

## Fluoridated hydroxyapatite coatings on human enamel using chemical deposition

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Investigation of fabricating enamel-like structure in vitro is of great interest in the fields of dentistry and material sciences. In this study the coatings with organized fluoridated hydroxyapatite crystals were obtained on etched human enamel (14 °C–45 °C) using a simple chemical approach. In the chemical approach phosphoric acid etched enamel slices were immersed in a solution for 10 days which contained NaH<sub>2</sub>PO<sub>4</sub>, Ca(NO<sub>3</sub>)<sub>2</sub>, EDTA-Na<sub>2</sub> and NaF to form fluoridated hydroxyapatite coatings. The coating was analyzed by SEM, EDS, XRD, FT-IR and nanoindentation tests. We found that the coating is uniform and dense, with enamel-like structure and the crystals of coating have a typical hexagonal structure and are about 200 nm in cross section and the shape of crystal. When the enamel slices were immersed for 10 days the Elastic modulus and hardness of the coating reached 85.3 ± 6.2 GPa and 3.91 ± 0.55 GPa respectively which are close to that of natural enamel. Compared to hydroxyapatite (HAP) coatings it is useful and demonstrates a potential application to repair enamel damage in dental clinics.

**Key words:** FAP, Enamel, Chemical deposition, Coating, Hexagonal structure.

### Introduction

Dental enamel is the outer most layer of teeth as well as the hardest mineralized tissue in the human body [1]. Mature enamel has more than 96 wt% mineral content, whereas in dentin the mineral content drop to 68 wt% [2]. Its structure is similar to hydroxyapatite (HAP), Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>, the crystals found in enamel are carbonated hydroxyapatite nanocrystals, 50–70 nm in width, 20–25 nm in thickness, with length to width aspect ratio over 1000 [3], which is arranged into a highly organized micro-architectural unit called an enamel prism [4, 5]. The highly organized hierarchical microstructure of enamel prism provides efficient protection to dental enamel against physical and biochemical attack [6, 7]. As mature dental enamel is not living tissue, such as dentin and bone, it is seldom remineralized by the body when it comes to demineralization [8]. Therefore attention has been focused on the formation of HA coating on the enamel surface which has enamel-like structure. Different methods have been used to form HA coating which mimic the biomineralization process of tooth using gelatin [9, 10], amelogenin [3, 11, 12], surfactants [13, 14], chelating reagents [8, 15–17], or calcium phosphate paste in simple chemicals method [18–20] or electrochemical deposition [21]. These approaches call for too severe conditions to apply in clinics and sometimes the structure of coating was not enamel-like structure.

It had been found that the presence of fluorine in teeth is helpful in preventing dental caries and pure HAP has a relatively lower acid resistance than fluoridated hydroxyapatite (FAH) [22, 23]. In order to reduce the dissolution of acid of HAP coatings, attentions have been turned to FAH coating [24–26], which could provide lower dissolution, better apatite-like layer deposition and better performance in the biological condition.

Based on previous studies we used an aqueous solution method to synthesize enamel-like structures directly on the phosphoric acid etched enamel surface. The solution contains EDTA-Na<sub>2</sub>, Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub> and NaF. In this report, the detailed preparation and characterization of FAH coating on the enamel surfaces were presented. The goal of the study is to prove aqueous solution route is effective in preparing FAH coating and possibly can restore the early enamel lesions.

### Materials and Methods

#### Tooth slices preparation

Human third molars were selected and cut longitudinally or transversely by low speed diamond saw cooled by water. The tooth slices with enamel were etched 30 s in 30% phosphoric acid and then washed with de-ionized water and dried in air.

#### Fluoridated hydroxyapatite coating preparation

The treated tooth slice was first immersed in a solution which contained 0.1 M Ca(NO<sub>3</sub>)<sub>2</sub>, 0.1 M EDTA-2Na, 0.06 M NaH<sub>2</sub>PO<sub>4</sub> and 0.01 M NaF. Then, solutions was put into a water bath at 14 °C, 25 °C,

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37 °C, and 45 °C for 10 days. Finally, the tooth slice with a newly-grown layer was rinsed with de-ionized water and dried in air.

