility product constant (K_{En}=5.5 × 10⁻⁵⁵) for human enamel.¹⁹ The solubility product constants used for FA²⁰ and CaF₂,²¹ were 2.63 × 10⁻⁶⁰ and 2.69 × 10⁻¹¹, respectively. DS values greater than one represent conditions of supersaturation, whereas values below one represent conditions of undersaturation.

Statistical Analyses

Comparisons of changes in mean mineral content at selected time points and as a function of time were made using un-paired t-tests. Significance was established at p<0.05. Conditions of unequal variance were taken into consideration, as required.

Results

Effect of Fluoride on the Demineralization of Sound Human Enamel Under *In Vivo*-Like Conditions

An example of the results obtained for sections of sound human enamel exposed to the control solution (S1) is shown in Fig. 3a, where % mineral is plotted versus depth from the enamel surface as a function of time. Progressive demineralization took place and resulted in the formation of a subsurface lesion over the 98-day period. Subsurface lesions of this type were observed in all eight sections studied. As illustrated in the example shown in Fig. 3b, mineral loss was reduced substantially in sections of enamel exposed to the demineralizing solution (S2) that contained 0.08 ppm F. Subsurface lesions like that shown in Fig. 3b were observed in six out of the eight sections, with the remaining two sections showing no measurable mineral loss. As can be seen in Fig. 4, the mean change in mineral loss increased linearly in sections exposed to these two solutions, after an initial slow phase of demineralization, such that the extrapolated linear curves intersect the abscissa at positive values; ~14 days for the control and at ~27 days in the presence of 0.08 ppm F. Although standard deviations are not shown for the sake of simplicity, the rate of mineral loss (the slope of the line) in the 0.08 ppm F-containing solution is significantly ($p < 0.001$) less (by a factor of 5) than that observed for the sections exposed to the control solution. However, as illustrated in the example shown in Fig. 3c, essentially no mineral loss was detected by microradiography for sections exposed to the demineralizing solution (S3) containing 0.19 ppm F over the 98-day period. Similar results were also obtained for sections exposed to the demineralizing solution (S4) containing 0.38 ppm F.

Effect of Fluoride on the Demineralization of Artificial Carious Lesions in Human Enamel Under *In Vivo*-Like Conditions

Artificial lesions utilized in most of experiments were similar with respect to initial lesion depth, with the exception of the sections exposed to the experimental solution L5, which had a mean lesion depth ($\pm$ SD) of $603 \pm 28 \mu\text{m}$. The mean initial lesion depths ($\pm$ SD) of sections exposed to the remaining solutions were L1 ($232 \pm 18 \mu\text{m}$), L2 ($220 \pm 15 \mu\text{m}$), L3 ($329 \pm 80 \mu\text{m}$), and L4 ($229 \pm 59 \mu\text{m}$).

Typical examples of the change in mineral content observed as a function of time following exposure of the pre-formed artificial incipient lesions to demineralizing solutions containing 1.0, 2.1 and 25.0 ppm fluoride are shown in Fig. 5a – Fig. 5c, respectively. As illustrated by the example in Fig. 5a, enamel demineralization progressed with time for lesions exposed to solution L1 that contained 1.0 ppm fluoride. Using solutions L2 (2.1 ppm fluoride), L3 (5.1 ppm fluoride), L4 (10.1 ppm fluoride) and L5 (25.0 ppm fluoride), significant remineralization of the outer portion of the subsurface lesions took place. However, demineralization was also observed to varying degrees in inner portions of the lesions exposed to solutions containing 2.1, 5.1 and 10.1 ppm fluoride, resulting in an increase in mineral loss in the inner portion of enamel and in lesion depth. An example of this phenomenon is shown in Fig. 5b. However, little to no demineralization (*i.e.*, almost exclusive remineralization) was observed in the sections exposed to solution L5 (Fig. 5c) with the highest fluoride concentration (25 ppm) and saturation levels with respect to FA and calcium fluoride.

