Development of fluorapatite cement for dental enamel defects repair

**Jie Wei · Jiecheng Wang · Wenpeng Shan ·
Xiaochen Liu · Jian Ma · Changsheng Liu ·
Jing Fang · Shicheng Wei**

Abstract In order to restore the badly carious lesion of human dental enamel, a crystalline paste of fluoride substituted apatite cement was synthesized by using the mixture of tetracalcium phosphate (TTCP), dicalcium phosphate anhydrous (DCPA) and ammonium fluoride. The apatite cement paste could be directly filled into the enamel defects (cavities) to repair damaged dental enamel. The results indicated that the hardened cement was fluorapatite [$Ca_{10}(PO_4)_6F_2$, FA] with calcium to phosphorus atom molar ratio (Ca/P) of 1.67 and Ca/F ratio of 5. The solubility of FA cement in Tris–HCl solution (pH = 5) was slightly lower than the natural enamel, indicating the FA cement was much insensitive to the weakly acidic solutions. The FA cement was tightly combined with the enamel surface, and there was no obvious difference of the hardness between the FA cement and natural enamel. The extracts of FA cement caused no cytotoxicity on L929 cells, which satisfied the relevant criterion on dental biomaterials, revealing good cytocompatibility. In addition, the results showed that the FA cement had good mechanical strength, hydrophilicity, and anti-bacterial adhesion properties. The study suggested that using FA cement was simple and promising approach to effectively and conveniently restore enamel defects.

1 Introduction

Dental enamel is the outmost layer of teeth and the hardest mineralized tissue in the human body [1]. Unlike other calcified tissues, such as dentin and bone, there are no living cells in mature enamel [2]. After make enamel, the ameloblasts cells are no longer present when enamel is formed [3]. Therefore, there are no cells to carry out the repair when the enamel is damaged. Demineralization of dental enamel will progress, leading to the deterioration of the composition and structure through the dental caries process when caused by acidogenic bacteria [4]. Dental caries is one of the most widespread and costly infectious diseases remaining to be overcome [5, 6].

The traditional treatment of dental caries (defects in enamel and the underlying dentin) involves mechanical removal of the affected part and refilling of the cavity with substitutes like amalgam, ceramics, or polymer composites to prevent tooth death [7, 8]. But the method is not ideal because a disproportionate amount of healthy tooth has to be removed to make the alloy or resin stick, and secondary caries frequently arises at the interfaces between the teeth and foreign materials. In previous studies, acellular regeneration of enamel included immergence by supersaturated solution [9, 10], plasma spray [11], sol–gel [12], electro-deposition [13], and apatite application [3, 5].

J. Wei · J. Wang · X. Liu · J. Fang (✉) · S. Wei (✉)
Center for Biomedical Materials and Tissue Engineering,
Academy for Advanced Inter-disciplinary Studies, Peking
University, Beijing 100871, People's Republic of China
e-mail: biomater2006@yahoo.com.cn

S. Wei
e-mail: weishicheng99@163.com

J. Wei · W. Shan · C. Liu
Key Laboratory for Ultrafine Materials of Ministry of Education,
East China University of Science and Technology,
Shanghai 200237, People's Republic of China

J. Ma
Hospital of Stomatology, Tongji University,
Shanghai 200072, People's Republic of China

S. Wei
School and Hospital of Stomatology, Peking University,
Beijing 100081, People's Republic of China

Among these techniques, apatite application has aroused much more interests, as fluorapatite (FA) is considered to be an ideal material for regeneration of enamels due to its excellent biocompatibility and bioactive [14]. It was proved that fluoride could not only improve the acid resistance of apatite crystals effectively but also a certain concentration of fluoride release from FA could inhabit metabolism of bacterial [15, 16]. Yamagishi et al. [17] reported a paste of fluoridated hydroxyapatite that could be used to repair early carious lesion. However, this may limit its application in restoration of the badly carious lesion.

The human tooth is protected by enamel that is composed of apatite crystals, acid-forming bacteria will cause microscopic damage of the enamel, creating cavities that are more than 50 μm deep [18, 19]. Such cavities cannot be repaired by the restorative materials (such as amalgam, ceramics, or polymer composites) because these materials do not adhere (bond) perfectly to the enamel surface owing to the difference in chemical composition and crystal structure. In order to further restoration of carious lesion, we have recently synthesized a white crystalline paste of fluorapatite (FA) using apatite cement and fluoride. We directly filled with FA cement pastes into the enamel defects (cavities) to repair damaged enamel (badly carious lesion).