### Structural and compositional analysis

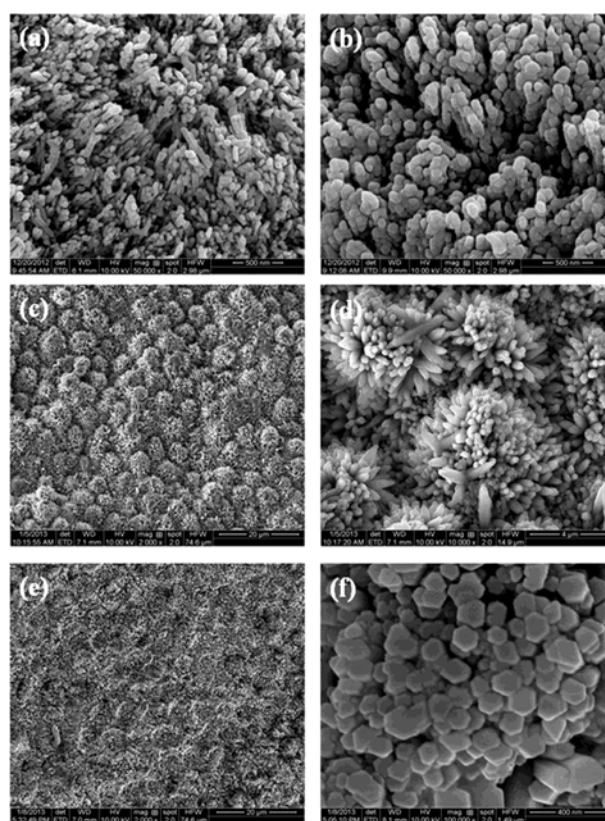
Secondary electron images and energy-dispersive X-ray spectroscopy of the FAH coatings were taken using field-emission scanning electron microscopy (JEM-5900LV, JEOL, Tokyo, Japan) at 15 kV. The crystal structures and orientation of the coating were determined by X-ray diffraction and data were collected from 10 ° to 60 ° with a step size of 0.02626 ° and a scanning speed of 0.02 s per step. Fourier transform infrared spectroscopy (FT-IR) of the sample with coating was conducted on a Bruker Vertex 70 spectrometer with Attenuated Total Reflection (ATR) DuraScope diamond accessory over a range of 500–4000  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  under about 30,000 psi.

### Mechanical properties analysis

The Agilent Nano Indenter G200 was used to perform nanomechanical testing with a Berkovich tip under continuous stiffness measurement for the coatings and dental enamel. Human third molars, extracted following the standard procedures for extraction at the Shandong University School of Dentistry, were embedded in phenol-formaldehyde resin and polished with 0.2  $\mu\text{m}$  diamond paste at the occlusal surface. For each sample at least 50 indents controlled in 1000 nm depth limit were made.

