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Preparation of fluoride substituted apatite cements as the building blocks for tooth enamel restoration

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ABSTRACT

Fluoride substituted apatite cement (fs-AC) was synthesized by using the cement powders of tetracalcium phosphate (TTCP) and sodium fluoride (NaF), and the cement powders were mixed with diluted phosphoric acid (H_3PO_4) as cement liquid to form fs-AC paste. The fs-AC paste could be directly filled into the carious cavities to repair damaged dental enamel. The results indicated that the fs-AC paste was changed into fluorapatite crystals with the atom molar ratio for calcium to phosphorus of 1.66 and the F ion amount of 3 wt% after self-hardening for 2 days. The solubility of fs-AC in Tris–HCl solution (pH 6) was slightly lower than hydroxyapatite cement (HAC) that was similar to the apatite in enamel, indicating the fs-AC was much insensitive to the weakly acidic solution than the apatite in enamel. The fs-AC was tightly combined with the enamel surface because of the chemical reaction between the fs-AC and the apatite in enamel after the caries cavities was filled with fs-AC. The extracts of fs-AC caused no cytotoxicity on L929 cells, which satisfied the relevant criterion on dental biomaterials, revealing good cytocompatibility. The fs-AC had potential prospect for the reconstitution of carious lesion of dental enamel.

1. Introduction

Dental caries is a prevalent chronic and world-wide oral disease [1]. As a non-living tissue, the main composition of mature enamel is inorganic apatite so dental enamel is scarcely self-repaired by living organisms after substantial mineral loss [2]. Traditional clinical practice has recommended complete removal of softened and discolored dentin (demineralized carious dentin) to eliminate infected tissue and create a hard foundation to support a proposed restoration such as composite resin or metal alloys, which result in poor adhesion between the repair materials and enamel at the interface during the restoration, and this technique is far from ideal, because excessive sound dentin is removed [3–5]. Obviously, reconstitution of carious dentin is a more desirable clinical approach than the traditional clinical practice. It has been proved that fluoride could not only improve the acid resistance of apatite crystals effectively (a certain concentration) but also inhibit metabolism of bacterial [6]. Yamagishi et al. reported a paste of fluoridated hydroxyapatite that could be used to repair early carious lesion, and the apatite paste could be bond to the surfaces of the dental enamel [7]. However, this method may limit its application in the restoration of badly carious lesion of dental enamel, such as carious cavities.

Hydroxyapatite biomaterials are promising candidates for reconstruction of calcified tissue, such as human tooth and bone, because they are the main inorganic components of dentin and bone minerals [8]. However, the native structure of enamel is too complex to be remodeled, and the synthesized apatite biomaterials (such as bioceramics) often have different from the natural ones [9]. The human tooth is protected by enamel that is composed of apatite crystals, acid-forming bacteria cause microscopic damage to the enamel, creating carious cavities, which cannot be repaired by the restorative materials (such as amalgam, ceramics, or polymer composites) because these materials without viscidity do not adhere (bond) perfectly to the enamel surfaces owing to the differences in chemical composition and crystal structure [10]. In order to further restoration of badly carious lesion of dental enamel, such as carious cavities, in this study, a paste of fluoride substituted apatite cement (fs-AC) with good viscosity was synthesized, and the fs-AC pastes could be directly filled into the carious cavities and adhered with dental enamel surfaces to repair the damaged enamel.

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2. Materials and methods

2.1. Preparation and characterization of fs-AC

The fluoride substituted apatite cement consisted of the cement powders and cement liquid. The cement powders were composed of tetracalcium phosphate ($Ca_4(PO_4)_2O$, TTCP) and sodium fluoride (NaF), and cement liquid was diluted phosphoric acid (H_3PO_4) with the concentration of 17% (v/v). TTCP was synthesized by a solid-to-solid reaction between calcium phosphate and calcium carbonate at a temperature of 1500 °C for 8 h. The TTCP was grounded in a planetary ball mill for 1 h, followed by sieving through 120 meshes to obtain TTCP powders [11].

The fs-AC with self-hardening (self-setting) property was prepared by mixing the cement powders (5 g TTCP + 0.46 g NaF) with cement liquid (3.2 mL diluted H_3PO_4) to form a cement paste. The fs-AC paste was placed into stainless steel molds with the size of Ø10 mm × 2 mm. After stored in beakers in a constant temperature oven at 37 °C and 100% relative humidity (r.h.) for 2 days, the pre-hardened fs-AC solid mass sample was obtained. We prepared hydroxyapatite cement (HAC) (as a control), which contained tetracalcium phosphate as cement powder and diluted phosphoric acid (17% H_3PO_4) as cement liquid without sodium fluoride. The HAC was prepared by mixing the cement powder with liquid to form cement paste, and the following process was similar to the preparation of fs-AC.

