



Effects of Different pH Conditions on Enamel Erosion Repair by Nano Fluorapatite Pastes

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The effects of different pH conditions on enamel erosion repair by nano fluorapatite (*n*-FA) pastes were evaluated in this study. Eighteen human dental enamel blocks with artificially-induced erosion were randomly divided into three groups that were coated with *n*-FA pastes with 3 different pH values (pH < 1, pH = 4.5 and pH = 7.5, respectively) for 15 minutes. SEM, XRD, XPS, Vickers micro-hardness test and mass measurement were performed for the enamels before and after treatment. A layer of enamel-like fluoride substituted hydroxyapatite was observed on the surface of all the samples. After treatment by *n*-FA pastes with 3 different pH values (pH < 1, pH = 4.5 and pH = 7.5), the Vickers micro-hardness value was respectively changed to 125.9 HV, 252.1 HV and 304.9 HV from 241.3 HV of the artificial enamel erosion, and mass loss was 0.75 mg/mm², 0.41 mg/mm² and 0.30 mg/mm², respectively. SEM analysis showed that the surface of the enamels treated by *n*-FA pastes with pH 7.5 and pH 4.5 was smoother than those treated by *n*-FA pastes with pH < 1. These results suggested that the pH value had significant effects for the repairment of enamel erosion with *n*-FA pastes. This study demonstrated that the *n*-FA paste with neutral pH value (7.5) for enamel erosion repair would not only significantly enhance the enamel surface hardness, but also avoid the enamel mass loss and increased surface roughness.

Keywords: Enamel, Erosion, Nano Fluorapatite, pH Value, Self-Assembly.

1. INTRODUCTION

Enamel is an exterior surface of the teeth in humans. Although the chemical composition of enamel is similar to hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2(\text{HA})$, excellent mechanical properties, e.g., hardness and excellent wear resistance,^{1,2} caused by a highly organized micro-architectural unit (referred as enamel prisms) allow enamels to perform physiological³ function of chewing and protecting soft tissues in the pulp. However, enamel is easy to be destroyed by acid produced by oral bacteria (e.g., *Streptococcus mutans*). The molecular mechanism of dental caries is the occurrence of HA decalcification in the acidic conditions.

Clinically, the enamel lesions cannot be self-repaired, no matter how small the lesion is. Conventional treatment for excavating decay is refilling the defect with artificial materials, e.g., amalgam, ceramics, or polymer composites.^{3,4} However, secondary caries arises frequently

after conventional treatment.⁵ For the initial enamel lesion that was present as a white spot, it is not worth sacrificing much health enamel to make a hole for retention. After the initial report on the situ repair of the enamel lesions in 2005,⁶ several studies on acellular regenerations with different methods, e.g., immersion by supersaturated solution,^{7–12} plasma spray method,¹³ sol-gel method,¹⁴ electrodeposition¹⁵ and apatite application,^{6, 16–19} have been reported.

Among these methods, apatite application has attracted more and more interests because nano fluorapatite $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$ (*n*-FA) has excellent biocompatibility and bioactivity, and thus becomes an ideal material for regeneration of enamels.²⁰ Fluoride has been widely used to prevent dental caries because it not only improves the acid resistance of apatite crystals, but also inhibits bacterial metabolism.^{21,22} Furthermore, our previous studies showed that application of fluorapatite/phosphate acid pastes induced a fast growth of new apatite crystals within 15 minutes on human teeth.⁶ The new apatite layer that was estimated to be approximately 30 micrometers in

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thickness grew on the enamels seamlessly with their face parallel to the tooth surface, which is identical to the structure of enamel apatite.⁸ Moreover, 1% of fluoride produced stronger acid resistance than the controlled natural enamel.⁶ However, the extreme acidity of the paste may damage the surrounding soft and hard tissues, and the poor bio-security and low mechanical properties of newly formed apatite layer limited its clinical applications.

In order to identify suitable conditions to synthesize enamel-like apatite crystals on tooth enamel, we investigated the effects of pH values (extreme acidic pH < 1, pH 4.5 and pH 7.5, which is close to the pH value in physiological environment) on the self-assembly of *n*-FA into enamel-like crystals *in vitro*. The detailed preparation and characterization of new fluoride-containing HA on the enamel surfaces were described and a possible relationship between unique solubility of *n*-FA at different pH conditions and growth of new apatite crystals was proposed.

