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Mechanical and rheological properties and injectability of calcium phosphate cement containing poly (lactic-co-glycolic acid) microspheres

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ABSTRACT

To enhance tissue ingrowth and promote rapid resorption, efforts were made to build macropores into calcium phosphate cement (CPC); however, this led to a decrease in its mechanical properties. In this study, poly (lactic-co-glycolic acid) (PLGA) microspheres were incorporated into CPC to impart macroporosity and maintain early strength. The influences of the content of PLGA microspheres on the mechanical strength, rheological properties, injectability, setting time, and microstructure of CPC were also systematically investigated. At the PLGA to CPC mass ratios of 20/80 and 30/70, the compressive strength of the composites was similar to that of CPC without PLGA microspheres. The rheological results indicated that PLGA microspheres/CPC pastes showed plastic and shear-thinning behaviors. The addition of PLGA microspheres to CPC resulted in the increase of viscosity and yield stress of the pastes. Simultaneously, the injectability of the pastes decreased with the addition of PLGA microspheres. When the PLGA to CPC ratio was 20/80, the injectability of the paste was still higher than 95%. The calcium phosphate cement containing 20 wt.% PLGA microspheres exhibited excellent injectability and satisfactory setting time without strength degradation. Obviously, such an in situ macropores-generable CPC should have potential prospects for the wider applications in orthopedics, oral, and maxillofacial surgery.

1. Introduction

Since the calcium phosphate cement (CPC) was first reported by Brown and Chow [1] in 1986, CPC has been widely used as bone substitutes in dentistry, orthopedics, and reconstructive surgery because of its satisfactory biocompatibility and the nature of set in situ [2–4]. However, the drawbacks of calcium phosphate cement, including low degradation rate, low strength and the lack of macroporosity for bone ingrowth, limited its clinical uses [5,6]. Therefore, great efforts were made to enhance tissue ingrowth and degradation rate by increasing the porosity of the materials [7,8]. But if the porosity was increased to a high value (e.g. higher than 80%), CPC would become too weak to adequately maintain their shape for use in vivo.

Poly (lactic-co-glycolic acid) (PLGA) degraded by random hydrolysis into lactic and glycolic acids and was considered to be non-toxic and biocompatible both in vitro and in vivo [9–11]. So the biodegradable PLGA microspheres were used to impart macroporosity to the cement and maintain early strength simultaneously. When implanted in vivo, the PLGA microspheres degraded to create macropores in situ for bony ingrowth because the degradation of the PLGA microspheres was generally faster than that of the surrounding calcium phosphate cement [12,13]. The strengthening of the graft from bony ingrowth and the deposition of new bone should offset the weakening of the graft due to polymer degradation [14]. In addition, the incorporation of degradable microparticles could be used for drug and growth factor delivery [15].

In clinical applications when there is a need for a precise placement of the cement paste to conform to a defect area, or when the minimally invasive surgical techniques are required, the injectability of CPC was desirable [16,17]. In order to achieve a satisfactory injectability, the rheological properties of CPC were quite important because they played a vital role in obtaining the required properties (low resistance to flow, no powder–liquid phase separation) during the injection process. Although some studies were devoted to investigating the rheological properties and injectability of CPC [18–20], to our knowledge, there was little research about such properties of CPC containing PLGA microspheres of various weight ratios.

The purpose of the present study was to systematically investigate the mechanical and rheological properties as well as injectability of CPC containing PLGA microspheres. The setting time of the cements

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was also determined. X-ray diffraction (XRD) and scanning electron microscope (SEM) were used to identify the interactions between the cement and the PLGA microspheres. In particular, the effects of PLGA to CPC ratio on the properties of composites were discussed.

2. Experimental

2.1. Materials and preparation

The CPC powder used in this study was prepared by mixing the partially crystallized calcium phosphate (PCCP) with dicalcium phosphate anhydrous (DCPA) at a mass ratio of 1:1. The PCCP was synthesized in our laboratory as described in our previous works [21,22]. The precursor of PCCP was synthesized from aqueous solution of $Ca(NO_3)_2 \cdot 4H_2O$ (0.36 mol/L) and $(NH_4)_2HPO_4 \cdot 12H_2O$ (0.15 mol/L) by chemical precipitation method in our laboratory. Then the deposit was centrifugally separated, freeze-dried and calcined at 450 °C for 2 h in a furnace to attain PCCP. The PCCP powder was milled in a planetary ball mill by using ZrO_2 balls at 400 rpm for 2 h. DCPA of analytical grade purity was commercially obtained from Shanghai No.4 Reagent & H.V. Chemical Co. Ltd, China.