Quantitative measures of the effect of fluoride on simultaneous demineralization (within the inner portion of lesions) and remineralization (within the outer portion of lesions) in all sections examined are shown in Fig. 6. In this figure, we attempt to illustrate the effect of fluoride on the processes of demineralization and remineralization separately, although these processes occur simultaneously and are clearly interrelated. Nevertheless, as shown, it is apparent that the rate of enamel demineralization decreases markedly with increasing fluoride concentrations

in the experimental solution, particularly with respect to the changes observed using solution L1 (1.0 ppm fluoride) where only demineralization was observed. Although a trend is clearly illustrated (lower portion of graph in Fig. 6), differences in mean mineral loss only approached significance in some cases (*e.g.*, at the longest time points examined, mean mineral loss of sections exposed to solution L4 (10.1 ppm fluoride) exhibited less mineral loss than those exposed to solution L2 (2.1 ppm fluoride) with a *p*-value of 0.074), possibly due to the limited number of sections employed and the fact that multiple processes are occurring at the same time. Using solution (L5) with the highest fluoride concentration (25.0 ppm), however, little to no demineralization was observed and could not be accurately calculated.

Simultaneously, the rate of enamel remineralization in the outer portion of the lesion was found to increase with increasing fluoride concentrations (upper portion of graph in Fig. 6), although observed differences between solutions were not all statistically significant. A significant ($p < 0.05$) increase was found in the mean mineral content of the outer portion of lesions in sections exposed to solution L4 (10.1 ppm fluoride), in comparison to that observed using solution L2 (2.1 ppm fluoride) at the longest time points examined, while observed differences between the mean mineral content of sections exposed to solution L5 (25.0 ppm fluoride) and L2 (2.1 ppm fluoride) approached significance ($p = 0.056$). All sections exposed to solutions L2 – L5 containing 2.1 – 25.0 ppm fluoride, respectively, exhibited measurable remineralization in the outer portion of the lesions, in comparison to sections exposed to solution L1 (1.0 ppm fluoride) that exhibited only demineralization.

Furthermore, a comparison of the net change in mineral content (*i.e.*, considering both observed demineralization and remineralization), as shown in Fig. 7, shows that fluoride concentrations of 5.1 ppm (L3) and greater result in a significant ($p < 0.05$) net increase in the mineral content of the incipient lesions, in comparison to that observed in sections exposed to solutions containing lower fluoride concentrations, where demineralization tended to predominate. As anticipated, when experiments (unpublished data not shown) were carried out using neutral experimental solutions that were supersaturated with respect to both fluorapatite and tooth enamel, only remineralization was observed (*i.e.*, without simultaneous demineralization).

Effect of Fluoride on the Demineralization of Artificial Lesions in Hydroxyapatite Pellets

In order to confirm the results obtained using human enamel, similar experiments were carried out using artificial lesions created in two hydroxyapatite pellets. An example of the change in mineral content observed as a function of time following exposure of such a lesion to demineralizing solution L2 is shown in Fig. 5d. As can be seen in this figure, initial lesions (0 h) produced in the HA pellets did not exhibit a more highly mineralized surface layer. Nevertheless, as seen in the human enamel sections, remineralization took place in the outer portion of the pellet lesions, while demineralization of the inner portion of the lesions progressed simultaneously.

Discussion

Results of previous *in vitro* studies designed to determine the efficacy of fluoride in the prevention of enamel demineralization clearly indicate that observed effects are highly dependent upon the experimental conditions used⁴. Thus, there is a significant need to carry out such studies under conditions that are most relevant to the caries process. In the present study, we have shown that the rate of demineralization of sound enamel surfaces *in vitro*, under conditions with DS_{En} values similar to those found in dental plaque fluid following sucrose exposure, is significantly reduced in the presence of as little as 0.08 ppm fluoride and inhibited by fluoride concentrations of 0.19 ppm and greater. In the absence of fluoride, as shown in Fig. 4, mineral loss was found to increase linearly with time after an initial induction period. This result is consistent with previous findings that suggest that the kinetics of enamel

demineralization is controlled by surface reactions^{7, 22}, even under *in vivo*-like conditions⁵. Mineral loss also increased linearly in the presence of 0.08 ppm of fluoride, although at a significantly reduced rate. No mineral loss was detected when fluoride concentrations of 0.19 ppm and 0.38 ppm were added to the demineralizing solution. Thus, in comparison to earlier findings¹ where we showed that a higher concentration of fluoride (1.0 ppm) was needed to prevent the demineralization of enamel exposed to a solution with a greater driving force for demineralization (*i.e.*, a significantly lower DS_{En} value of 0.12) than that used in this study, the present findings confirm our hypothesis that the amount of fluoride needed to inhibit enamel demineralization is inversely related to the driving force for demineralization. This hypothesis is based on experimental evidence that supports a working model¹ that fluoride ion in solution inhibits enamel demineralization by enhancing the deposition of less soluble fluoridated hydroxyapatite phases within the enamel surface (Fig. 1). When the rate of this deposition exceeds the rate of demineralization, the sections appear as if there is no net loss of enamel mineral.

