

Effect of Fluoride on the Morphology of Calcium Phosphate Crystals Grown on Acid-Etched Human Enamel

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Key Words

Biomineralization · Enamel · Erosion · Fluoridated hydroxyapatite · Nanostructure

Abstract

The aim of this study was to examine the effect of fluoride ion concentration on the morphology of calcium phosphate crystals grown on acid-etched enamel as a model for tooth enamel erosion. Samples were immersed in calcification solution for 16 h and changes in crystal morphology were monitored by field emission scanning electron microscopy. Without fluoride, plate-like octacalcium phosphate crystals (20 nm thick, 2–10 μ m wide) were formed. With 1–10 mg/l fluoride, arrays of denser needle-like nanocrystals (20–30 nm wide, >500 nm in length) were formed. We conclude that there is a minimal fluoride concentration (1 mg/l) that dramatically affects the morphology of calcium phosphate crystals grown on etched enamel *in vitro*.

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The hierarchical structure of dental enamel contains organized nanocrystals of carbonated fluoridated hydroxyapatite, which make the tooth surface highly abrasion-resistant with an interesting combination of wear and fracture resistance [Boyde, 1997]. However, exposure

of dental enamel to extreme acidic pH in the oral cavity, either from extrinsic sources (diet) or intrinsic factors (reflux disorders), leads to dental erosion [Meurman and ten Cate, 1996; Lussi et al., 2004; Honório et al., 2008]. Dental erosion, which is the loss of enamel mineral due to acidic exposure not involving bacteria, has been directly associated with disorders such as bulimia nervosa and chronic gastroesophageal reflux [Nunn, 1996].

In the last five decades, fluoride has been used for the treatment of dental erosion and caries prevention through different modalities [ten Cate, 1997]. In addition, the effect of fluoride on the nanostructure of remineralized apatite has been well documented [Aoba, 1997; Robinson et al., 2004]. However, the optimal dose of fluoride resulting in a positive effect on enamel hydroxyapatite remineralization with minimum risk of toxicity has not been well documented.

A future alternative approach to treat affected enamel would be strategies to reconstruct a synthetic enamel-like structure of hydroxyapatite nano-rods from supersaturated calcium phosphate solution [Yamagishi et al., 2005; Zhan et al., 2005; Chen et al., 2006; Ye and Wang, 2007]. Methods used in the past by various investigators involved extreme conditions such as the application of high temperature, high pressure, or extreme acidic conditions, which are not practical in the clinical setting. The presence of 0.1–2 mg/l fluoride in the mineralization solution

is known to promote hydrolysis of octacalcium phosphate (OCP) to apatite [Moriwaki et al., 1983; Iijima and Moradian-Oldak, 2005]. It has also been shown that at 0.1–1 ppm F the ribbon-like OCP changes to an interlayered structure, and at 2 mg/l needle-shaped apatite crystals were produced [Iijima, 2001]. During enamel development, F may facilitate the fast deposition of mineral [Robinson et al., 2004]. However, direct evidence of the effect of F ion on the morphology and nanostructure of crystal growth at a nanoscale level on an enamel surface has not been reported.

In the present study, we investigated the effect of fluoride ion concentration on the morphology of calcium phosphate crystals grown on an acid-etched enamel surface as a model for tooth enamel erosion. We applied a biomimetic strategy in which supersaturated calcification solutions were used to initiate the growth of calcium phosphate crystals on the etched enamel surfaces [Wang et al., 2004]. Our long-term objective is to develop strategies for application of fluoride in reconstructing tooth enamel [Fan et al., 2009].

Materials and Methods

Tooth Preparation

Human third molars without fillings (extracted following the standard procedures at the University of Southern California School of Dentistry and used with Institutional Review Board approval) were selected. The teeth were treated with 3% sodium hypochlorite and sliced into 0.2- to 0.5-mm-thick samples as previously described [Fan et al., 2009]. Each tooth slice was first etched with a 3% HNO₃ solution for 50 s, rinsed with about 20 ml running deionized water, and then immediately immersed in 12 ml freshly prepared biomimetic calcification solution.