2. MATERIALS AND METHODS

2.1. Materials

The *n*-FA with a size of approximately 40 nm was synthesized in the laboratory. Briefly, solution A was made by adding 23.6 g analytical grade $\text{Ca}(\text{NO}_3)_2$ into 200 mL of analytical grade *N,N*-dimethylacetamide under stirring condition. Solution B was made by dissolving 0.74 g analytical grade NaF and 7.9 g $(\text{NH}_4)_2\text{HPO}_4$ in 2000 mL deionized water. Subsequently, solution A was slowly added into solution B in a condition of constant stirring, when solution B was heated to 70 °C. Afterwards, stirring was lasted for additional 4 hours at the temperature of 90 to 100 °C. The precipitate was separated by centrifugation and washed for three times after the suspension sat at room temperature for 24 hours. Finally, the *n*-FA precipitate was dried in the oven for 24 hours, and the powder samples were characterized by the transmission electron microscopy (TEM, TEM-1000, Japan) and X-ray diffraction (XRD, D/max-rA, Japan).

2.2. Methods

2.2.1. Measurement of Fluoride Release

In order to measure the solubility of *n*-FA, 0.1 g of *n*-FA powders were dispersed into a series of distilled water of 25 mL. The pH of the water was adjusted with HCl (1 mol/L) or NaOH (1 mol/L) to 4.5, 5.5, 6.5 and 7.5, respectively. The samples were then incubated in the water bath at 37 °C for 30 minutes. Subsequently, the suspensions were filtrated twice using a filter paper, and aliquots of 8 mL filtered fluid of each sample were mixed with 2 mL of total ionic strength adjustment buffer (TISAB, pH 5.23). The fluoride release was determined using a fluoride ion-specific electrode, which was pre-calibrated.

Each sample was measured twice, and each test was repeated for 3 times.

2.2.2. Preparation of Enamel Samples

In this study, 18 human premolars without visible evidence of caries were obtained from orthodontic treatment. The samples were stored in physiological solution after removal of their roots and pulps. In order to obtain uniform experimental area and flat surface, the samples were made into enamel blocks using a low speed diamond saw (SYJ-150, Shenyang Kejing, China), and the enamel surfaces of each specimen was mechanically polished using silicon carbide papers. Then initial enamel lesions were produced by immersing the samples into 17% phosphoric acid (H_3PO_4) for 1 minute. The samples were then randomly divided into three groups (each group contained 6 samples) for treatment with different pH of *n*-FA pastes.

2.2.3. Preparation of the Pastes and Treatment

The *n*-FA pastes were prepared by mixing 0.75 g *n*-FA with 1 mL 17% H_3PO_4 solutions at three different pH conditions (pH < 1, extreme acidic environment, pH = 4.5, and pH = 7.5, which is close to the pH value in physiological environment). The pH of 17% H_3PO_4 was lower than 1, but the precise pH could not be measured. To obtain pH 4.5 and pH 7.5 paste, 17% H_3PO_4 solutions were adjusted with 5 M NaOH solution before mixing with *n*-FA powder.

Firstly, 17% H_3PO_4 solution was brushed on enamel samples. Subsequently, *n*-FA pastes with different pH were applied on the sample surfaces before the acidic solution was dried. The repairing process was lasted for 15 minutes at 37 °C. In order to remove the residue pastes on the enamel surfaces, all of the samples were washed with distilled water and treated with the ultra sonication for 20 minutes. The schematic representation of the sample treatment was illustrated in Figure 1.

2.2.4. Characterization of the Enamel Samples Before and After Treatment with *n*-FA Paste

The enamel samples were cut in half. The cross-section was mechanically polished using silicon carbide papers of 2000 grits and was slightly etched by acid. The morphologies of the surface and cross-section were observed by using a high-resolution field emission scanning electron microscope (SEM, FEI NanoSEM 430). Crystal structures of the sample surfaces were characterized and analyzed by thin film X-ray diffractometer (XRD, X'pert PRO, Panalytical, Netherlands). Elements on the sample surfaces were determined by X-ray photoelectron spectroscopy (XPS Axis Ultra made in Kratos Analytical Ltd.). The hardness of the specimen surfaces was measured using a Vickers micro-hardness tester (Shanghai Materials Tester Machine