Based on the present findings, concentrations (0.04 – 0.3 ppm) of fluoride found in dental plaque fluid^{10–15} could potentially reduce or inhibit the rate of enamel demineralization *in vivo*, despite these seemingly low fluoride levels. However, this conclusion would only apply to sound enamel surfaces, since we have found that significantly higher concentrations of fluoride are required to prevent the demineralization of pre-formed artificial incipient caries lesions under similar *in vivo*-like conditions (*i.e.*, similar DS_{En} values). Our results clearly show that a demineralizing solution (L1) containing 1 ppm fluoride induces further demineralization of artificial *incipient* lesions, whereas fluoride present at significantly lower concentrations (as little as 0.19 ppm) can prevent the dissolution of *sound* enamel surfaces under similar demineralizing conditions. Although higher concentrations of fluoride (*i.e.*, 2.1, 5.1 and 10.1 ppm) present in the acidic experimental solutions resulted in the partial remineralization of the outer enamel surface, inner portions of the enamel underwent simultaneous demineralization, resulting in increased mineral loss and deeper lesions. With increasing concentrations of fluoride, the tendency for enamel demineralization within inner enamel was significantly decreased (Fig. 6). At concentrations of 25.0 ppm fluoride little to no demineralization of the inner portion of the lesion was observed (*i.e.*, essentially only remineralization of the incipient lesions took place).

Our results also clearly show that enamel remineralization and/or demineralization occurs to varying degrees and at different depths within artificial incipient lesions in human tooth enamel exposed to the acidic experimental solutions containing fluoride. These findings can be explained by two possible mechanisms. First, the observation of simultaneous remineralization and demineralization within outer and inner regions of enamel, respectively, is consistent with the suggestion that the effective composition of the experimental solution changes as ions diffuse and precipitate within the partially demineralized outer enamel region, as illustrated in Fig. 8b. As a result of remineralization of the outer enamel region, the remineralization potential of the resultant solution that penetrates the inner enamel pore volume is reduced, due to the loss of constituent ions. This suggestion would require that diffusion of bulk solution ions is rate-limiting. Accordingly, the solution within the inner enamel pore volume acquires an enhanced demineralization potential, relative to the bulk solution, since the bulk solution is already undersaturated with respect to tooth enamel. The magnitude of such an effect will depend upon the extent of mineral deposition in the outer enamel region and the initial composition of the bulk solution. As illustrated in Fig. 8a–Fig. 8c, and supported by our experimental findings, the onset, extent and prevention of enamel demineralization within artificial caries-like lesions are a function of both the DS_{FA} and the fluoride content of the demineralizing solution. Observed effects on remineralization and demineralization may further depend upon the nature of the lesion with respect to initial porosity, tortuosity and depth. Tooth type (*e.g.*, premolar *vs.* third molar), which has been shown to influence *in vitro*

demineralization, presumably due in part to differences in structure, may also influence these competing processes²⁷.

A second possible explanation for simultaneous remineralization and demineralization is that the inner portion of the enamel lesion is more susceptible to demineralization, due to gradients in enamel solubility with inner enamel being more soluble relative to outer enamel.^{23,24} Although a partial influence of gradients in enamel solubility on the observed results can not be ruled out entirely, the fact that simultaneous remineralization and demineralization was observed (Fig. 5d) in compressed hydroxyapatite pellets that lack solubility gradients indicates that the present observations in human enamel may be best explained by the first mechanism described in the preceding paragraph. Hydroxyapatite pellets have been previously used to gain insight into the dynamics of enamel reactions.^{9,25}