2 Materials and methods

2.1 Preparation and characterization of FA cement

Fluorapatite (FA) cement consists of powders and cement liquid (water). The FA cement powders are composed of tetracalcium phosphate ($Ca_4(PO_4)_2O$, TTCP), dicalcium phosphate anhydrous ($CaHPO_4$, DCPA), and ammonium fluoride (NH_4F) in a molar ratio of 1:1:1. TTCP was synthesized by a solid-to-solid reaction between calcium phosphate and calcium carbonate at a temperature of 1,500°C for 8 h. Dicalcium phosphate dehydrate (DCPD, $CaHPO_4 \cdot 2H_2O$) was prepared from ammonium hydrogen phosphate [$(NH_4)_2HPO_4$] and calcium nitrate [$Ca(NO_3)_2$] in the acidic environment. DCPA was obtained by removing the crystallization water in DCPD at 120°C for 5 h [20]. The mixed powders of TTCP and DCPA were grounded in a planetary ball mill for 30 min, followed by sieving through 140 meshes.

The FA cement powder was prepared by adding the NH_4F powder into the mixed powders of TTCP and DCPA. The FA cement paste was prepared by mixing the FA cement powder and water with different ratio of powder to liquid (P/L). The FA cement powders reacted with water to form cement pastes. The FA cement pastes were placed into stainless steel molds with the size of Φ 10 × 2 mm.

After stored in the beakers in a constant temperature oven at 37°C and 100% relative humidity for 2 days, the hardened FA cement samples were obtained. The hardened FA cement samples were characterized by X-ray diffraction (XRD), and Fourier transform-infrared spectroscopy (FT-IR).

2.2 Solubility of FA cement

The solubility of the FA cement was characterized by the weight loss ratio in Tris–HCl solution at different time, and teeth samples were used as controls. After setting for 2 days and dried at 60°C for 6 h, the FA cement samples (Φ 10 × 2 mm) with initial weight W_0, were put into 500 ml of Tris–HCl solution (pH = 5, adjusted by diluted HCl) with a weight-to-volume ratio of 0.3 g ml^{-1}. The solution was continuously shaken in a water bath at 37°C. At different time point, the FA cement samples were removed from the Tris–HCl solution, cleaned with water, dried at 60°C for 6 h and its new weight W_t was recorded. It was then re-immersed into a fresh Tris–HCl solution at the same weight -to-volume ratio followed by continuous shaking. The weight loss ratio of the FA cement samples at different time was calculated according to the Eq.:

$$\text{Weight loss ratio } (\%) = (W_0 - W_t)/W_0 \times 100$$

Three samples of each kind of FA cement were tested and the average value was recorded.

2.3 Cytotoxicity of FA cement

L929 cells were used to test the cytotoxicity of FA cement, which was carried out by using FA cement extracts in contact with L929 cells according to International Standard Organization (ISO/EN 10993). To prepare the FA cement extracts, a stock solution of 200 mg ml^{-1} was first prepared by adding 5 g hardened FA cement (after setting for 2 days and dried at 60°C for 6 h) into DMEM culture medium. After incubation at 37°C for 24 h, the mixture was centrifuged and the supernatant was collected. The serial diluted extracts (200, 100, 50, and 25 mg ml^{-1}) were prepared by diluting the stock solution with serum-free DMEM. Subsequently, these extracts were sterilized by filtration through 0.2 μm filter membrane for cell cultured experiments.

The cells were seeded on a 96-well plate with the density of 3×10^4 cells/well and cultured for 24 h. Then, the culture medium was removed and replaced by 50 μl of extracts and 50 μl of DMEM medium supplemented with 20% FCS. The DMEM without extract supplemented with 10% FCS was used as a blank control. After incubation for 24 h, MTT test was carried out to determine the cell viability. In brief, 100 ml of 0.5 mg ml^{-1} 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl

tetrazolium bromide (MTT) solution was added into each well. After additional incubation for 4 h, dimethyl sulfoxide (DMSO) was added to stop the reaction between MTT and cells. The optical density (OD) value was measured at the wavelength of 490 nm using an enzyme linked immuno-adsorbent assay plate reader.

2.4 Repair of enamel defects

Human teeth with big enamel defects (cavities) were etched with 17 wt% phosphate acid for about 30 min, then, the FA cement pastes were filled into the enamel defects immediately before the acid solution dried. After the teeth samples with enamel defects were filled with the FA cement pastes, the as-prepared teeth samples were stored in beakers in a constant temperature oven at 37°C and 100% relative humidity (r.h.) for 2 days. The teeth samples with FA cement repair the defects were sectioned perpendicular to the dental crown using a diamond saw at interface between the enamel and FA cement. The surface morphology and microstructure of the enamel and FA cement samples were examined with scanning electron microscope (SEM).The hardness and elastic modulus of the enamel defects repaired with FA cement were determined by nanoindentation. The compressive strength of the enamel defects repaired with FA cement was measured using a universal testing machine (AG-2000A, Shimadzu Autograph, Shimadzu Co. Ltd., Japan). In addition, the water contact angle of the hardened FA cement samples was measured using a sessile drop method at room temperature with the contact angle equipment (DSA 100, KRUSS, Germany). In this study, the natural enamel samples were used as controls.