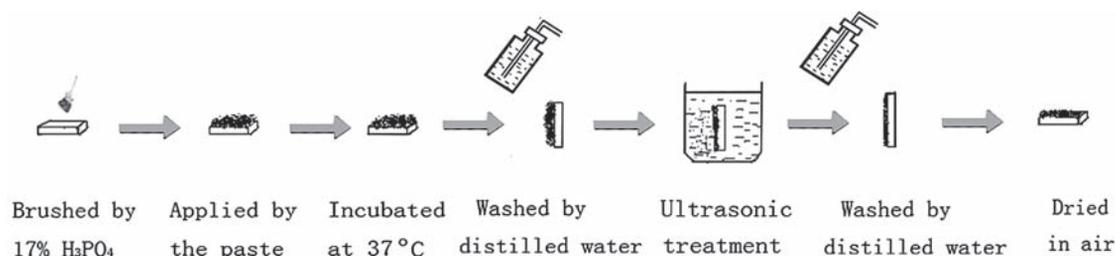


Fig. 1. Schematic representation for the treatment of the enamel with *n*-FA pastes at different pH values.

Co., China). In order to avoid the lag effect, each sample was subjected to at least six measurements under the same condition. One-way ANOVA was performed to compare the micro-hardness of the samples treated by the *n*-FA pastes at different pH conditions. The mass loss per unit area of the samples treated by different pH of *n*-FA pastes was determined using electronic balance.

3. RESULTS

3.1. Characterization of *n*-FA by TEM and XRD

TEM analysis showed that the synthetic nano fluorapatite particles exhibited a rod-like shape with a size of approximately 10 nm in diameter and 40 nm in length (Fig. 2(a)). XRD analysis (Fig. 2(b)) showed that the main peaks of *n*-FA particles at $2\theta = 25.9^\circ, 31.9^\circ, 40^\circ, 47^\circ$ and 50° were ascribed to apatite, indicating that the synthetic *n*-FA had a structure similar to apatite. The intense sharp peaks at 002 and 004 reflections indicated that the synthetic FA particles had high crystallinity.

3.2. Release of F⁻ ion from *n*-FA into Solutions

The release of F⁻ ion into solution at different pH conditions was determined after *n*-FA was dissolved. The results showed that the percentage of F⁻ ion in the solution with a pH of 4.5, 5.5, 6.5 and 7.5 was 0.074 ppm, 0.031 ppm, 0.022 ppm and 0.076 ppm, respectively (Fig. 3). These results indicated that *n*-FA had a relatively high solubility in both weak acidic and neutral conditions.

3.3. Formation of FA Layer on the Surface of Enamel

SEM analysis showed that a coating layer was formed on the surface of enamels of all the 3 groups after 15 min of treatment with *n*-FA pastes (Fig. 4). On the micrometer's scale, the surface of enamels treated with extreme acidic *n*-FA pastes (pH < 1) (Fig. 4(a)) was much rougher than that treated with *n*-FA with a pH of 4.5 (Fig. 4(c)) and 7.5 (Fig. 4(e)). In addition, honeycomb-like structures were observed on the surface of enamels treated with extreme acidic *n*-FA pastes (Fig. 4(a)). On the nanometer's scale, the newly formed crystals tended to be aligned in parallel to each other and were arranged into ordered needle-like

structures that were similar to those of enamel prisms in human enamels (Figs. 4(b), (d) and (f)). Figure 5 showed the SEM images of a longitudinal and polished section of the repaired tooth. In order to reveal the underlying structure, a slight acidic etching was applied. There were no boundaries between the repaired layer and the enamel substrate in all the 3 groups. The thickness of the newly formed layers was estimated to be approximately 30 micrometers (boundary is shown by arrows) according to the slight