## Results and Discussion

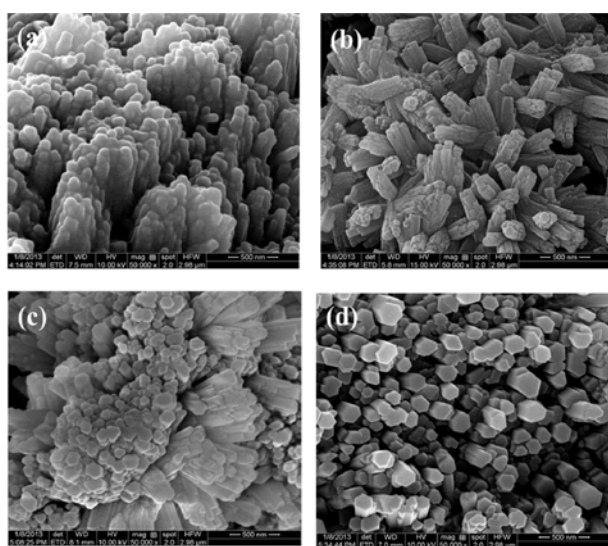
The morphology of synthetic FAP coating varies depending on the growing time and temperature of the water bath. Fig. 1 shows the SEM images of the newly-grown layers at different growing stages at 37 °C. After etching by 30% phosphoric acid (Fig. 1(a)), the activated enamel surface was covered with a type of spherical shaped crystals (Fig. 1(b)), which is obviously different to the enamel crystals. Besides, the coating of these crystals was porous. The image shows that these crystals are no more than 100 nm in cross section. As its growing, the crystals transformed from a spherical shape to a shuttle-like structure (Figs. 1(c,d)). Fig. 1(c) shows the typical SEM image of the regenerated enamel layer for 6 days, which has an obvious enamel prism-like structure and similar size of about 7  $\mu\text{m}$  in cross section, and the rod-like crystals are about 200 nm in cross section (Fig. 1(d)). The microstructures are like those of natural enamel. After growing for a long time, the crystals changed from shuttle-like structures to well-defined crystals with a typical apatite hexagonal structures (Fig. 1(e,f)). The possible reason for the change of morphology of fluoridated hydroxyapatite crystals is most likely that the crystals with spherical shape are amorphous fluoridated calcium phosphate



**Fig. 1.** SEM images of (a) enamel surface after acidic etching; (b) sample (a) immersed in the solution for 1 day; (c, d) sample (a) immersed in the solution for 6 days; (e,f) sample (a) immersed in the solution for 10 days.

which is less thermodynamically stable than hexagonal cross section structure [17]. The newly-grown crystals on the activated kinks, steps and defects on the etched enamel crystals which have different orientations between prism and inter-prism would appear to form a similar size and orientation to those native enamel prisms and interprisms.[27] When the adjacent crystals with different growth direction connected with others they would fuse and finally only the parallel crystals could be seen which positioned perpendicular to the enamel surface [8]. In the end, a newly formed fluoridated hydroxyapatite coating with a typical apatite hexagonal structure was produced.

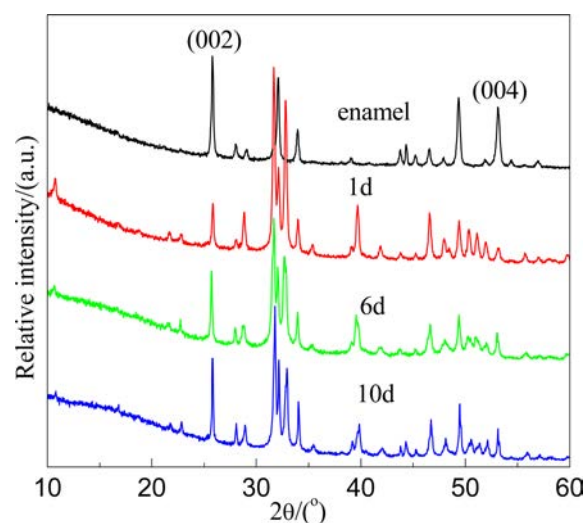
Fig. 2 exhibits the distinct morphologies of the grown layer in water bath at different temperature. With the growing temperature rising, the shape and size of rod-like crystals changed. At lower growing temperature such as 14 °C, the growth of crystals was limited and resulted in rod-like structure (Fig. 2(a)), while the crystals change (Fig. 2(b)) when temperature increased to 25 °C. The well-defined crystals with a typical apatite hexagonal structure (Fig. 2(c)) were produced when the enamel slices immersed in the above mentioned solution at 37 °C for 10 days. The cross section of the crystals raised with the temperatures of water bath while enamel prism-like structure still remained about