Calcification Solution

Coatings were obtained by immersing the tooth slices in the calcification solution containing 2.58 mM calcium (CaCl₂·2H₂O, >74.4% CaCl₂, Curtin Matheson Scientific, Houston, Tex., USA), 1.55 mM phosphate (KH₂PO₄, >99%, EM Science, Gibbstown, N.J., USA) and 180 mM NaCl at 37°C, buffered by 50 mM Tris-HCl. The pH of the calcification solution was adjusted with 1 M HCl to 7.60 using a 718 STAT Titrimo (Metrohm, Herisau, Switzerland). The relative supersaturation values (σ) were calculated by the SPEC96 speciation program with the Davies extension of the Debye-Hückel equation, using mass balance expressions for total calcium and total phosphate with appropriate equilibrium constants as previously described [Wang et al., 2006]. Fluoride, from NaF (>99%, Sigma-Aldrich, St. Louis, Mo., USA), was added to obtain the final desired concentrations of fluoride just before immersing the tooth slice. The mineralization solution containing the tooth slice was sealed in a 20-ml scintillation vial and incubated at 37°C statically for 2–30 h.

Characterization of the Crystals in Coatings

Tooth slices with the coating were sputtered with carbon to a thickness of 5 nm for conductivity, and secondary electron images were acquired by a field emission scanning electron microscope (FE-SEM) at 5 kV (Leo, 1550 VP). Elemental analysis was achieved by means of an Oxford INCA Energy 300 X-ray Energy Dispersive Spectrometer system attached to the SEM. Attenuated total reflection Fourier transformed infrared (ATR-FTIR) was obtained from a diamond accessory (Gladi-Diamond ATR, Pike Technology, Madison, Wisc., USA) attached to a Jasco 2000 FTIR system. Infrared spectra were taken at 4 cm⁻¹ intervals over the 4,000–500 cm⁻¹ range for a total of 30 scans. The mineralized samples for FTIR were prepared freshly and air-dried 3 h before the test. The coating on the enamel was carefully brought into contact with the diamond crystal surface and compressed by the clamp tip at 30,000 psi. The spectra were converted to transmission mode by the FTIR software.

Results

The effect of fluoride concentration on the morphology of calcium phosphate crystals formed on acid-etched enamel surfaces was tested after 16-hour deposition, in the presence of 0, 0.1, 0.5 and 1 mg/l fluoride in the supersaturated calcification solution (fig. 1a–c). Relative supersaturation values (σ) were calculated to be 3.08 for OCP, 24.7 for hydroxyapatite and 25.4 for FAP after addition of 52.6 nM NaF (1 mg/l F). The blue shift in the ATR-FTIR spectrum of the ν_4 band from 561 cm⁻¹ (typical of hydroxyapatite) to 557–555 cm⁻¹ (typical of OCP) indicated that the mineral phase of the coating formed without fluoride was mainly OCP (fig. 1d) [Fowler et al., 1993; Wang et al., 2004]. The morphology of the OCP crystals was flake-like with 5–10 μ m width and 20 nm thickness (fig. 1a). This morphology was also found in previous biomimetic mineralization studies [Abe et al., 1990; Wen et al., 1998]. The concentration of fluoride affected the morphology of crystals formed on the enamel surface dramatically. At 0.5 mg/l fluoride, the majority of the crystals had a plate-like morphology (fig. 1b). Careful examination of the OCP (100) crystal plane at higher magnification revealed the presence of short plates, 5–10 nm in width and length, growing on the large (100) plane (fig. 1b, inset). The coating deposited in the calcification solution with 1 mg/l fluoride became denser (fig. 1c) and the majority of crystals had a needle-like shape over 500 nm in length with a 20–30 nm diameter (fig. 1c, inset). Similar needle-like crystals were found in the coating deposited at 2–10 mg/l fluoride (data not shown). Observations at other time periods showed that the coatings had begun to form at 2 h and even at this stage the morphol-