PLGA (50/50 lactide to glycolide mol ratio, MW: 60000) was purchased from Jinan M.K. Biotechnology Co. Ltd, China. PLGA microspheres were made by a solvent evaporation method. PLGA (15 g) was dissolved in 100 ml methylene chloride (CH_2Cl_2) to form a homogeneous suspension, which was subsequently poured into 800 ml 0.5% methyl cellulose (M20, Sinopharm Chemical Reagent Co. Ltd.) solution. The solution was stirred at 500 revolutions per minute with an overhead stirrer for 8 h at room temperature, allowing the solvent to evaporate. After stirring, the solution was allowed to stand 4 h and the liquid was decanted. Then the microspheres were washed with deionized water for three times, centrifuged and lyophilized. Microspheres with a diameter ranging in 100–300 μm were separated by sieving for use in this study.

The CPC powder was uniformly mixed with PLGA microspheres at PLGA/CPC weight ratios of 0/100 (as a control), 10/90, 20/80, 30/70, and 40/60, respectively. Then the mixtures were homogeneously mixed with deionized water at liquid to CPC powder ratio of 0.4 mL/g for 1 min to obtain the cement pastes. All processes were carried out at 25 °C ± 2 °C.

2.2. Phase analysis and microstructure observation

The phases presented were identified by XRD (X'Pert Pro, PANalytical Co., the Netherlands). Data were collected for 2θ ranging between 10° and 70° with a step size of 0.033°. The morphology and microstructures of the specimens were observed by using SEM (H-800, Hitachi CO., Japan). The specimens were coated with gold before observed.

2.3. Mechanical properties tests

The compressive mechanical properties of the specimens were measured by using a universal material testing machine (Instron 5567, Instron Co., Britain) at a crosshead speed of 0.5 mm/min. Steel cylindrical molds with an inner diameter of 6 mm and a height of 12 mm were used to prepare specimens for compressive tests. The specimens were stored in an incubator at 37 °C and 97% humidity for 24 h. Then the samples were ready for the mechanical testing. The compressive modulus was calculated as the slope of the initial portion of the stress vs. strain curve. Each measurement was repeated 6 times and the average value was calculated.

2.4. Setting time measurements

The setting time of the calcium phosphate cement containing PLGA microspheres was measured according to ASTM C191-03. The samples were tested using a Vicat apparatus which had a movable rod of 300 ± 0.5 g in mass and a removable needle of 1 ± 0.05 mm in diameter. The Vicat needle fixed at the end of the rod was carefully lowered vertically onto the surface of the newly shaped cement samples and was allowed to remain there for 5 s. The indentation was repeated at intervals of 30 s until the cement was hardened. Initial setting time was calculated as the difference between the time at which the needle penetrated 25 mm into cement paste and the time at which the powder initially contact the water. Final set occurred when there was no visible penetration. Each measurement was repeated 6 times and the average value was calculated.

2.5. Rheological properties tests

The rheological properties, including viscosity and yield stress, of the injectable pastes were tested by a rheometer (R/S-SST, Brookfield, USA). Viscosity (Pa s) is a measurement of a fluid's resistance to flow, describing the internal friction of a moving fluid. Yield stress (Pa) was the critical stress that must be applied to a material to make it began to flow. The bigger the yield stress was, the harder the cement was injected. The yield stress measurement was gained by plotting the shear stress values for a range of shear rates, fitting a curve to the data, and extrapolating through the stress axis. The intersect on the stress axis gave the yield stress value. For each test, 6 g of the mixture of CPC and PLGA microspheres with different ratios were mixed with deionized water at the L/P ratio of 0.4 to form pastes. Immediately prior to the start of each test, the paste was mixed in an agate mortar for 1 min to form a homogeneous suspension. The slurry was then transferred to the cylinder (spindle number: CC14 DIN) of the rheometer. The viscosity was tested under the shear rate control mode. In this mode, the shear rate was increased linearly from 0 to 100 s^{-1} in 60 s. The yield stress was tested under the shear stress control mode. In this mode, the shear stress was increased linearly from 0 to 1000 Pa in 100 s.

2.6. Injectability tests

The injectability of the calcium phosphate cement containing PLGA microspheres was tested by a syringe, which was fitted with a needle of 1.6 mm inner diameter. The liquid to CPC powder ratio was 0.4, with 2.5 g of CPC powder and 1 ml of liquid. After mixing the mixture of CPC and PLGA microspheres with liquid for two minutes, the as-prepared paste was poured into the syringe. Then a 5 kg compressive load was mounted vertically on the top of the plunger for 2 min. The mass of the paste before and after injecting was measured and the injectability was calculated according to Eq. (1). Each test was repeated for 6 times and the average value was calculated.

$$\text{Injectability } \% = \text{Mass expelled from the syringe} / \text{Total mass before injecting} \quad (1)$$

2.7. Statistical analysis

Quantitative data were presented as mean ± standard deviation (SD) and statistical analysis was performed by using a one-way analysis of variance (one-way ANOVA). A comparison between two means was made by using the Tukey's test with statistical significance set at $p<0.05$.

3. Results

The morphology and dimensions of the PLGA microspheres are presented in Fig. 1. The microspheres were smooth in surface morphology and the sizes of the microspheres were in the range of 100–200 μm.