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Novel injectable calcium phosphate/chitosan composites for bone substitute materials

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Abstract

In this study, a novel injectable bone substitute material was developed which consists of chitosan, citric acid and glucose solution as the liquid phase, and tricalcium phosphate powder as the solid phase. This material was moldable because of its paste consistency after mixing. We used four groups of cement to investigate the mechanical properties and biocompatibility of the new biomaterial in vitro, which were named group A (10% citric acid), B (15% citric acid), C (20% citric acid) and D (25% citric acid). The setting times of the cements were 5–30 min. X-ray diffraction analysis showed that the products were hydroxyapatite (HA) and dicalcium phosphate anhydrous. When the concentration of citric acid was increased, the compressive strength of specimen increased. Through the simulated body fluid test, we observed the material was bioactive. Group D could induce Ca and P ions to deposit the surface group D quickly. These results indicated that the concentration of citric acid in the liquid component affected the mechanical properties and bioactivity of cements. The cell cultivation test showed that the cytocompatibility of the new biomaterial was good. The method for preparing the novel bone substitute material is simple. The starting material is more readily available and cheaper than HA, poly(methyl methacrylate), and so on. The cement could have good prospects for medical application.

Keywords: Calcium phosphate; Chitosan; Citric acid; Bone substitute material; Bioactivity

1. Introduction

Bone defects occur in a wide variety of clinical situations, and their reconstruction to provide mechanical integrity to the skeleton is a necessary step in the patient's rehabilitation. The current gold standard for bone reconstruction, the autogenous bone graft, works well in many circumstances. However, autograft reconstruction has some disadvantages, such as the need for a second surgery at the donor site, the limited quantity and shape of available bone, and the resorption of the graft [1–3]. To reduce or eliminate the need for bone grafting, substantial effort has been invested in finding a suitable artificial bone substitute.

Recently, with the increasing popularity of minimally invasive techniques, one of the major thrusts in orthopedics has been to develop injectable systems that can mould to the shape of a bone cavity and polymerize when injected in situ. Such devices should shorten the surgical operation time, minimize the damaging effects of large muscle retraction, reduce the size of the scars and lessen post-operative pain, allowing patients to achieve rapid recovery in a cost-effective manner. The most commonly used injectable bone cement is poly(methyl methacrylate) (PMMA), but it suffers from the fact that it does not degrade and that its high curing temperatures can cause necrosis of the surrounding tissue [4]. Therefore, further development of alternative injectable materials is necessary. Calcium phosphate cement (CPC) self-hardens to form hydroxyapatite, has excellent osteoconductivity and bone-replacement ability [5–18], and is promising for use in craniofacial and

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orthopedic repair. CPC consists of a powder containing one or more solid compounds of calcium and/or phosphate salts and a cement liquid that can be water or an aqueous solution [19,20]. If the powder and the liquid are mixed in an appropriate ratio, they form a paste that at room or body temperature sets by entanglement of the crystals precipitated within the paste [6,20,21]. The material can be shaped for several minutes and is, depending on the liquid/powder ration, injectable via a syringe [22,23].

Chitosan, an amino-polysaccharide obtained by alkaline deacetylation of chitin, a natural component of shrimp or crab shells, is biocompatible and biodegradable [24], and osteoconductive [25]. It has been used in surgical interventions for the reduction of periodontal pockets [25–28]. Several studies reported that chitosan solution could be used as the liquid phase of CPC [29–33].

In the present study, we developed a novel injectable calcium phosphate cement that could be molded into any desired shape in an appropriate time after mixing. The liquid component of the cement consisted of chitosan, citric acid and glucose solution. The powder component consisted of tricalcium phosphate (TCP). X-ray diffraction showed that the products were hydroxyapatite (HA) and dicalcium phosphate anhydrous (DCPA). The mechanical property tests showed that the compressive strength increased when the concentration of citric acid increased. The compressive strength increased with time. The cell cultivation test showed that the bioactivity of the new material was good. The method is simpler than the procedures for preparing bone-filling paste reported in Refs. [29–33].

2. Materials and methods

2.1. Materials

In this study, the cement consisted of both liquid and powder components. The liquid component was a solution of chitosan, citric and glucose. Chitosan was obtained from Shanghai Bio Life Science & Technology Co. Ltd. (Shanghai, China). The degree of deacetylation was 94% (evaluation method as per Ref. [34]); M_w was 942,000 Da (obtained from viscosity measurements and the Mark–Houwink relationship [35]). The mass fraction of chitosan was 2% in liquid phase. Citric acid and glucose were purchased from Guangzhou Chemical Reagent. After parallel experiments, four types of liquid components were chosen in this research to investigate the effects of the concentration of citric acid on mechanical properties and bioactivity. The composition of the liquid phase is shown in Table 1. The powder component was TCP, which was purchased from Shanghai No. 4 Reagent & H.V. Chemical Co. Ltd. The mean particle size of TCP powder was 6.70 μm (measured using the Brookhaven laser particle analyzer, BI200SM, USA) after milling for 8 h. In this study, the liquid-to-powder (L/P) ratio was 1:0.6 (ml:g). Other reagents were all commercially available. The four types of cement were named groups A–D.

Table 1
Composition of liquid phase

Sample name	Concentration of liquid component (wt.%)		
	Chitosan	Citric acid	Glucose
A	2	10	20
B	2	15	20
C	2	20	7
D	2	25	20

2.2. Setting time measurement

Setting times of the cements were measured according to the international standard ISO 9917 for dental silicophosphate cement [36]. Setting times of the samples were measured by using the Gilmore needle method. A cement specimen kept in 98% relative humidity at 37 °C was considered set when a 400-g mass loaded to a needle with a tip diameter of 1 mm failed to make a perceptible circular indentation on the surface of the cement.

2.3. Morphology observation of cements

After setting, the cements were observed under a scanning electron microscope (SEM, XL-30 ESEM, Philips Co., The Netherlands).

2.4. X-ray diffraction analysis

The X-ray diffraction patterns of the cements were recorded by X-ray powder diffraction analysis (MSAL-XD2, Bulaige Science & Technology Co. Ltd., Beijing, China) using the following parameters: 40 kV, 20 mA, 8°/min.

2.5. Compressive strength measurement

Samples of each of the four types of cement were molded into columns (9.0 mm in diameter and 18.0 mm in length, shown in Fig. 1). The specimens were incubated in air at

Fig. 1. Photo of set samples.

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