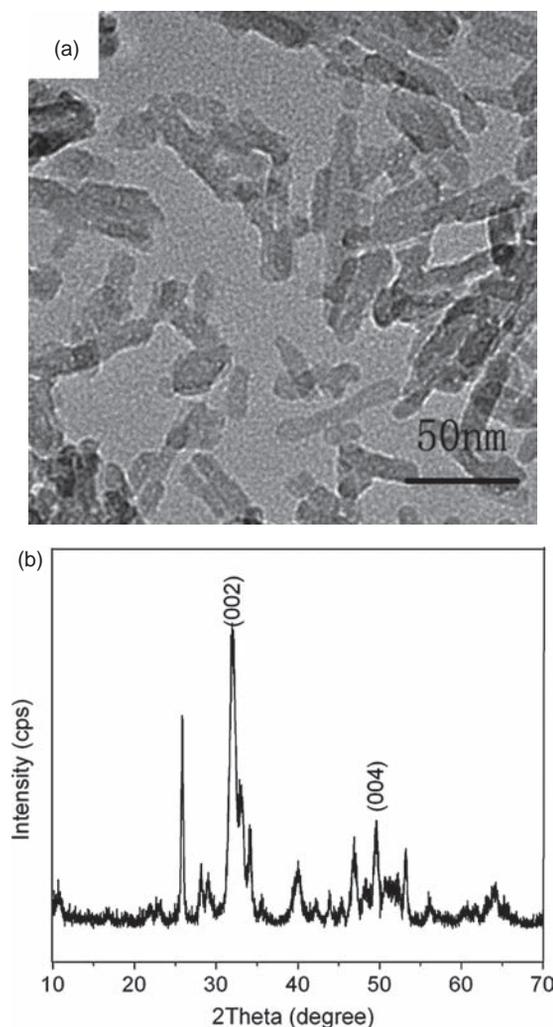


Fig. 2. TEM image (a) and XRD spectrum (b) of *n*-FA.

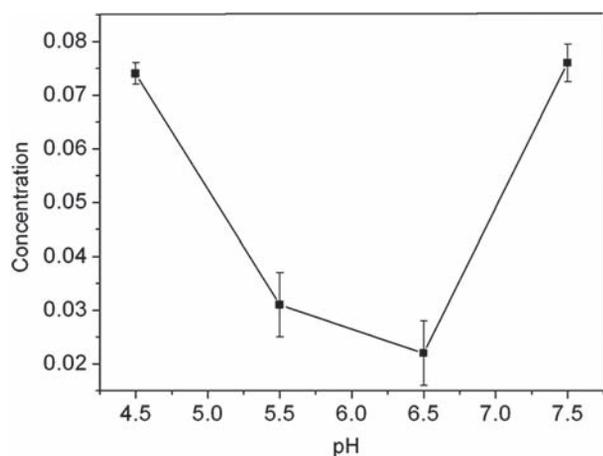


Fig. 3. Changes of F⁻ ion concentration of the solution at different pH value after *n*-FA soaking.

difference in orientation between the repaired layer and enamel. In contrast to the groups of enamels treated with *n*-FA at pH of 4.5 (Fig. 5(b)) and pH of 7.5 (Fig. 5(c)), the outlines of single enamel prime were much clearer in the groups treated with extreme acidic *n*-FA paste (pH < 1 (Fig. 5(a))).

3.4. Characterization and Chemical Analysis of New Crystal Layers

XPS analysis showed that the surface layers of all the 3 groups treated with *n*-FA at different pH conditions (pH < 1, pH = 4.5 and pH = 7.5) contained Ca²⁺, P⁵⁺ and small amount of F⁻ ions (Figs. 6(b)–(d)) that were nearly identical to those in the enamel part (Fig. 6(a)). The ratio between F⁻ ion and Ca²⁺ ion in the newly formed layers of enamels treated with 3 different pH of *n*-FA was 2.84%, 2.41%, 2.27%, respectively. These ratios were significantly higher than that in the natural enamel (0.24%). Figure 7 showed the XRD patterns of the dental enamel surfaces before and after *n*-FA treatment under different pH conditions (pH < 1, pH = 4.5 and pH = 7.5). All the treated or untreated samples contained apatite with similar crystal structures and lattice constants. The crystallinity of the samples was increased as the pH was increased and the intense sharp peaked at 002 and 004 reflections (Fig. 7(d)), indicating that the crystallinity of fluoride apatite was higher than that of hydroxyapatite (Fig. 7(a)).

3.5. The Effect of pH on the Vickers Micro-Hardness of the New Layer

The Vickers micro-hardness of the enamel surfaces was 241.3 ± 34.2 HV, 259.8 ± 13.4 HV and 271.8 ± 13.4 HV after acid etching for artificially-induced enamel erosion (Fig. 8). The Vickers micro-hardness was changed to 125.9 ± 37.5 HV, 252.1 ± 42.4 HV and 304.9 ± 28.6 HV, respectively, after treatment with *n*-FA at pH < 1,

pH = 4.5 and pH = 7.5 (Fig. 8). These results indicated that the pH of the solution had significant effects on the Vickers micro-hardness of the samples treated by the *n*-FA pastes. One-way ANOVA was performed to compare the micro-hardness of the samples treated by the *n*-FA pastes at different pH conditions, which showed a significant difference between any two groups ($p < 0.05$).