2.5 Bacterial adhesion test

Escherichia coli strain was used to test the bacterial adherence on FA cement samples (natural enamel samples as controls). The *E. coli* strain was cultured at 37°C overnight in Trypto-Soy broth (TSB). The mixture was diluted at a ratio of 1:1,000 in TSB with 0.25% glucose, and 1 ml of the bacterial cell suspension (10^5 CFU) was inoculated into 24-well tissue culture plates, and the FA cement samples were placed in the wells. After cultivation for 24 h, the samples were rinsed twice with 2 ml phosphate buffered saline (PBS) to eliminate the non-adherent bacteria. After rinsing, the samples were transferred into new tubes with 10 ml PBS, and were then ultrasonically washed with water for 5 min to remove adherent bacteria. The number of viable bacteria in the solution was counted using the cultural method and Pearlcore *Staphylococcus* medium.

3 Results

3.1 XRD analysis

In order to further confirm the phase composition and crystallinity of the synthetic FA cement, the powder XRD pattern analysis was carried out. The XRD pattern of FA cement after hardening for 2 days is shown in Fig. 1. It was found that the main detected peaks at $2\theta = 25.8$, 28.1, 29.2, 31.9, 32.6, 34.1, 40.2, 46.6, 49.7, and 53.2° were ascribed to apatite. The results indicated that the hardened FA cement was apatite structure.

3.2 IR analysis

The IR spectrum of the hardened FA cement is shown in Fig. 2. The absorption bands at 1,071 and 951 cm^{-1} were ascribed to PO_4^{3-} (phosphate group). The two specific
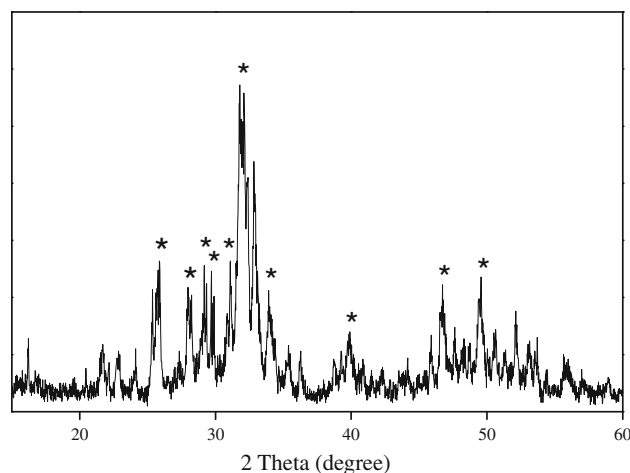

Fig. 1 XRD pattern of FA cement after hardening for 2 days, *asterick* represents FA peaks
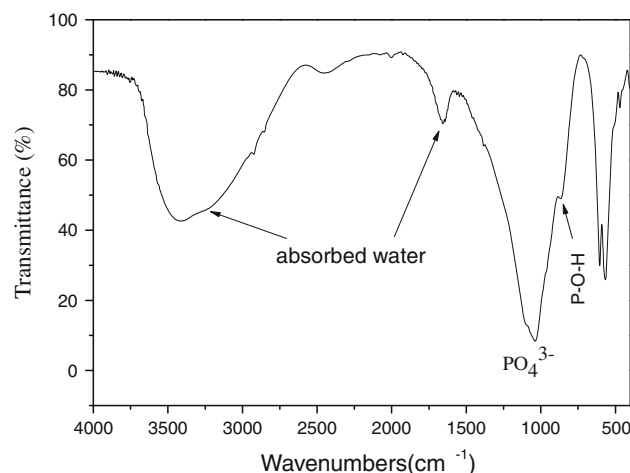

Fig. 2 IR pattern of FA cement after hardening for 2 days

peaks at 1,431 and 1,752 cm^{-1} and the broad band from 2,800 to 3,473 cm^{-1} were attributed to the absorbed water. The band at 879 cm^{-1} might correspond to the vibration of P–O–H from PO_4^{3-}. There was no -OH group specific peaks at 1,571 and 631 cm^{-1}, indicating that no hydroxyapatite appeared in the finally hardened FA cement products. -OH group specific peaks might be replaced by F group to form FA. The results of the IR were in accordance with the XRD of FA cement.

3.3 Elemental composition

The elemental composition of the FA cement was further characterized by EDS. The EDS spectrum (Fig. 3) showed that the FA cement contained Ca, P, and F elements. Moreover, the EDS results also revealed that the FA cement had an average Ca/P atom molar ratio of 1.67, which was in agreement with the theoretical ratio of apatite (Ca/P = 1.67). The Ca/F atom mol ratio of the FA cement was five, indicating that the amount of F ions in the FA cement was similar to fluorapatite (FA, $Ca_{10}(PO_4)_6F_2$). Combined with the XRD, IR, and EDS results, it can be suggested that the produced FA cement was fluorapatite (FA).