Simultaneous remineralization and demineralization has been observed in studies on the remineralization of artificial lesions in *bovine* enamel using acidic remineralization solutions.^{17,25} Unlike the present findings, however, reported remineralization did not occur near the outer surface, but mainly at intermediate depths of the lesions. It was also reported that demineralization progressed simultaneously within deeper portions of the lesion, although quantitative time-course data were not presented. The authors of these studies suggested that the ion product for FA within this intermediate region of *bovine* enamel was higher than that of the outer and inner areas due to a combination of remineralizing ions diffusing inward and dissolved ions diffusing out toward the enamel surface, resulting in an enhanced remineralization potential within this mid-region. Although similar findings were not obtained in the present study, our results also support the idea that the rates of simultaneous demineralization and remineralization are controlled by local variations in relevant ion products. Investigators have also observed the formation of a “double surface layer” in studies on the effect of fluoride in solution on the demineralization of *bovine* enamel³, although lesion depths were found to decrease with increasing fluoride concentrations, contrary to our present findings using human enamel. These authors hypothesized that a more resistant FA layer may form at intermediate lesion depths during enamel dissolution. The present report, to the best of our knowledge, contains the first quantitative description of time-dependent changes in mineral content associated with simultaneous remineralization and demineralization in human enamel lesions.

Thus, our results are consistent with the conclusion that simultaneous mineral deposition (remineralization) and dissolution (demineralization) within caries-like lesions in human enamel *in vitro*, induced by solutions supersaturated with respect to FA and undersaturated with respect to tooth enamel, is a result of rate-limiting diffusion of mineral ion constituents. Incipient lesions extend into enamel and are significantly more porous than sound enamel, thus providing for a greater penetration of solution ion constituents and a larger surface area of enamel mineral for subsequent reaction. Hence, the marked difference in the mineral structure of incipient lesions, compared to that of sound enamel, gives rise to the phenomenon of simultaneous remineralization and demineralization of incipient lesions observed here. Within the more porous enamel lesion that extends several hundred μm s, differential effects of diffusion and reaction on local driving forces for mineral precipitation and dissolution of enamel are very apparent (Figs. 5). In general, as shown in Figs. 6, higher fluoride concentrations resulted in an increase in remineralization (outer enamel) and a decrease in demineralization (inner enamel), resulting in a significant increase in mineral gain (Fig. 7).

Higher concentrations of fluoride (and greater DS_{FA} values) in bulk solution are required to prevent the demineralization of incipient caries-like lesions in comparison to sound enamel. The reason for this is again likely to be related to the marked difference between sound and caries-like enamel structures. For example, consistent with our proposed model (Fig. 8a), the

findings shown in Fig. 5a suggest that the lowest fluoride concentration (*i.e.*, 1.0 ppm) used is critically reduced within the very outer porous enamel surface of the caries-like lesion through the precipitation of a fluoridated hydroxyapatite phase (*e.g.*, FA). This results in a lowering of the driving force for remineralization and in an increase in the driving force for demineralization throughout the entire lesion, to a point where the rate of demineralization exceeds the rate of remineralization. Thus, similar to that which takes place under conditions where remineralization of the outer enamel lesion is observed using higher concentrations of fluoride (2.1 – 10.1 ppm), simultaneous demineralization in inner enamel results due to the preferential deposition of fluoridated phases within the outer porous enamel surface. Due to induced reductions in fluoride concentration and in the resulting remineralization potential, and enhancement of the demineralization potential within more porous enamel surfaces, relative to those factors in the bulk solution, higher bulk solution concentrations of fluoride are required to prevent the demineralization of caries-like lesions than those needed to prevent the demineralization of sound enamel. For sound enamel surfaces, relatively lower concentrations of fluoride are required to prevent its demineralization, since fluoride concentrations and the driving force for remineralization are not reduced at the surface, because solution ions are replenished rapidly and diffuse into the dense enamel surface to a very limited degree. Consistent with this overall mechanism, it has been shown using bovine tissues that higher concentrations of fluoride are required to inhibit the demineralization of the more permeable dentin tissue *in vitro*, relative to amounts of fluoride required to inhibit the demineralization of sound enamel.²⁶

The fact that relatively low concentrations of fluoride are required to prevent the demineralization of sound enamel may also be related to the initial slow phase of enamel demineralization observed here and previously^{5,7,22} to precede the linear increase in mineral loss with time (Fig. 4). The authors of the latter cited study suggested that the slower phase of demineralization may be related to the properties of surface enamel or to the development of the more highly mineralized surface layer. Limited findings (Fig. 4) relevant to this point were obtained here and suggest that low levels of fluoride can extend this induction period (*e.g.*, from 14 to 27 days) and slow down the onset of a more rapid phase of enamel demineralization. Since this induction period was only observed in studies using sound enamel, the influence of fluoride on this process may also be of importance in explaining the observed differences between sound and caries-like enamel.