3.6. The Effect of pH on the Mass Loss of Enamel

The mass loss per unit area of human dental enamel after treatment with *n*-FA paste at different pH conditions was summarized in Figure 9. The results showed that the mass loss per unit area of all the samples was 0.75 ± 0.12 mg/mm², 0.41 ± 0.03 mg/mm², 0.30 ± 0.03 mg/mm², respectively, after treatments by *n*-FA paste at pH < 1, pH = 4.5 and pH = 7.5. Statistical analysis showed that the mass loss of the sample treated by *n*-FA paste at pH < 1 was significantly higher than that treated by *n*-FA at pH = 4.5 and pH = 7.5. There was no statistical difference between the mass loss of sample treated by *n*-FA at pH = 4.5 and that treated by *n*-FA at pH = 7.5.

4. DISCUSSIONS

Synthetic apatite was commonly used to repair the enamel defects in dental researches. However, these chemically analogues of enamel have not been widely applied in clinic practices. The native structure of enamel is too complex to be remodeled and the synthesized apatite crystallites are quite different from the native structures, which results in poor adhesion during the restoration. In order to further repair the carious lesion, we synthesized *n*-FA, which could be directly applied onto the enamel surfaces to repair the damaged enamels. Our results showed that the FA cement crystals could be formed on the surfaces of human teeth with tight contact to the enamel (*n*-FA closely bond to the enamel apatite). These results indicated that *n*-FA paste under different pH values played a significant role in the repair of enamel in terms of the improvement in micro-hardness, surface structures in micrometer's scale and mass loss. However, obvious differences in crystals' structure and chemical composition were not observed. The important finding in this study was that neutral *n*-FA paste (pH = 7.5) could not only be self-assembled into enamel-like crystals, but also significantly reduced the mass loss and micro-hardness of enamel. In addition, neutral *n*-FA can keep the enamel surface smoother, in comparison to the acidic paste (pH < 1). The expectation that neutral *n*-FA could repair the physical defect, improve the mechanical hardness and ensure the security of clinical application has been achieved.

When *n*-FA is applied on the enamel surfaces, two reactions occur, which are the dissolution/crystallization and self-assembly of apatite.^{6, 17, 18, 23} Previous studies emphasized the importance of extreme acidic condition during