3.4 Solubility of FA cement

The solubility of the FA cement in Tris–HCl solution was characterized by the weight loss ratio. Fig. 4 showed the weight loss ratio of the FA cement and natural enamel samples immersing in the acidic Tris–HCl solutions for various time periods. It was found that the weight loss ratio of the natural enamel was slight higher than FA cement. At 15 days, the weight loss ratio of the natural enamel was around 1.2 wt% while FA cement was 0.75 wt%, indicating the dissolution of FA cement was slightly lower than
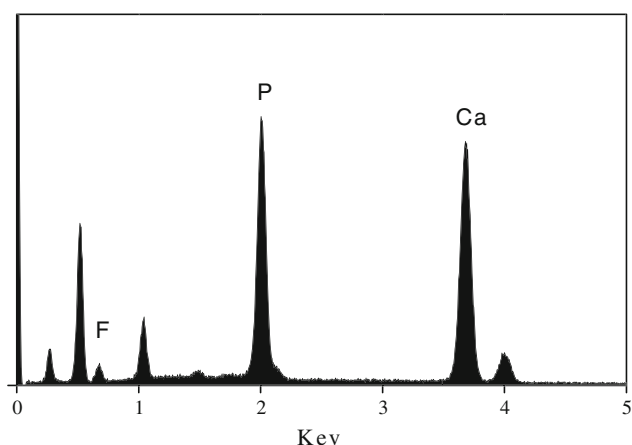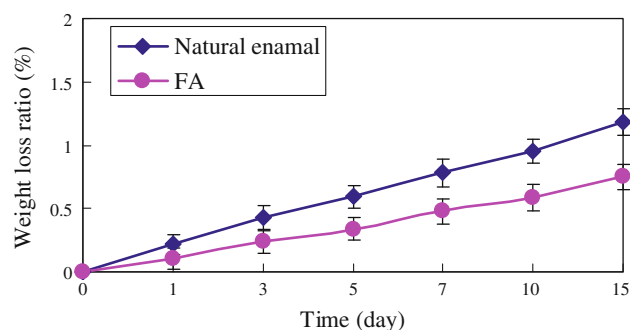

Fig. 4 Weight loss ratio of FA cement and natural enamel samples after immersing in Tris–HCl solution with time

the natural enamel. The results suggested that the FA cement was much insensitive to the acidic solutions (pH = 5) than the natural enamel.

3.5 Cytotoxicity of FA cement

The effects of the extracts of FA cement with different concentrations on L929 cells are shown in Fig. 5. It could be seen that, in a broad extract concentration range (25–200 mg ml^{-1}), cell viability all exceeded that of blank control. The results showed that the extracts of FA cement caused no cytotoxicity on L929 cells that satisfied the relevant criterion on dental biomaterials, indicating FA cement had good cytocompatibility.

3.6 Repair of enamel defect

The photos of the human dental enamel defects filled with the FA cement pastes are shown in Fig. 6. Figure 7 exhibited the distinct interface morphologies of the dense FA cement and enamel by SEM examination. The microstructure of the restored enamel revealed no obvious structural gap at the interface between the FA cement and the enamel region. This showed that the newly formed FA cement had properly integrated with the teeth enamel (FA tightly bond with the enamel surface without interstice).
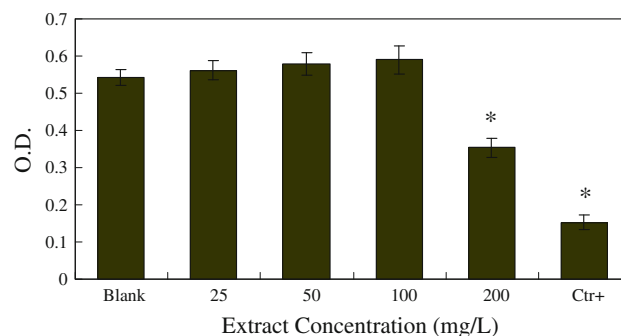

Fig. 5 Effects of the extracts of FA cement with different concentrations on L929 cells after culture for 24 h. The experimental group compared with blank control group, $P < 0.05$