In conclusion, regardless of the mechanism, our results clearly indicate that although fluoride concentrations typically found in dental plaque fluid may be effective in preventing caries development in sound smooth surface enamel, significantly higher concentrations of fluoride would be required to prevent the progression of incipient lesions. Such higher levels may be attainable, at least transiently, for example, following an oral rinse of sufficiently high fluoride concentration¹⁴. Importantly, the observed prevention of lesion progression in incipient-like lesions by fluoride is associated with enhanced remineralization.

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Figure 1.

A working model based on experimental evidence¹ that supports the hypothesis that fluoride ion in solution inhibits enamel demineralization by enhancing the deposition of less soluble fluoridated hydroxyapatite phases (*e.g.*, FA) within the enamel surface. When the solution is undersaturated with respect to enamel (*i.e.*, $DS_{En} \ll 1$), the demineralization of enamel is induced, resulting in the loss of mineral ions from the enamel surface. In the presence of increasing concentrations of fluoride, the demineralizing solution becomes supersaturated with respect to FA (*i.e.*, $DS_{FA} \gg 1$), enhancing the rate of FA deposition within the enamel surface. When the rate of this deposition exceeds the rate of demineralization, net loss of enamel mineral is prevented. DS_{En} and DS_{FA} are defined in Materials and methods.

Figure 2.

Overall experimental design used to assess the effect of fluoride on the demineralization of sound human enamel and on the demineralization of artificial carious lesions. Experimental solutions (S1–S4 and L1–L5) are described in Table 1. Experimental details are provided in Materials and methods.

a. To confirm findings found in human enamel, two compressed pellets of hydroxyapatite containing artificial lesions were exposed to solution L2.

Figure 3.

a. An example of progressive demineralization of a thin section of human enamel exposed to experimental solution S1 containing no fluoride, resulting in subsurface demineralization. Subsurface demineralization was observed in all eight sections studied. Mineral content profiles like this were obtained from scans of microradiographs of single sections of teeth as a function of time of exposure (days) to experimental solutions, as described in Materials and methods. The abscissa represents the depth (μm) from the enamel surface. The ordinate represents the relative mineral content (% mineral) normalized with respect to the mineral content of the plateau region observed in each microradiograph. **b.** Using the procedure described in Fig. 3a, an example of progressive demineralization of a thin section of human

enamel exposed to experimental solution S2, containing 0.08 ppm fluoride. Mineral loss was reduced substantially, relative to that found using experimental solution S1 that did not contain fluoride, and was observed in six out of eight sections studied. **c.** an example of results obtained for thin sections of human enamel exposed to experimental solution S3 containing 0.19 ppm fluoride. As shown with this example, no mineral loss was observed for all sound enamel sections exposed to this solution. Similarly, no mineral loss was observed in sections exposed to solution S4, containing 0.38 ppm fluoride.

Figure 4.

Mean change of mineral loss of sections exposed to control solution S1 (n=8) without fluoride and experimental solution S2 (n=6) containing 0.08 ppm fluoride. As can be seen, the mean change in mineral loss increased linearly in both solutions, after an initial slow phase of demineralization. Extrapolated linear curves intersect the abscissa at ~14 days for the control and at ~27 days for experiments carried out in the presence of 0.08 ppm F. The rate of mineral loss (the slope of the line) in the 0.08 ppm F-containing solution is significantly ($p < 0.001$) lower than that observed for the sections exposed to the control solution.

Figure 5.

Examples of progressive changes in the mineral content of artificial lesions in human enamel thin sections and hydroxyapatite pellets as a function of time (hours) following exposure to selected experimental solution, obtained using the same microradiographic procedures described for Fig. 3 and in Materials and methods. Scans of initial lesions are presented in bold.

a. An example of the results using human enamel sections following exposure to experimental solution L1 with 1.0 ppm fluoride, where only demineralization was observed in the artificial lesion; **b.** An example of the results using human enamel sections following exposure to experimental solution L2 with 2.1 ppm fluoride, where simultaneous remineralization and demineralization were observed; **c.** An example of the results using human enamel sections following exposure to experimental solution L5 with 25.0 ppm fluoride, where only remineralization was observed. This was the case for two of the three sections examined. In

the remaining section exposed to solution L5 simultaneous remineralization and demineralization were observed; **d.** An example showing simultaneous remineralization and demineralization of an artificial lesion in a hydroxyapatite pellet following exposure to experimental solution L2 with 2.1 ppm fluoride.