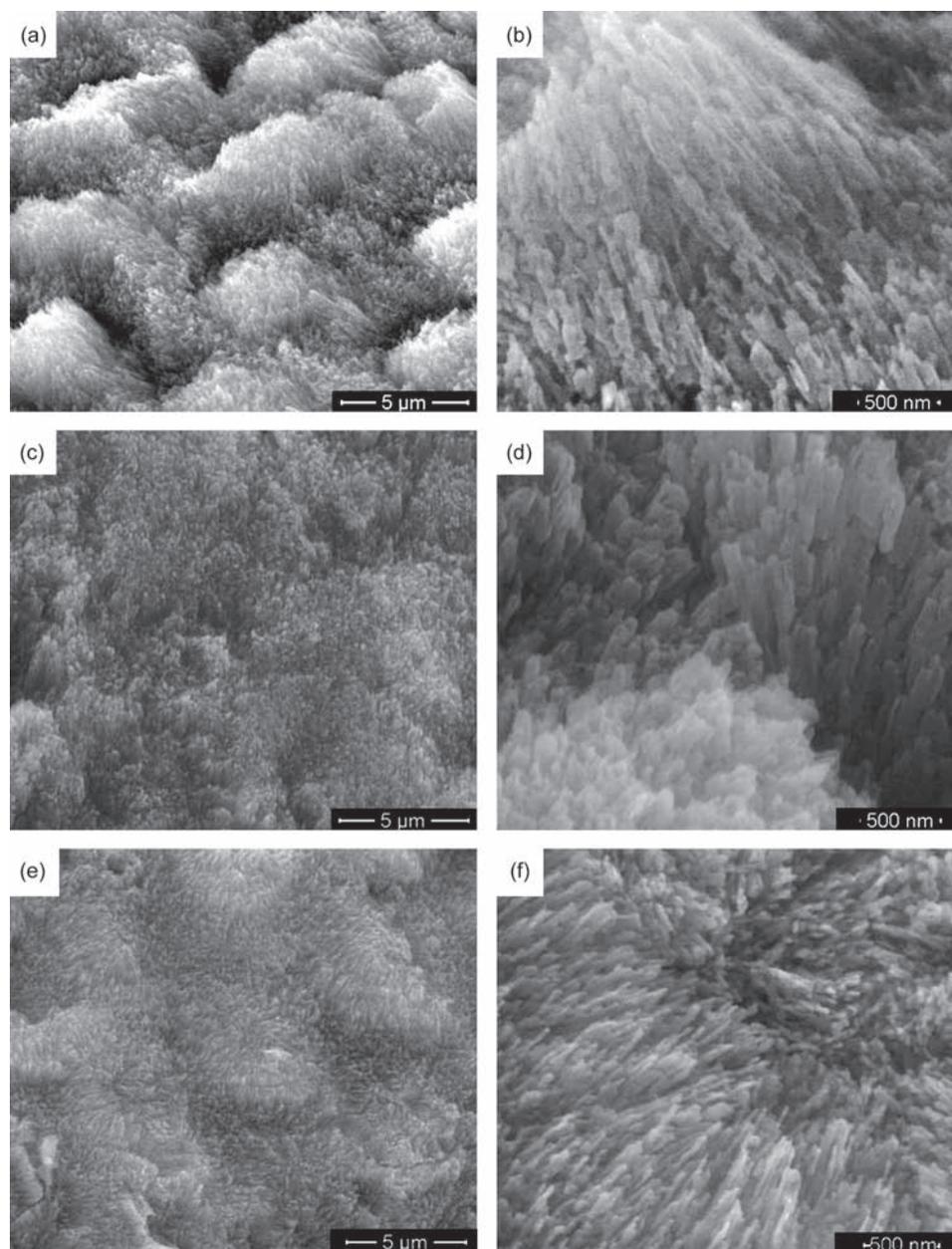


Fig. 4. SEM images of enamel surfaces treated by *n*-FA pastes under different conditions: pH < 1 (a), (b), pH = 4.5 (c), (d) and pH = 7.5 (e), (f).

the growth of new apatite crystals on the enamel surfaces, and proposed that the major apatite growth unit was the cluster of calcium deficient apatite $\text{Ca}_9(\text{PO}_4)_6$ dissolving from FA in the dense paste. When these apatite clusters are diffused toward the interface in the dilute acidic mother solution, they are dissolved into ionic species around the interface, leading to the growth of large high-crystalline apatite crystals on enamel apatite. In other words, new apatite crystals' growth was a process of dissolution/crystallization of *n*-FA in the solution.

We speculated that the water solubility of *n*-FA were the key factors affecting the nucleation and crystal growth. The fluoride release experiments showed that a lot of

F^- ions were released from *n*-FA into solutions under both pH 4.5 and pH 7.5 conditions. Because the crystals structure and chemical composition of *n*-FA was similar to those of HA, *n*-FA had the analogous physical and chemical properties to *n*-HA.^{24–26} it was reasonable to assume that *n*-FA had much higher solubility or dissociation degree under neutral condition. When ionic species reached super-saturation, nucleation and crystallization occurred on the enamel surface.

It should be pointed out that the formation of new apatite crystals on the enamel surfaces cannot completely rely on the dissolution/crystallization of *n*-FA in the dense paste. To obtain the lowest free energy, the short