Fig. 3 EDS pattern of the FA cement after hardening for 2 days

Fig. 6 Photos of the human dental enamel defect before (**a**) and after (**b**) filled with FA cement
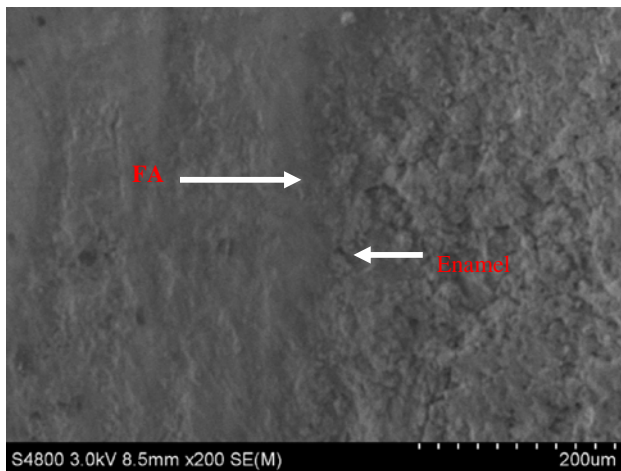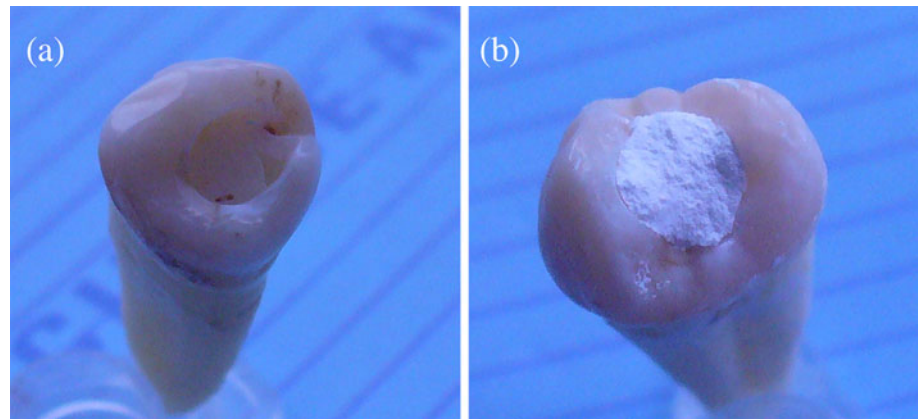

Fig. 7 SEM images of the transversely section of the FA cement applied to human dental enamel defects (enamel cavity). The *arrows* point the interface between the FA cement (*left*) and the enamel region (*right*)

3.7 Morphology and microstructure of FA cement

The morphology and microstructure of FA cement filled into the enamel defects after hardening for 2 days were examined by SEM as shown in Fig. 8. It was found that the dense FA cement layer on the tooth enamel surface exhibited the homogeneous apatite crystals as shown in Fig. 8a. A higher magnification examination showed a conspicuous enamel prism-like structure on the tooth surface composed of bundles of crystals as shown in Fig. 8b. The rod-like crystals have a typical apatite hexagonal cross section of approximately 400 nm in length and 50 nm in diameter.

3.8 Mechanical strength and bacterial adhesion

The mechanical strength of elastic modulus and hardness of the FA cement were examined by using nanoindentation, which was a key parameter of the restored enamel defects
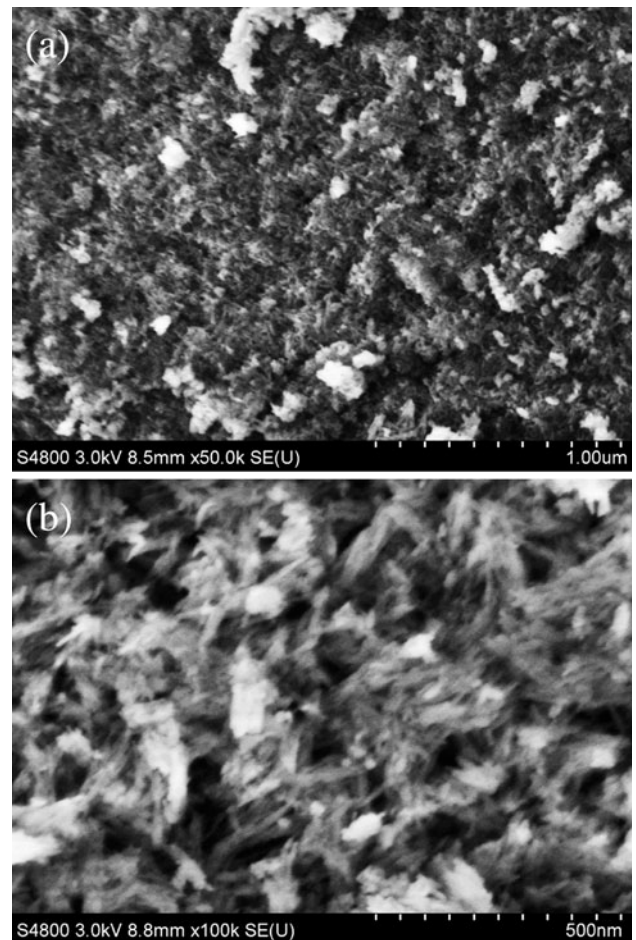