Figure 6.

Mean change in mineral content in thin sections of artificial caries-like lesions as a function of time (h) of exposure to experimental solutions L1(♦), L2 (▪), L3 (▲), L4 (○) and L5 (X) (Table 1), containing 1.0, 2.1, 5.1, 10.1 and 25.0 ppm fluoride, respectively. Mineral gain and loss were assessed as described in Materials and methods. Vertical bars for each point represent the standard deviation. The upper half of this graph shows % mineral gain (remineralization) observed in the outer portion of examined lesions, while the lower half of this graph shows % mineral loss (demineralization) seen in the inner portion of examined lesions. In general, higher fluoride concentrations resulted in an increase in the rate of remineralization (outer enamel) and a decrease in the rate of demineralization (inner enamel).

Figure 7.

Mean change in the net mineral content of thin sections of artificial caries-like lesions at the longest time point examined following exposure to experimental solutions L1(63 h), L2 (760 h), L3 (760 h), L4 (735 h) and L5 (723 h) (Table 1), containing 1.0, 2.1, 5.1, 10.1 and 25.0 ppm fluoride, respectively. Mineral gain and loss were assessed as described in Materials and methods. Vertical bars for each point represent the standard deviation. Significant ($p < 0.05$) differences in comparison to results obtained using solution L1 (#), L2 (&) and L3 (@) are indicated. Fluoride concentrations of 5.1 ppm and greater resulted in a significant net increase in mineral content of the incipient lesions, in comparison to that observed in sections exposed to solutions L1 and L2 containing lower fluoride concentrations, where demineralization tended to predominate.

Figure 8.

A schematic representation illustrating a proposed mechanism to describe the observed effects of experimental solutions undersaturated with respect to tooth enamel ($DS_{En} = \sim 0.22$) and supersaturated with respect to fluoridated hydroxyapatites (*e.g.*, FA) on mineral changes in artificial caries-like lesions in human enamel. As the fluoride concentration and DS_{FA} of the bulk experimental solutions increase, a net remineralization potential of the resultant solutions that penetrate the enamel pore volume increases (represented by the color intensity and size of the gray gradient) resulting in the deposition of FA (remineralization) in progressively deeper regions of the incipient lesions. As seen under intermediate conditions (**b**), the preferential deposition of mineral ions in the outer enamel region results in a gradual decrease in the remineralization potential of the solution within inner enamel and in a corresponding increase in its demineralization potential, resulting in simultaneous demineralization. The extent of observed remineralization and demineralization within inner enamel is a function of the fluoride concentration and the DS_{FA} of the bulk solution. When the fluoride concentration (*i.e.*, 1.0 ppm F), and DS_{FA} are relatively low, FA deposition is restricted to the very outer enamel surface and only demineralization is observed (**a**). When the fluoride concentration (*i.e.*, 25.0 ppm F) and DS_{FA} are relatively high, remineralization predominates throughout the lesion (**c**).

Table 1.

Composition of Experimental Solutions

No.	Concentration ^a				pH	Degree of Saturation ^b		
	Ca	P	Lactic Acid	F		En	FA	CaF ₂
S1	3.2	7.2	65	0	5.02	0.241	-	-
S2	3.3	7.0	65	0.08	5.02	0.243	2.31	0.07
S3	3.2	6.6	65	0.19	5.04	0.243	2.54	0.12
S4	3.2	6.7	65	0.38	5.03	0.240	2.70	0.18
L1	3.1	14.7	50	1.0	4.79	0.214	2.87	0.37
L2	3.1	14.8	50	2.1	4.80	0.219	3.17	0.61
L3	3.1	15.2	50	5.1	4.79	0.216	3.47	1.10
L4	3.0	14.8	50	10.1	4.80	0.215	3.70	1.72
L5	3.1	15.1	50	25.0	4.80	0.219	4.19	3.17

^a All concentrations are expressed in mmol/l, except for fluoride that is expressed in ppm. All solutions also contained 3 mmol/l sodium azide as a bacteriostat.

^b The degree of saturation with respect to enamel (En), fluorapatite (FA) and calcium fluoride (CaF₂), as defined in "Materials & methods".