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Abalone water-soluble matrix for self-healing biomineralization of tooth defects

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ABSTRACT

Enamel cannot heal by itself if damaged. Hydroxyapatite (HAP) is main component of human enamel. Formation of enamel-like materials for healing enamel defects remains a challenge. In this paper, we successfully isolated the abalone water-soluble matrix (AWSM) with 1.53 wt% the abalone water-soluble protein (AWSPro) and 2.04 wt% the abalone water-soluble polysaccharide (AWSPs) from abandoned abalone shell, and self-healing biomineralization of tooth defects was successfully achieved in vitro. Based on X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), hot field emission scanning electron microscopy (HFESEM) and energy dispersive spectrometer (EDS) analysis, the results showed that the AWSM can efficiently induce remineralization of HAP. The enamel-like HAP was successfully achieved onto etched enamel's surface due to the presence of the AWSM. Moreover, the remineralized effect of eroded enamel was growing with the increase of the AWSM. This study provides a solution to the resource waste and environmental pollution caused by abandoned abalone shell, and we provides a new method for self-healing remineralization of enamel defects by AWSM and develops a novel dental material for potential clinical dentistry application.

1. Introduction

Dental enamel is the hardest tissue in mammals [1]. It is the outermost layer of human tooth that helps to prevent tooth decay. The main component of human enamel is 95–97% enamel-like hydroxyapatite (HAP) and a little organic matter [2]. The highly-ordered structure of enamel-like HAP forms the hardest tissue in the human body [3]. The enamel matrix proteins (EMPs) can induce the formation of enamel [4,5]. However, the EMPs only appear in the early formation of enamel. Therefore, enamel is different from other tissues of human, which cannot heal by itself when tooth defects generate.

At present, there are three kinds of methods to heal human tooth. The first method is dental filling, which is mainly suitable for a small area of the enamel wear. The second method is inlay repair, which is more suitable for serious enamel wear. And the third one is full crown restoration. In any case, to heal the defects of the damaged teeth, they all need to synthesize the enamel-like materials. HAP is the most stable and slightly soluble in water among the calcium phosphates [6,7]. In general, due to the excellent bioactivity, biocompatibility and osteoconductivity, synthetic HAP has been widely applied as a biomaterial to repair or substitute human hard tissues [8–14], such as bones and teeth [15]. So far, various methods for HAP have been developed, including solid-phase method [16], sol-gel method [17], hydrothermal method [18–22], electrophoresis method [23], etc. However, as reported in the literature, these methods usually require harsh reaction conditions such as extremely high temperature and high pressure and so on. This will limit the clinical applications. Recently, a biomimetic method of remineralization has drawn great attention among the scholars, which uses mild and biocompatible conditions to obtain samples. Roveri et al. [24] has reported a biomimetic method for remineralization onto the surface of enamel. It is easy to control reactive conditions. So, biomimetic method is a good choice for remineralization of tooth defects.

Abalone is a gastropod mollusk. Owning to the delicious taste and high nutrition, abalone meat is a rare traditional Chinese food ingredient and one of necessary dishes for Chinese banquet. However, the abalone shell brings a great burden to the environment after eating the abalone meat. Every year, thousands of tons of discarded abalone shell bring a

Abbreviations: HAP, hydroxyapatite; AWSM, abalone water-soluble matrix; AWSPro, abalone water-soluble protein; AWSPs, abalone water-soluble polysaccharide; HFESEM, hot field emission scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; EDS, energy dispersive spectrometer; UV, ultraviolet absorption spectra; EMPs, enamel matrix proteins; SBF, simulated body fluid; DDW, deuterium depleted water; PBS, phosphate buffer solution.

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serious threat to environment all over eastern China. This not only resulted in waste of natural resources, but also polluted the environment. Abalone shell is a biocomposite consisting of both over 95 wt% calcium carbonate and less than 5 wt% organic matters [25–28]. The enamel is also a biocomposite consisting of 95–97% enamel-like HAP and a little organic matter. So the proportion of the organic matter and inorganic mineral of abalone shell is very similar to enamel. The organic matrix was divided into abalone water-soluble matrix (AWSM) and abalone insoluble matrix [29,30]. It is undeniable that the AWSM plays an important role in the process of biological remineralization [31,32]. Many studies have shown that the AWSM can facilitate the formation of crystal [33,34]. Ni et al. [35] have reported that the successful formation of HAP on the nacre's surface with AWSM. Whereafter, Lamghari et al. [36] suggested the AWSM can activate the osteogenic bone marrow cells and lead to bone formation in vitro under simulated body fluid (SBF) condition. However, it's reported by a few literatures that the AWSM can make contributions to remineralization of tooth defects. So, it is still a challenge to achieve desirable materials for clinical dentistry.

Hence, in this paper, we isolated the AWSM from abalone shell, and self-healing biomineralization of tooth defects was successfully achieved in vitro. Moreover, enamel-like HAP was successfully achieved onto the surface of etched enamel in the presence of the AWSM. The crystal mineral phase and morphology of the remineralized enamel with AWSM were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), hot field emission scanning electron microscopy (HFESEM) and energy dispersive spectrometer (EDS) analysis. The protein and polysaccharide content of the AWSM were detected by ultraviolet (UV) absorption spectra.