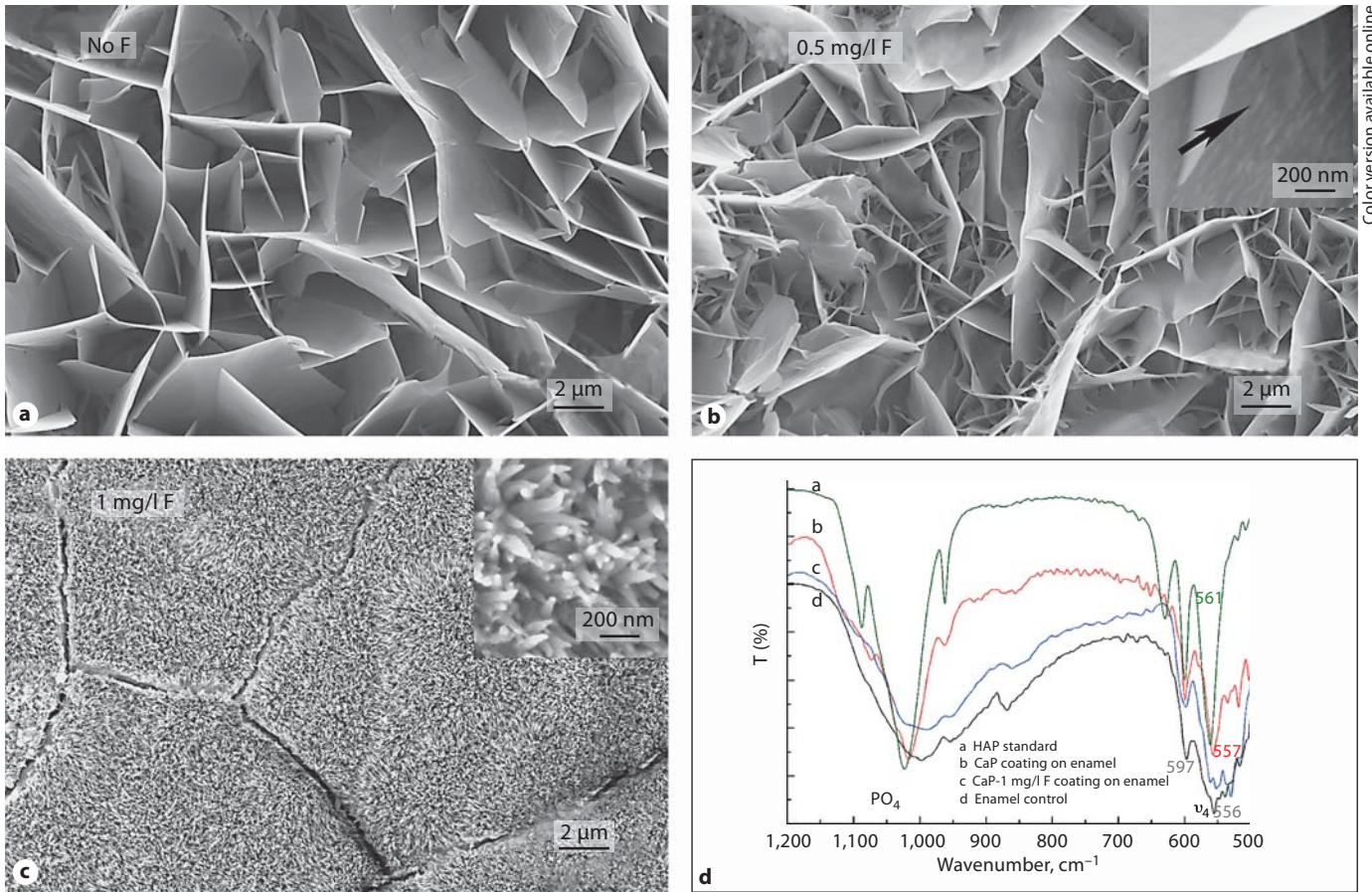


Fig. 1. a–c SEM images of the coating deposited on enamel during 16 h in the presence of 0 (a), 0.5 (b), or 1 (c) mg/l fluoride. Coating topography and crystal morphology progressively change from porous flake-like apatite to needle-like fluoridated hydroxyapatite nanocrystals array with increasing fluoride concentration. Inset in b shows short nano-needles (arrow) formed on the (001)

face at high magnification. Inset in c shows the nano-needle array at high magnification. d ATR-FTIR spectra from a hydroxyapatite (HAP) standard, coatings formed in mineralization solution with and without fluoride, and a native enamel surface. The HAP standard has an OH⁻ band at 629.8 cm⁻¹, and ν₄ bands at 599.5 and 560.8 cm⁻¹.