Fig. 8 SEM images of the morphology/microstructure of FA cement filled the defects of enamel after hardening for 2 days

as shown in Fig. 9. The measurement was performed by using FA cement filled the defects of enamel. The results showed that the surface hardness and elastic modulus of the FA cement were 3.8 GPa and 87.1 GPa, respectively. On the other hand, the surface hardness and elastic modulus of the natural enamel were 4.1 and 92.6 GPa, respectively.
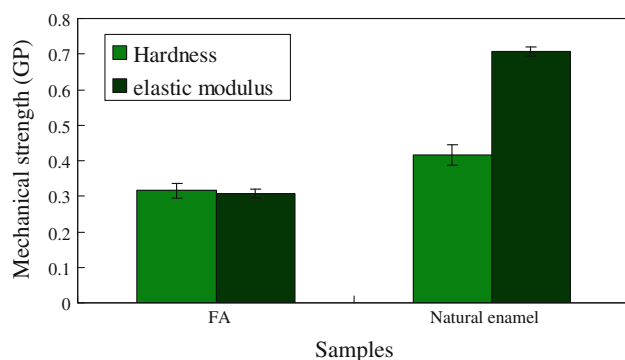
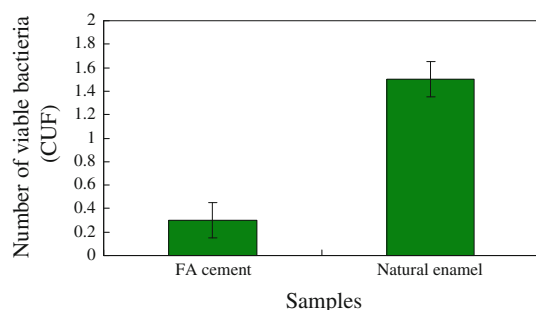**Fig. 9** Elastic modulus and hardness of FA cement (natural enamel as a control)

Fig. 10 Number of viable bacteria (*E. coli*) adherence on the FA cement (natural enamel as a control) after 24 h (10^7 CFU)
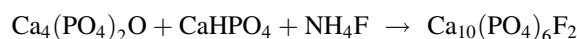
The compressive strength of the hardened FA cement was also examined; it was found that the compressive strength of the FA cement was of 112 MPa compared with the natural enamel of 117 MPa. The results indicated that the compressive strength of FA cements was almost close to those of the natural ones. In addition, the results showed that the water contact angles of the FA cement and natural enamel were 57 and 61°, respectively, indicated that FA cement surfaces had good hydrophilicity. Figure 10 showed the number of viable bacteria (*E. coli*) adherence on the surfaces of the FA cement and natural enamel samples, it was found that the number of viable bacteria on the FA cement was obviously lower than that on the natural enamel samples, indicating FA cement could inhibit bacteria attachment.

4 Discussions

Apatite bioceramic is always considered as a model compound of enamel due to the similarity in chemical composition [21, 22]. Therefore, the repair of enamel defects by using synthetic apatite was always suggested in dental research. Unfortunately, these chemically analogous compounds of enamel are not widely applied in clinic practices

[23]. The native structure of enamel is too complex to be remodelled and the synthesized apatite crystallites often have different from the natural ones, which result in poor adhesion and mechanical strength during the restoration [24].

In order to further repair the badly carious lesion, we have synthesized a white crystalline paste of fluorapatite (FA) cement, which could be directly filled into the enamel defects (cavities) to restore damaged enamel. It was found that the FA cement crystals could be formed on the surfaces of human teeth with tight contact to the enamel (FA closely bond to the enamel apatite). According to the analysis of XRD, IR, and EDS, the results indicated that the hardened product of FA cement paste was fluorapatite, which had an average Ca/P ratio of 1.67 and Ca/F ratio of five. The mixed powders of TTCP (basic) and DCPA (acidic) reacted with water to form cement paste that first changed into hydroxyapatite (acidic-basic neutralization reaction), then, hydroxyapatite reacted with ammonium fluoride to form fluorapatite (FA). Therefore, fluorapatite was formed based on the chemical reactions as follows:

$$Ca_4(PO_4)_2O + CaHPO_4 + NH_4F \rightarrow Ca_{10}(PO_4)_6F_2$$

The enamel cavities could be repaired by the setting of the FA cement because the restorative material could adhere perfectly to the enamel surface owing to the similarity of FA cement in chemical composition and crystal structure to enamel apatite.

Xiaokun Wang et al. [25] explored a method to repair caries of direct growth of human enamel-like structures on tooth using fluorapatite/phosphoric acid pastes. The results showed that the paste was fluoridated $CaHPO_4 \cdot 2H_2O$ (F-DCPD) with a Ca/P ratio of 1.0. However, F-DCPD had obviously high solubility than apatite, thus F-DCPD was not suitable for repair enamel defects. A study showed that the variation in the molar ratio of calcium to phosphate greatly affected the solubility of the phosphate; the lower the Ca/P ratio, the higher the solubility of calcium phosphate [26]. Others suggested that the solubility of apatite with Ca/P of 1.67 was significantly lower than that of 1.50 [27]. In this study, the dense FA cement used to repair enamel defects had the Ca/P ratio of 1.67, which was similar to hydroxyapatite with low solubility. In addition, many studies have shown that the solubility of fluorapatite was lower than hydroxyapatite owing to the incorporation of F ions into the apatite crystals [28].

