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Setting behavior, mechanical property and biocompatibility of anti-washout wollastonite/calcium phosphate composite cement

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ABSTRACT

In this study, a calcium phosphate-based composite cement was fabricated by incorporating wollastonite (WS) into calcium phosphate cement (CPC). The setting behavior, microstructure, injectability, porosity, compressive strength, anti-washout property, in vitro degradation, and cell behavior of the WS/CPC composite cement were systematically investigated. The results revealed that the addition of WS promoted the hydration reaction but without affecting the hydration product of CPC. The injectability of the WS/CPC composite cement declined with the incorporation of WS to a certain extent, especially when the content of WS was higher than 20 wt%. By incorporating appropriate amount of WS into CPC, the composite cement obtained feasible setting time, enhanced compressive strength, improved anti-washout performance, and favorable biocompatibility. On the basis of its improved comprehensive application-relevant properties, the WS/CPC composite cement is prospective to be a promising biomaterial for bone defect repairing.

1. Introduction

Calcium phosphate cement (CPC) has attracted much attention of scientists worldwide because of its excellent biological performance, arbitrary shaping, isothermal self-setting properties, etc [1]. CPC consists of powder phase, which is composed of single or multiple calcium phosphate compounds and liquid phase that can be water or aqueous solution. The moldable paste of CPC is obtained by mixing the powder and liquid phase at an appropriate ratio, and hardens at room or body temperature through the dissolution-precipitation mechanism. Owing to the favorable performances, CPC has been widely used for orthopedic and dental applications [2].

However, the further clinical application of CPC is limited due to its insufficient mechanical performance and poor anti-washout property. Therefore, substantial efforts have been devoted to solve the above problems. Additives such as fibers [3], particles [4], polymers [5] were introduced to reinforce the CPC matrix. Nevertheless, most reinforcement methods deteriorate the injectability of CPC and are incapable of improving the anti-washout property. Previous studies showed that the anti-washout property of CPC was mainly promoted by introducing specific organic adhesives such as sodium alginate, chitosan and hydroxypropyl methylcellulose [6]. These organic additives increased the viscosity of the cement pastes, so as to enhance its cohesion. Nonetheless, the organic additives, especially naturally derived additives, may deteriorate other properties of CPC. For example, the compressive strength of CPC will be generally reduced and the setting time will be prolonged by the addition of organic adhesives. The development of versatile additives which can improve the comprehensive performance of CPC but without starkly compromising other properties is important.

In this work, wollastonite ($CaSiO_3$, WS) was selected to improve the physical and chemical properties and bioactivity of CPC. Silicon, which uniquely localized in active calcification sites of new bone, can improve cell proliferation and activate the gene expression related to bone [7,8]. The silicon and calcium ions released from WS can stimulate cell function and enhance osteogenesis [9]. It is shared that WS-based biomaterials possess good biodegradability and bioactivity and can quickly induce the precipitation of bone-like hydroxyapatite (HA) on their surface after soaking in simulated body fluid (SBF) [10,11], making the bioactive materials closely bond to soft/hard tissues [12]. In addition, WS is usually used as reinforced filler in composite materials because of

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its excellent mechanical performance. Greish and Brown [13] reported that the tensile strength and bioactivity of HA-Ca polycarboxylate composites were improved by incorporation of WS fibers. Encinas-Romero et al. [14] developed HA-WS composite ceramic, which exhibited enhanced hardness and reduced elastic modulus with increasing the amount of WS in the composites. Furthermore, the composite with higher WS amount had faster deposition rate of apatite on the surface.

Considering the benefits of WS, the calcium phosphate composite cement incorporating wollastonite (WS/CPC) was developed in this study. The effects of WS on the setting behavior, compressive strength, injectability, anti-washout property, degradability, and biocompatibility of the composite cement were systematically investigated.

2. Materials and methods

2.1. Materials preparation

The chemical raw materials including dicalcium phosphate anhydrous ($CaHPO_4$, DCPA), $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2HPO_4$, and Na_2SiO_3, were commercially obtained from Shanghai No. 4 Reagent & H.V. Chemical Co. Ltd, China. The CPC powder used in this study was prepared by mixing partially crystallized calcium phosphate (PCCP, median diameter of 16.5 μm) and DCPA (median diameter of 3.7 μm) at a weight ratio of 1:1. PCCP was synthesized from an aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ (0.36 mol/L) and $(NH_4)_2HPO_4$ (0.15 mol/L) by chemical precipitation method in our laboratory, as described in our previous article [15]. The hydrous precipitate was centrifugally separated, washed, freeze-dried, and calcined at 450 °C for 2 h in a furnace to partially crystallize.