ogy of the deposited crystals was the same as at 16 h, for all fluoride concentrations. The FTIR spectrum of the mineral phase formed in the presence of 1 mg/l fluoride was similar to that of apatite (fig. 1d). FTIR spectra also showed the similarity between native enamel and the coating formed in the presence of 1 mg/l F (fig. 1d). The absorbance band at 900–1,100 cm⁻¹ was attributed to a PO₄ group. A ν₄ band of PO₄³⁻ at 550–650 cm⁻¹ indicated that the coating was mostly composed of hydroxyapatite. Elemental analysis by Energy Dispersive Spectrometer of the coating containing needle-like crystals prepared with 1 mg/l F revealed a Ca/F ratio of 9.8 and a Ca/P ratio of 1.45. Since no diffraction band for CaF₂ was detected in the XRD spectra (data not shown) we concluded that the crystals were fluoridated hydroxyapatite.

face at high magnification. Inset in c shows the nano-needle array at high magnification. d ATR-FTIR spectra from a hydroxyapatite (HAP) standard, coatings formed in mineralization solution with and without fluoride, and a native enamel surface. The HAP standard has an OH⁻ band at 629.8 cm⁻¹, and ν₄ bands at 599.5 and 560.8 cm⁻¹.

Discussion

Fluoride ion-containing reagents have been applied routinely in caries prevention and treatment of dental erosion. However, the optimal F concentration to facilitate effective enamel remineralization is unknown. Clinically, fluoride concentration can be as high as 2.26% (w/w) F to treat etched enamel [Beltrán-Aguilar et al., 2000]. Whether such a high concentration of F can promote sufficient fluoridated hydroxyapatite formation is, however, not clear.

The treatment of apatite crystals with fluoride solution can cause mineral loss and the replacement of phosphate with fluoride ions [Malaowalla and Myers, 1962]. It is well known that exposure to a fluoride-containing so-

lution can cause spherical CaF_2 precipitates on enamel surfaces. Such CaF_2 precipitation was thought to promote fluoride release and remineralization of enamel hydroxyapatite [Francis et al., 1968; Caslavská et al., 1975]. Our results, however, show that under controlled conditions fluoride can be incorporated directly into fluoridated hydroxyapatite grown on an etched enamel surface without the formation of spherical CaF_2 particles. Due to increasing concerns over the side effects from exposure to high concentrations of fluoride [Aoba and Fejerskov, 2002; Marthaler, 2004], the application of a low concentration of fluoride would be more appropriate to promote fluoridated hydroxyapatite mineralization for caries prevention or erosion treatment.

Detailed studies on the seeded growth of apatite from supersaturated solution have suggested that OCP may be a precursor of the apatite phase in vitro [Nancollas and Tomazic, 1974]. Fluoride can accelerate epitaxial growth of apatite crystals on the OCP precursor, changing the crystal morphology [Moriwaki et al., 1983; Iijima et al., 1992]. Changes in the morphology of apatite crystals grown from unstable supersaturated solution as the result of fluoride addition have also been reported previously by Eanes and Meyer [1977], who reported that the presence of fluoride eliminated the formation of the intermediate OCP phase. Fluoride was hypothesized to reduce growth of (100) of OCP and to cause needle-like crystal structure formation [Iijima, 2001]. In view of this accumulating knowledge on the effect of fluoride on calcium phosphate mineral phase and morphology, we adopted a biomimetic strategy to develop a technique for

enamel remineralization in the presence of fluoride. We found that as little as 1 mg/l fluoride promoted formation of an array of 20–50 nm diameter needle-like nanocrystals on enamel. Such crystals are similar to native enamel crystals in width and thickness and therefore have the potential to possess similar mechanical properties [Fan et al., 2007]. Although the etched enamel surface used in our study does not model subsurface lesions, the biomimetic calcification system developed could be applied to artificial subsurface lesions used as a model for dental caries [Yamazaki and Margolis, 2008].

In conclusion, fluoride had a dose-dependent effect on crystal morphology in the supersaturated calcification solution used in our in vitro experiments. A uniform coating with organized fluoridated hydroxyapatite crystals was grown in the presence of 1–10 mg/l fluoride. Introducing as little as 1 mg/l fluoride was effective in altering the crystal nanostructure from porous plate-like OCP to a needle-like array of fluoridated hydroxyapatite nanocrystals. The biomimetic mineralization system used here is a first step towards developing a clinically applicable means of forming an enamel-mimicking mineralized layer, under minimal fluoride exposure, on eroded or damaged tooth enamel [Fan et al., 2009].

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