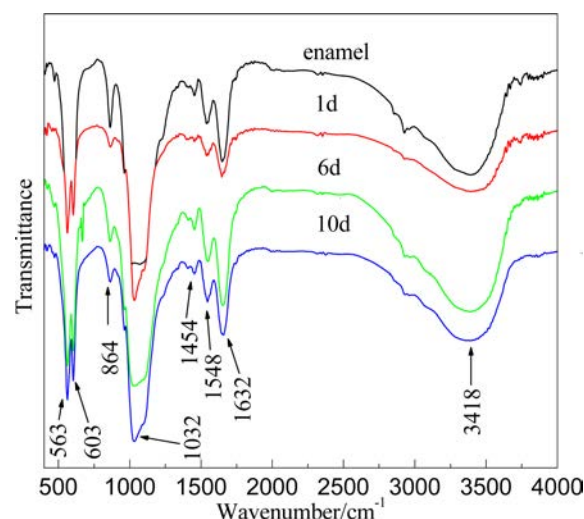
**Fig. 2.** SEM images of FAH coating grown on human enamel at different temperatures for 10 days: (a) 14 °C; (b) 25 °C; (c) 37 °C; (d) 45 °C.

5–8  $\mu\text{m}$ . From the SEM image at 25 °C it could be seen that the rod-like structure were composed of many small crystals aligned parallel to each other and exhibited different orientation. When the temperature raised to 45 °C, the crystals began to parallel to each other. Besides, the length to width aspect ratio was higher than that of crystals grown at 37 °C.

The XRD (Fig. 3) and ATR FT-IR (Fig. 4) spectrum of the mineral in the coating indicated the presence of FAH crystals. Fig. 3 shows the XRD patterns of enamel slices with and without the newly growth layer. The reflections from the substrate enamel were not readily detected due to the densely packed structure of the coating. The relative intensity of (002) peak and (004) peak of the natural enamel slices are stronger than those of the coating, which indicated that the crystals of natural enamel align were more orderly along the c-axis than that of the coating. From the SEM image (Fig. 1(d)) we can see some rod-like crystals grown between bundled structures which have different orientations and are just like inter-prisms in the natural enamel, which resulted in that (002) peak and (004) peak were relatively weak. With the growing time

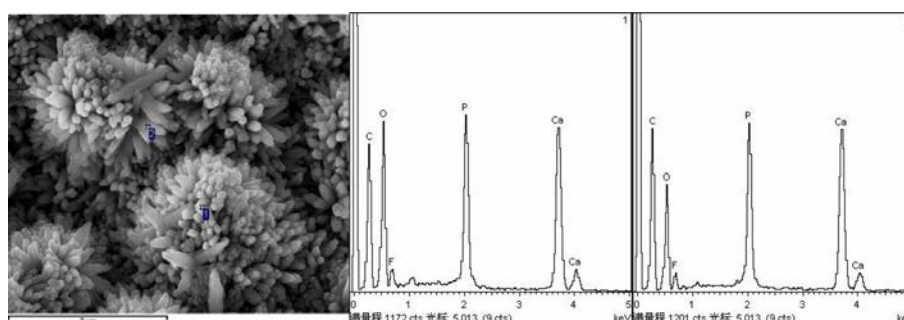


**Fig. 3.** XRD patterns of FAH coating for different growing time at 37 °C compared with enamel control.



**Fig. 4.** FT-IR spectrum of the FAH coating for different growing time compared with enamel control.

raised only parallel crystals could be seen in the SEM image (Fig. 1(f)). Therefore, (002) peak is stronger again. The relative intensity of (002) peak is stronger when growth time is 10 days than 1 day which indicated that the crystals grown for 10 days align were