The human tooth is protected by dental enamel consisting of apatite. Acid-forming bacteria will cause damage to the enamel, creating cavities in the enamel [29]. In this study, the solubility of the hardened FA cement in acidic environment (pH = 5) was determined by using Tris–HCl solution. The results revealed that the weight loss ratio of the FA cement was slightly lower than that of natural

enamel, indicating the FA cement was much insensitive to the acidic solutions than the natural enamel. It could be suggested that the underlying enamel surface would be well protected under the acidic condition if the FA cement covered on the enamel surface.

As a biomaterial used for repair dental defects, the biocompatibility is very important. Therefore, in this study, the cytotoxicity of the FA cement was determined by using the extracts of hardened FA cement with different concentrations on L929 cells. The results suggested that the extracts of FA cement caused no cytotoxicity on L929 cells, indicating good cytocompatibility, which was satisfied with the relevant criterion on dental biomaterials. Furthermore, the result showed that the newly-formed FA crystals were firmly bonded to the surface of natural enamel after the cement filled into the enamel cavities. According to the forming process, a possible mechanism could be proposed. Before the enamel defects (holes) were filled with the FA cement, the application of phosphoric acid to the tooth enamel surface would cause the dissolution of original enamel layer, which produced activated kinks, steps, and defects on the enamel surface. Once the FA cement pastes applied would lead to the reaction of FA cement with apatite of enamel layer. Therefore, the FA cement would be tightly combined with the enamel surface. This process will help repairing the damaged enamel. The human dental enamel defects were filled with the FA cement, and the microstructure of the restored enamel revealed no obvious structural gap at the interface between the FA cement and the enamel region. This showed that the newly-grown FA cements had properly integrated with the tooth enamel.

We have compared the mechanical properties of compressive strength, hardness, and elastic modulus of the hardened FA cement with natural enamel. The results indicated that the mechanical strength of FA cement was very close to those of the natural ones. FA cement had good hydrophilicity with the water contact angle almost similar to the natural enamel. In addition, the number of viable bacteria attached to the FA cement was evaluated using the bacterial adhesion test. The results showed that the FA cement greatly reduced *E. coli* adherence as compared with natural enamel, indicating the FA cement could inhibit *E. coli* attachment, which presumably depended on the F ions. As mature enamel is not living tissue, it is only scarcely repair after substantial mineral loss (caries). Currently, defects in enamel and the underlying dentin were usually refilled with unstructured substitutes like amalgam, ceramics, or polymer composites to prevent tooth death [30]. Our experimental results revealed a directly filled method to repair dental enamel defects using a cement technique. The results opened up new possibilities for the remodeling of complex biological minerals in vitro, as well as the repair of enamel defects with the advantage of lower solubility of fluorapatite cement in acidic environment. This study demonstrated the potential of applying FA cement to repair human enamel defects.

5 Conclusions

Fluorapatite cement with chemically and structurally resembles natural enamel was developed to repair the human dental enamel defects (carious lesions) by direct filling into enamel cavities. The simplicity of this repair approach has potential in dentistry that might find many novel applications in dental clinics as it offered a new method of repair teeth and provide with a material (FA) close to nature enamel than any restorative material (such as amalgam, ceramics, or polymer composites) used to date. Furthermore, the less soluble fluorapatite cement filling into enamel cavities should offer considerable protection against caries. The results showed that the FA cement had good mechanical strength, hydrophilicity and anti-bacterial adhesion properties. This study confirmed the possibility of applying FA cement pastes to repair caries of dental enamel. It is suggested that the FA cement could be used as a substitute for the conventional dental restorative materials to directly repair damaged tooth enamel.

References

1. Taji S, Seow WK. A literature review of dental erosion in children. Aust Dent J. 2010;55:358–67.
2. Chen HF, Tang ZY, Liu J, Sun K, Chang SR, Peters MC, et al. Acellular synthesis of a human enamel-like microstructure. Adv Mater. 2006;18:1846–51.
3. Chen HF, Clarkson BH, Sun K, Mansfield JF. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J Colloid Interface Sci. 2005;288:97–103.
4. Fowler CE, Li M, Mann S, Margolis HC. Influence of surfactant assembly on the formation of calcium phosphate materials-A model for dental enamel formation. J Mater Chem. 2005;15:3317–25.
5. Onuma K, Yamagishi K, Oyane A. Nucleation and growth of hydroxyapatite nanocrystals for nondestructive repair of early caries lesions. J Cryst Growth. 2005;282:199–207.
6. Shellis RP, Wilson RM. Apparent solubility distributions of hydroxyapatite and enamel apatite. J Colloid Interface Sci. 2004;278:325–32.
7. Wang LJ, Guan XY, Yin HY, Moradian-Oldak J, Nancollas GH. Mimicking the self-organized microstructure of tooth enamel. J Phys Chem C. 2008;112:5892–9.