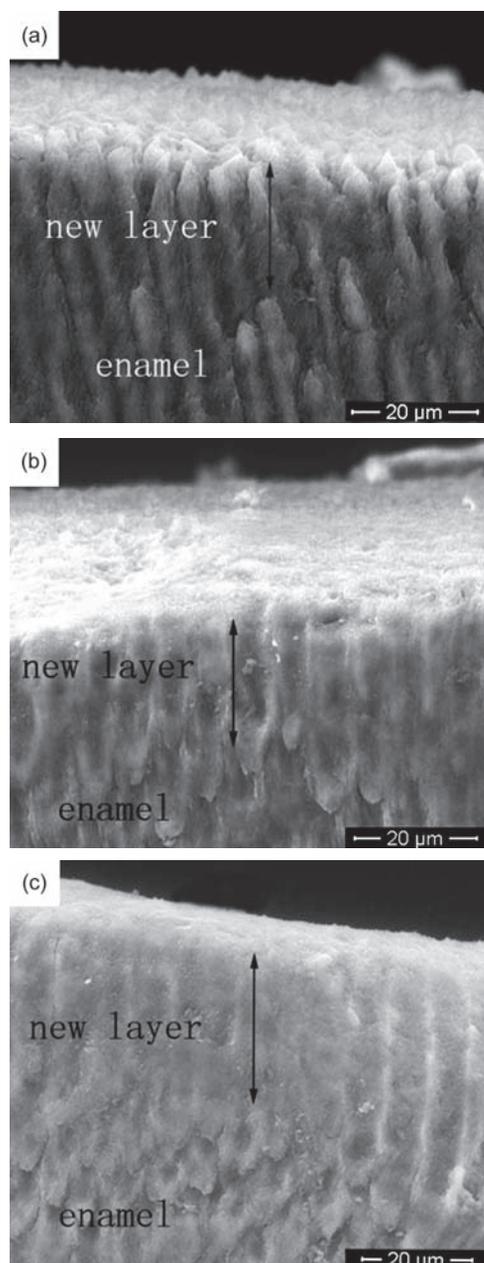


Fig. 5. SEM images for the cross-section of enamel treated by the *n*-FA pastes under different conditions, (a): pH < 1, (b): pH = 4.5 and (c): pH = 7.5.

rod-like synthetic *n*-FA was self-assembled into enamel-like crystals spontaneously due to the internal driving force.^{23, 27–29} During the process of solvent evaporation, the capillary force was the main force to bring the crystals together. Because of the rod-like structure of the FA, the capillary force along the long axis of the rods was much stronger than that at the rod ends, leading to the alignment of the nanorods in parallel to each other and arrangement of ordered needle-like structures similar to those enamel prisms in human enamel. It might be easier for the shorter rods to form a more densely packed structure with rods

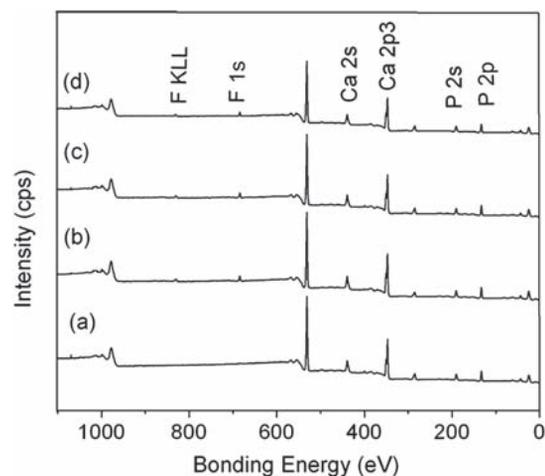


Fig. 6. XPS of dental enamel surfaces before (a) and after treatment with *n*-FA pastes at different pH conditions: pH < 1 (b), pH = 4.5 (c) and pH = 7.5 (d).

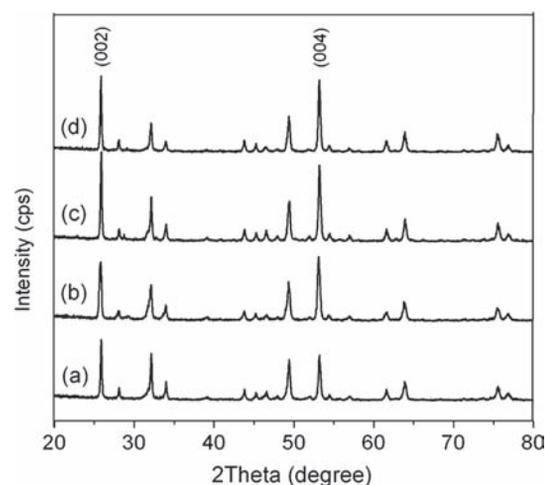


Fig. 7. XRD of dental enamel surfaces before (a) and after treatment with *n*-FA pastes at different pH conditions: pH < 1 (b), pH = 4.5 (c) and pH = 7.5 (d).