In order to confirm that the F ion entered into the apatite crystal lattice while did not adsorb on the surfaces of apatite cement, the experiments were done as follows: the pre-hardened fs-AC and HAC samples were grounded into powers, respectively. The power samples were then immersed in deionized water for 24 h and the water was changed one time at 12 h. Finally, these samples were dried at 120 °C for 24 h to obtain fs-AC and HAC power samples, which were characterized by X-ray diffraction (XRD; Rigaku Co., Japan), and Fourier transform-infrared spectroscopy (FT-IR; Magna-IR 550, Nicolet, American).

2.2. Solubility of fs-AC

The solubility of the fs-AC was characterized by the weight loss ratio (wt%) in Tris–HCl solution at different time, and the hydroxyapatite cement (HAC) was used as a control. After setting for 2 days and dried at 50 °C for 24 h, the fs-AC and HAC samples (Ø10 mm × 2 mm) with initial weight (W_i), were put in 400 mL of Tris–HCl solution (pH 6, adjusted by diluted HCl) with a weight-to-volume ratio of 0.5 g/mL. The solution was continuously shaken in a water bath at 37 °C. At different time, the fs-AC and HAC samples were removed from the Tris–HCl solution, cleaned with water, dried at 50 °C for 24 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 fs-AC and HAC samples at different time was calculated. Three samples of each kind of cement were tested and the average value was recorded.

2.3. Cytotoxicity of fs-AC

L929 cells were used to test the cytotoxicity of the fs-AC, which was carried out by using the fs-AC extracts in contact with L929 cells according to International Standard Organization (ISO/EN 10993-5), and hydroxyapatite cement (HAC) was used as a control. To prepare the fs-AC extracts, a stock solution of 200 mg/mL was first prepared by adding 5 g fs-AC (after setting for 2 days and dried at 50 °C for 24 h) into DMEM culture medium. After incubation at 37 °C for 24 h, the mixture was centrifuged and the supernatant was collected. Subsequently, the extracts were sterilized by filtration through 0.2 μm filter membranes for cell cultured experiments.

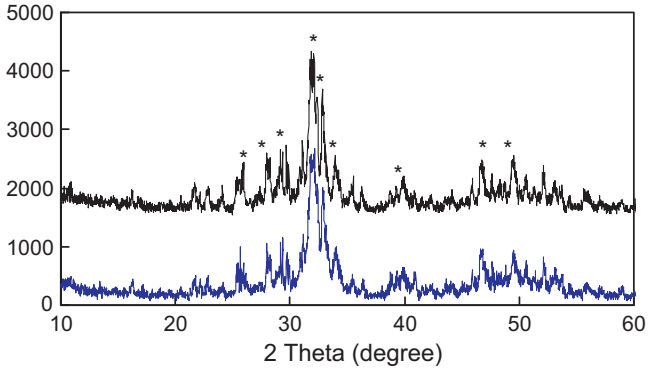

Fig. 1. XRD of fs-AC (a) and HAC (a) after hardening for 2 days, * represents apatite.

The cells were seeded on a 96-well plate and incubated for 24 h. Then, the culture medium was removed and replaced by 50 μL of extracts and 50 μL of DMEM medium supplemented with 10% FCS. The DMEM with 10% FCS (without extract supplement) was used as a blank control. After incubation for 24 h, MTT test was carried out to test cell viability. In brief, 100 mL of 0.5 mg/mL 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-dipheny-tetrazoliumromide (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) was measured by a microplate reader at the wavelength of 492 nm.

2.4. Restoration of enamel carious cavities

Human teeth with big enamel carious cavities were degreased with absolute ethanol, and etched with 17% phosphoric acid for about 30 min. The fs-AC pastes were filled into the enamel carious cavities immediately before the phosphoric acid solution dried. After the tooth samples with enamel carious cavities were filled with the fs-AC pastes, the as-prepared tooth samples were stored in beakers in a constant temperature oven at 37 °C and 100% relative humidity (r.h.) for 2 days. The tooth samples with fs-AC repair the enamel carious cavities were sectioned perpendicular to the dental crown using a diamond saw, and interface between the enamel and fs-AC was examined by using scanning electron microscope (SEM, JSM-6360LV, JEOL). The surface morphology and microstructure of the fs-AC filled into the dental enamel carious cavities were also examined with SEM.

3. Results

3.1. XRD analysis

The phase compositions and crystal structure of the hardened fs-AC and HAC were characterized by powder XRD as shown in Fig. 1. It can be seen that the diffraction peaks of the two samples at $2\theta = 25.7°, 28°, 29°, 31.8°, 32.4°, 33.5°, 39°, 46.8°, 49.5°$ and $52.6°$ were ascribed to apatite both in Fig. 1a and b. Clearly, all of the peaks can be readily indexed to a pure hexagonal phase, which was in accordance with apatite structure. The XRD results indicated that the hardened fs-AC and HAC were all apatite structure. The presence of HAC could be attributed to the chemical reactions as follows [12]:

$$5Ca_4(PO_4)_2O + 2H_3PO_4 \rightarrow 2Ca_{10}(PO_4)_6(OH)_2 \quad (HA)$$