WS powder was synthesized by a deposition-precipitation method using $Ca(NO_3)_2 \cdot 4H_2O$ and Na_2SiO_3 as starting materials. Na_2SiO_3 solution (0.1 mol/L) was added dropwise to stirring $Ca(NO_3)_2$ solution (0.1 mol/L) to produce a white precipitate. The precipitate was filtered, washed with distilled water and anhydrous ethanol several times, dried at 80 °C for 24 h, and then calcined at 800 °C for 2 h. Subsequently, the calcined WS powder was ground and sieved. The particles (size of 53–106 μm) were used for preparing the WS/CPC composite cement.

The WS/CPC powder was prepared by mixing the CPC powder with WS powder at the mass fraction of 0, 10, 20, 30, 40, and 50 wt%, respectively, which were named as nCPC (neat CPC), 10WS/CPC, 20WS/CPC, 30WS/CPC, 40WS/CPC and 50WS/CPC, respectively. The mixtures were well mixed with deionized water at a liquid to powder (L/P) ratio of 0.4 mL/g to obtain pastes with workable consistency at room temperature.

2.2. Setting time test

The setting time of the cements was measured according to the method of Gilmore needle [16]. The initial setting occurred when the light needle (113.4 g in mass, and 2.13 mm in diameter) failed to indent the surface of the sample, while the final setting time was determined by using the heavy needle (453.6 g in mass, 1.06 mm in diameter). Each measurement was performed six times and the average value was calculated.

2.3. Injectability test

The injectability of the cements was tested with a syringe, which was fitted with a needle of 1.6 mm inner diameter. After mixing the cement powder with deionized water for 1 min, the as-prepared paste was poured into the syringe, and then a weight of 5 kg was mounted vertically on the top of the plunger for 2 min. The mass of the paste before and after injection was measured and the injectability was calculated according to Eq. (1). Each test was performed six times and the average value was calculated.

$$\text{Injectability}(\%) = (\text{Mass expelled from the syringe}) / (\text{total mass before injection}) \times 100\% \quad (1)$$

2.4. Anti-washout assessment

The premixed paste was manually injected into the deionized water immediately. Then the samples were put into a shaker at 37 °C and shaken at the speed of 120 r/min. The anti-washout property was proved if the paste did not visibly disintegrate after immersed in solution for 60 min.

2.5. Compressive strength test

The cement pastes were poured into the cylindrical steel molds with an inner diameter of 6 mm and a height of 12 mm, and then pressed under a stress of about 700 kPa for 5 s to eliminate the big air bubbles in the pastes. Then the samples were demoulded and stored in a humidified incubator at 37 °C and 97% humidity for 72 h. After that, the samples were taken out and freeze-dried. The cement specimens were polished for compressive strength test. The compressive strength of the cement specimens were measured using a universal material testing machine (Instron 5567, Instron, USA) at a crosshead speed of 0.5 mm/min. Each measurement was repeated 6 times and the average value was calculated.

2.6. Porosity determination

The cements were incubated at 37 °C and 97% humidity for 72 h. The porosity of the samples was determined by the Archimedes technique with ethanol as the displacement liquid. The weight of a dried sample was recorded as G_0, and the weight of a specific gravity bottle full of ethanol was recorded as G_1. Then the sample was put into the bottle and evacuated under vacuum for 0.5 h to remove the air in the sample. The weight of the bottle full of ethanol and with the cement sample was recorded as G_2. Then the sample was taken out from the bottle and weighed, and the weight was recorded as G_3. The porosity of the cement sample was calculated using Eq. (2).

$$\text{Porosity}(\%) = (G_3 - G_0)/(G_3 + G_1 - G_2) \times 100\% \quad (2)$$

2.7. Phase and microstructure characterization

The hydrated samples were milled into powders and analyzed using X-ray diffraction (XRD; X'Pert PRO, PANalytical, Netherlands). IR transmittance spectra were recorded by Fourier transform infrared spectroscope (FTIR; Avatar 360, Nicolet, USA) in the 4000–400 cm^{-1} range. The sample was homogeneously mixed with spectroscopic grade KBr, and then pressed into thin disk using a press machine. The microstructure of the cement specimens was observed by a scanning electron microscope (SEM; Nova NanoSEM 430, FEI, USA), equipped with an energy dispersive X-ray spectrometer (EDS; INCA X-act, Oxford, U.K.). The pH variation of the cement slurry (with the L/P ratio of 10 mL/g) was tested by a pH meter (Jingke Leici Co. Ltd., China) [15]. To investigate the evolution of phase and microstructure during hydration reaction, the samples were removed and quenched in cold ethanol to stop the hydration reaction after incubating for 0.5, 1, 4, 12, 24, and 72 h, respectively, and then freeze dried for the XRD analysis and SEM observation.