2. Materials and methods

2.1. Extraction of the AWSM

Firstly, to remove surface substance of the abalone shell, the abalone shell was soaked with 10 vol.% acetic acid solution for 10 h. The shell was dried naturally after cleaning. Secondly, the dried shell was crushed and grinded to abalone shell powders by universal grinder (SF-130, China). The powder (10 g) was dissolved into 120 ml 10 vol.% acetic acid solution with stirring until no longer to produce bubbles. Then, the suspension was centrifuged at 10,000 rpm for 10 min to obtain the supernatant, and the supernatant was dialyzed and desalted using a dialysis tube (MWCO 500, USA) for 24 h (every 8 h to replace deionized water) to obtain the purified solution, which containing the AWSM. Finally, the solution was freeze-dried to achieve the AWSM.

2.2. Preparation of enamel slices

Collect human normal premolars (approved by unidentified donors visiting at the Affiliated Stomatological Hospital, Fujian Medical University, Fuzhou, China). After cleaning, the teeth were cut longitudinally into about 0.5–1 mm thick slices using a low speed saw (IsoMet, USA). Then, the slices were cleaned by deuterium depleted water (DDW). Finally, all the teeth slices were stored in phosphate buffer solution (PBS) at 4 °C for further experiment.

Table 1
Preparation of the samples under different treatment conditions.

Sample name	Acid-etching time/s	AWSM/g	Simulated body fluid/ml	Remineralized temperature/°C	Remineralized time/h
a	0	0	40	37	72
b	20	0	40	37	72
c	20	0.005	40	37	72
d	20	0.010	40	37	72
e	20	0.015	40	37	72

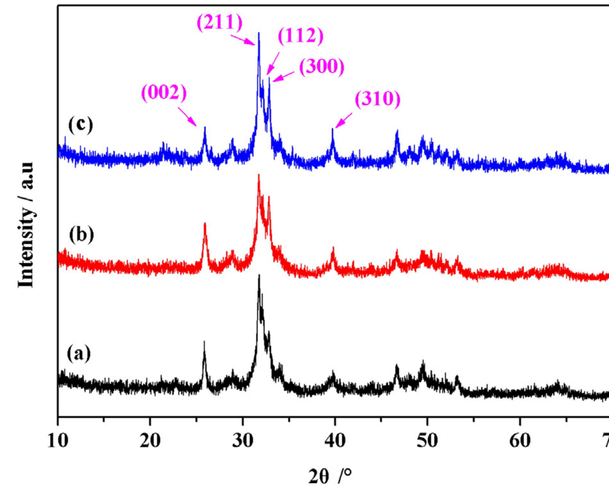

Fig. 1. XRD patterns of the (a) nature enamel, (b) remineralized enamel without AWSM and (c) remineralized enamel with 0.01 g AWSM.

2.3. Remineralization of eroded enamel slices

To imitate the enamel defects, the surface of enamel slices was artificial etched with 37% phosphoric acid for 20 s, and rinsed with DDW. Then, the etched enamel was immersed in 40 ml freshly prepared mineralization solution (containing SBF and AWSM) and remineralized at 37 °C for 72 h. After the remineralization, the samples were taken out the mineralization solution, rinsed with DDW and dried at room temperature. After that, the samples were obtained. For the control, the enamel slices were prepared without acid-etching and the remineralization of the acid-etching enamel slice was prepared without the AWSM. As shown in Table 1.

2.4. Characterization of remineralized enamel slices

The phase of the remineralized enamel was examined by X-ray diffraction (XRD, X'Pert PRO Panalytical diffractometer) with Cu Kα (λ = 0.154056 nm) incident radiation at working voltage of 40 keV ranging from 10° to 70° in a 0.02° step size. The phases were identified by comparison with the data reported in the Joint Committee of Powder Diffraction Standards database (JCPDS No. 09-0432).

The characteristic groups of solids were detected by attenuated total reflectance method (ATR of Fourier transform infrared

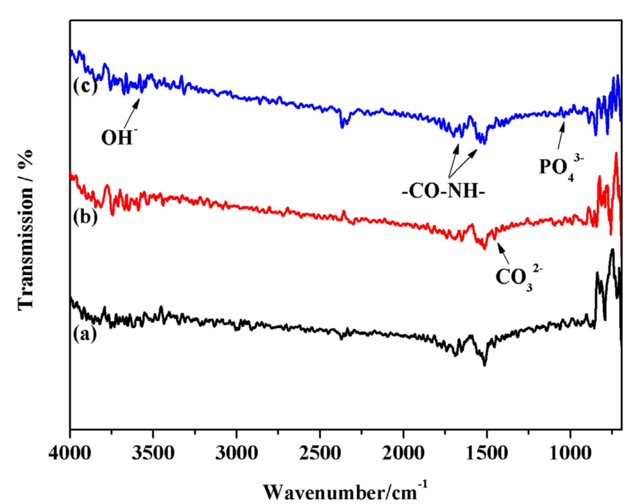

Fig. 2. FTIR spectra of the (a) nature enamel, (b) remineralized enamel without AWSM and (c) remineralized enamel with 0.01 g AWSM.