8. Yin YJ, Yun S, Fang JS, Chen HF. Chemical regeneration of human tooth enamel under near-physiological conditions. Chem Commun. 2009;39:5892–4.

9. Li H, Huang WY, Zhang YM, Zhong M. Biomimetic synthesis of enamel-like hydroxyapatite on self-assembled monolayers. Mater Sci Eng C. 2007;27:756–61.

10. Fan Y, Sun Z, Moradian-Oldak J. Effect of fluoride on the morphology of calcium phosphate crystals grown on acid-etched human enamel. Caries Res. 2009;43:132–6.

11. Fogarassy P, Gerday D, Lodini A. Agglomerated nanostructured particles disintegration during the plasma thermal spraying process. Mech Res Commun. 2005;32:221–9.

12. Busch S, Schwarz U, Kniep R. Morphogenesis and structure of human teeth in relation to biomimetically grown fluorapatite-gelatine composites. Chem Mater. 2001;13:3260–71.

13. Liao YM, Feng ZD, Li SW. Preparation and characterization of hydroxyapatite coatings on human enamel by electrodeposition. Thin Solid Films. 2008;516:6145–50.

14. Agathopoulos S, Tulyaganov DU, Marques PAAP, Ferro MC, Fernandes MHV, Correia RN. The fluorapatite-anorthite system in biomedicine. Biomaterials. 2003;24:1317–31.

15. Wegehaupt FJ, Solt B, Sener B, Wiegand A, Schmidlin PR, Attin T. Influence of fluoride concentration and ethanol pre-treatment on the reduction of the acid susceptibility of enamel. Arch Oral Biol. 2009;54:823–9.

16. Nakajo K, Imazato S, Takahashi Y, Kiba W, Ebisu S, Takahashi N. Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci. Dent Mater. 2009;25:703–8.

17. Yamagishi K, Onuma K, Suzuki T, Okada F, Tagami J, Otsuki M, et al. A synthetic enamel for rapid tooth repair. Nature. 2005;433:819.

18. Fan Y, Sun Z, Moradian-Oldak J. Controlled remineralization of enamel in the presence of amelogenin and fluoride. Biomaterials. 2009;30:478–83.

19. Kirkham J, Firth A, Vernals D, Boden N, Robinson C, Shore RC, et al. Self-assembling peptide scaffolds promote enamel remineralization. J Dent Res. 2007;86:426–30.

20. Liu CS, Shao HF, Chen FY, Zheng HY. Effects of the granularity of raw materials on the hydration and hardening process of calcium phosphate cements. Biomaterials. 2003;24:4103–13.

21. Fu B, Shen Q, Qian W, Zeng Y, Sun X, Hannig M. Interfacial interaction of tartaric acid with hydroxyapatite and enamel. J Mater Sci Mater Med. 2005;16:827–31.

22. Gao SS, Huang SB, Qian LM, Yu HY, Zhou ZR. Wear behavior of early carious enamel before and after remineralization. Wear. 2009;267:726–33.

23. Li L, Pan HH, Tao JH, Xu XR, Mao CY, Gu XH, et al. Repair of enamel by using hydroxyapatite nanoparticles as the building blocks. J Mater Chem. 2008;18:4079–84.

24. Shibata Y, He LH, Kataoka Y, Miyazaki T, Swain MV. Micromechanical property recovery of human carious dentin achieved with colloidal nano-beta-tricalcium phosphate. J Dent Res. 2008;87:233–7.

25. Wang XK, Xia CJ, Zhang ZH, Den XL, Wei SC, Zheng G, et al. Direct growth of human enamel-like calcium phosphate microstructures on human tooth. J Nanosci Nanotechnol. 2009;9:1361–4.

26. Gbureck U. Tetracalcium phosphate: synthesis, properties and biomedical applications. Acta Biomater. 2010;6:3815–23.

27. Guo H, Su JC, Wei J, Kong H, Liu CS. Biocompatibility and osteogenicity of degradable Ca-deficient hydroxyapatite scaffolds from calcium phosphate cement for bone tissue engineering. Acta Biomater. 2009;5:268–78.

28. Zhang HG, Zhu QS. Surfactant-assisted preparation of fluoride-substituted hydroxyapatite nanorods. Mater Lett. 2005;59:3054–8.

29. Busch S. Regeneration of human tooth enamel. Angew Chem Int Ed. 2004;43:1428–31.

30. Yoon BH, Kim HW, Lee SH, Bae CJ, Koh YH, Kong YM, Kim HE. Stability and cellular responses to fluorapatite-collagen composites. Biomaterials. 2005;26:2957–63.