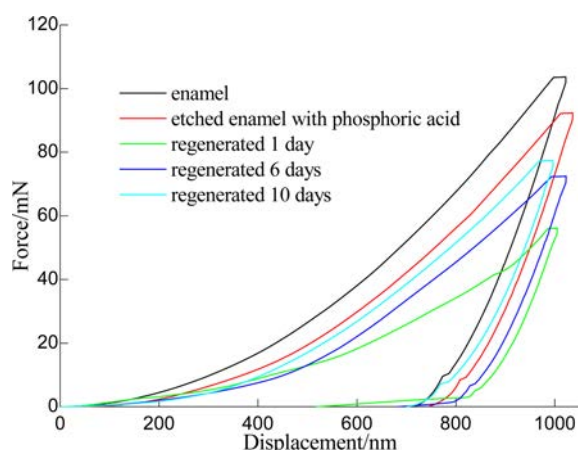


**Fig. 5.** EDS analysis of the FAH coating for 6d at 37 °C.

more orderly and parallel to each other than that of 1 day. FT-IR in Fig. 4 demonstrated the presence of FAP and the spectrum of FAH coating was close to that of native enamel. The strong absorption peak at  $3418\text{ cm}^{-1}$  and weak peak at  $1632\text{ cm}^{-1}$  and  $1548\text{ cm}^{-1}$  were attributed to O-H. The absorption peak at  $1454\text{ cm}^{-1}$  and  $864\text{ cm}^{-1}$  were ascribed to the characteristic band of the  $\text{CO}_3^{2-}$  group which incorporation into  $\text{PO}_4^{3-}$  or OH- in the growth of apatite lattice. The bands appeared at  $1032\text{ cm}^{-1}$ ,  $603\text{ cm}^{-1}$  and  $563\text{ cm}^{-1}$  assigned to the  $\text{PO}_4^{3-}$  group [3, 28].

The chemical composition of the newly grown layer was further characterized by EDS analysis (Fig. 5) which demonstrated that the rod-like structures contain fluoride. EDS data revealed that the coating has an average Ca/P ratio of 1.39 and that the amount of F- ions is approximately 18 at% against  $\text{Ca}^{2+}$  ions. This indicated that the produced crystals are calcium deficient fluoridated hydroxyapatite.

In addition, the mechanical properties of the coating by means of a nanoindenter were investigated (Fig. 6). Due to the unique hierarchical crystal organization, dental enamel has excellent properties of elastic modulus, hardness and fracture toughness when compared with pure apatite mineral. The differences in nanomechanical properties of the coatings were compared with natural enamel as seen in the elastic modulus-displacement curves in Fig. 6 and the calculated elastic modulus and hardness in Table 1. The coatings were measured as



**Fig. 6.** Loading-unloading curves of nanoindentation tests at the maximum distance of 1000 nm.

**Table 1.** Mechanical properties of enamel and FAP coating by nanoindentation.

|                         | Elastic modulus (GPa) | Hardness (GPa)  |
|-------------------------|-----------------------|-----------------|
| Enamel                  | $113.6 \pm 6.05$      | $5.97 \pm 0.48$ |
| Enamel etched with acid | $106 \pm 6.2$         | $4.87 \pm 0.52$ |
| FAP coating for 1d      | $64.3 \pm 7.6$        | $2.45 \pm 0.45$ |
| FAP coating for 6d      | $76.9 \pm 6.5$        | $2.87 \pm 0.63$ |
| FAP coating for 10d     | $85.3 \pm 6.2$        | $3.91 \pm 0.55$ |

about 400-800 nm in thickness. The data reveal that the elastic modulus and hardness of enamel decrease when it was etched with acid. It could be seen from the curves that the elastic modulus and hardness of coating would rise with the growing time prolonged.

## Conclusions

Based on the effectiveness of chelating reagents and F- ions on the formation of rod-like apatite crystals, we choose EDTA- $\text{Na}_2$  as the  $\text{Ca}^{2+}$  ions chelating reagents and when EDTA-Ca formed it would act as  $\text{Ca}^{2+}$  ions source for the formation of fluoridated hydroxyapatite. The simple chemical method could produce enamel-like structure with prism and inter-prism under near-physiological conditions ( $14^\circ\text{C}$  to  $45^\circ\text{C}$ ). The newly growth layer is quite close to the natural enamel in chemical components, microstructure, and nanomechanical properties. The newly grown crystals have bigger size and more F content than the crystals of enamel, so we concluded that the newly grown layer have the ability to resist against stronger acidic solution than the natural enamel. For the reason that fluoridated hydroxyapatite has excellent biocompatibility, we propose that reconstructed the early caries lesion by this simple chemical method may be a promising approach.

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