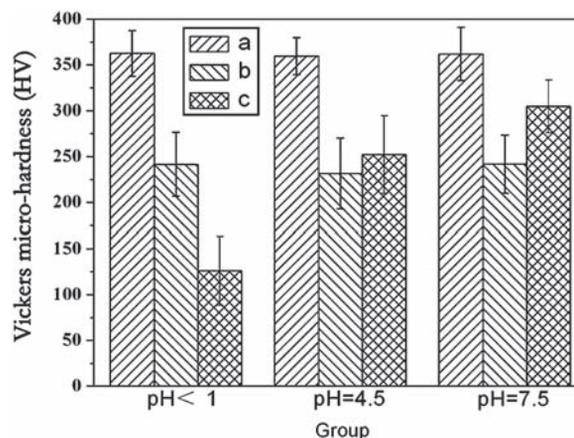


Fig. 8. Vickers micro-hardness of the enamel surfaces before and after treatments with the *n*-FA pastes at different pH conditions, (a): before acid pretreatment, (b): after acid pretreatment, and (c): after formation of new layers.

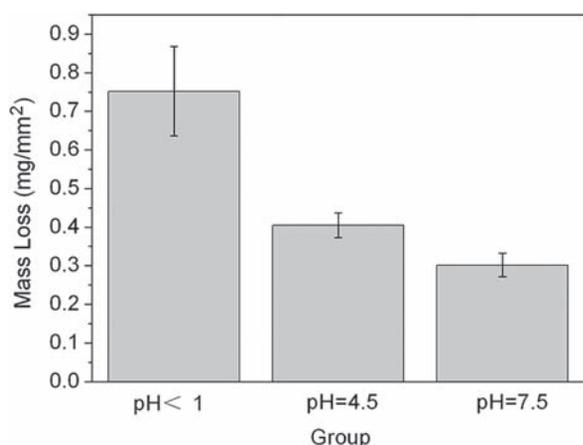


Fig. 9. Mass loss per unit area of enamel after treatment with *n*-FA pastes at different pH conditions.

aligned end-to-end. However, once the apatite crystals are aggregated together, the forces that maintain the structure are mainly from the van der Waals attraction and hydrophobic forces. The ordered prism-like structure might be the most stable structure for the rod-like crystals because the hydrophobic force would only allow the formation of such highly compacted aggregates on the water surface.

It should also be noted that the synthetic apatite crystals are much shorter than those enamel crystals. The short crystals appear to be easier to form compact enamel prism-like structures. Therefore, it is possible that the enamel crystals form enamel prism-like structure in the early stages of enamel development, while all the crystals are small and then become longer either by crystal re-growth and/or fusion of multiple small crystals.^{30–34}

In addition, our results demonstrated that application of phosphoric acid and acidic *n*-FA paste to the tooth enamel surface would cause the dissolution of enamel layer, which directly resulted in the loss of mass and reduction of micro-hardness. In contrast, the neutral *n*-FA (pH = 7.5) could result in the re-precipitation of apatite onto the enamel surface and avoided damage to enamels. Furthermore, new apatite crystal layer had the potential better acid resistance, in other words, better anti-caries capability than the natural enamel, due to their higher content of fluoride (over 2%) than that in natural enamels (0 to 0.59%). The positive correlation between fluoride content and anti-acid dissolution has been demonstrated in previous studies.

The results in this study opened up new possibilities for the remodeling of complex biological minerals *in vitro*, as well as the repair of enamel defects by taking the advantages of lower solubility of nano fluorapatite in acidic environment. This study demonstrated the potential of applying *n*-FA to repair human enamel defects.

5. CONCLUSIONS

In summary, this study demonstrated that *n*-FA paste at pH 4.5 could be self-assembled into enamel-like crystals

on artificial caries within 15 minutes. The FA coatings bond the substrate tightly and displayed a prism-like structure. The Vickers micro-hardness of the enamel-like crystals was approached to that of the normal enamel. Adjusting the pH of the paste to 7.5 was efficient for the rapid repair of early caries. In addition, neutral pH of *n*-FA facilitates the morphological reconstruction, improves the micro-hardness and ensures the security of its clinical applications. Moreover, neutral pH can avoid acid-etching on enamels caused by extreme acidic condition. The new crystal layers have potential excellent acid-resistance, which was also referred as caries-resistance in clinical applications. Therefore, *n*-FA could be considered as a promising novel material to achieve enamel regeneration.

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