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Immersion behavior of gelatin-containing calcium phosphate cement

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Abstract

Calcium phosphate cements (CPCs) have many favorable properties that support their clinical use as bone defect repair. However, it is difficult to deliver to the required site and hard to compact adequately due to inherently low ductility of ceramics. The aim of this study focused on the effect of the gelatin content on properties of CPCs. The diametral tensile strength, morphology, and weight loss of gelatin cements were evaluated after immersion in physiological solution, in addition to setting time. The results indicated that the setting time significantly increased with increasing gelatin amount. The 2 wt.% gelatin could make CPCs attain the maximum strength value of 2.1 MPa at 15-day immersion, while 1.6 MPa for the cement without gelatin. It is concluded that the presence of gelatin improved mechanical properties of CPCs; in particular, 2 wt.% gelatin. CPCs containing 2 wt.% gelatin hardened in an acceptable time recommended for clinical applications.

Keywords: Calcium phosphate; Bone cement; Gelatin; Biodegradability

1. Introduction

Over the past 40 years, the essential material of bone cement in clinical applications is polymethylmethacrylate (PMMA) [1]. However, this material could release methylmethacrylate monomer to result in necrosis of cells around the repair site [2]. In addition, PMMA cannot directly bond to bone tissue through a chemical bonding [3]. Therefore, a variety of bone cements have been made to aim at displacing PMMA. A self-setting cement composed of only calcium phosphate compounds that are similar with the mineral component of bone tissue was developed in the early 1980s [4] and has gained much popularity. Calcium phosphate cements (CPCs) are good bioactive materials for bone defect repair in orthopedic and dental surgery because of its excellent bioactivity and mechanical properties [5–9]. Self-setting CPCs can be handled by the surgeon in paste form and injected into bone cavities or defects. They then set to form a mineral matrix at the contact of which healing bone tissue can form. Although CPC has many favorable properties that support its clinical use, it has proved problematic. For example, it is difficult to deliver to the required site and hard to compact adequately due to relatively poor brittleness resistance. Efforts have been made in recent years to overcome the disadvantages. The polymeric materials such as chitosan [7,10], alginate [11], and gelatin [5,6] have the potential to improve the handling properties of CPCs. For example, Yokoyama et al. developed a chitosan-containing CPC that could be moulded into any desired shape due to its chewing-gum-like consistency [7]. In a study by Tajima et al., the addition of sodium alginate could enhance anti-washout property of Biopex® cement [11]. However, addition of sodium alginate

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into the liquid phase of Biopex® resulted in a slower transformation to apatitic phase. As a result, diametral tensile strength of set cement specimens decreased when it was hardened in an incubator kept at 37 °C and 100% relative humidity for 7 days.

Gelatin is a natural polymer, which is obtained from the bovine bone by thermal denaturation or physical and chemical degradation of collagen, and had been widely employed as a scaffold material in the tissue engineering. The research combining natural gelatin with bioactive CPC [5,6], attempting to achieve elastic bone cement, attracts much attention and is little studied. Fujishiro et al. found that addition of gelatin gel to α-tricalcium phosphate cement resulted in the formation of a porous solid with pores of 20–100 μm in diameter whose pore diameter increased with increasing gelatin gel content [5]. The compressive strength after one week soaking in tris(hydroxymethyl)-aminomethane buffer solution (pH 7.4) increased from 9.0 to 14.1 MPa with increasing gelatin content up to 5 wt.% and thereafter decreased.

The aim of this study is to examine the effect of gelatin amount on immersion behavior of calcium phosphate cements. The calcium carbonate ($CaCO_3$) and monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2 \cdot H_2O$) in a 2:1 molar ratio is selected for the intention to formulate stoichiometric composition of tricalcium phosphate. The major techniques used for characterizing the cement specimens included scanning electron microscopy (SEM) and diametral tensile strength (DTS). Effect of immersion time in the physiological solution on the properties of the various gelatin cements was studied, in addition to setting time.

2. Materials and methods

2.1. Preparation of the gelatin cement

Type B gelatin (isoelectric point of 4.7–5.2) from bovine skin (Sigma, St. Louis, MO) was weighed and dissolved in deionized distilled water at 60 °C until a homogeneous 10 wt.% gelatin solution was attained. First, $CaCO_3$ (Showa, Tokyo, Japan) was added to gelatin solution and dried at 60 °C in an oven. After that, $Ca(H_2PO_4)_2 \cdot H_2O$ (Showa, Tokyo, Japan) was added to the mixture using a conditioning mixer (ARE-250, Thinky, Tokyo, Japan). Herein, gelatin-containing calcium phosphates were obtained by mixing of gelatin-containing $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$ in the molar ratio of 2:1 for matching formulation of tricalcium phosphate in an attempt to form a calcium deficient apatite. The mixture was further ball-milled and sieved to obtained particles with sizes between 44 and 20 μm. Eventually, the gelatin/($CaCO_3$ + $Ca(H_2PO_4)_2 \cdot H_2O$) ratios of 2, 5, and 10 wt.% were obtained. The cement without gelatin was as the control.

The setting liquid was 1 M Na_2HPO_4 (J.T. Baker, Phillipsburg, NJ). The cement specimens were prepared using a liquid-to-powder (L/P) ratio of 0.4 mL/g. After mixing the specimens were placed into a cylindrical stainless steel mould to form the specimen dimension of 6 mm (diameter) × 3 mm (height), and stored in an incubator at 100% relative humidity and 37 °C for hydration.

2.2. Setting time and phase composition

The setting time of the cements was tested at various time intervals by using the 400-g Gillmore needle with a diameter of 1 mm according to the international standard ISO 9917-1 for water-based cements [12]. The setting time was recorded when the needle failed to create an indentation of 1 mm in depth in three separate areas. Six parallel experiments were carried out for the data of every group.

To further investigate the relationship between phase composition and ageing time for setting, after the predetermined periods of time the specimens were immediately placed in a tube containing absolute ethanol for 1 h for dehydration and dried at 60 °C. Thus, the setting reaction would be stopped at the specific time period. The as-dried specimens were ground to fine powders for characterization using X-ray diffractometer (XRD, Shimadzu XD-D1, Kyoto, Japan) operated at 40 kV and 40 mA at a scanning speed of 2°/min to determine phase composition of the products.

2.3. Diametral tensile measurement

As for mechanical performance testing, after mixing, the cements were molded in a stainless steel mould under a pressure of 0.7 MPa for 1 min using a uniaxial press. Then, these moulds were incubated in a 37 °C and 100% humidity environment and allowed to set for different predetermined periods of time to examine the set reaction of the cement specimens through diametral tensile testing. The testing was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 0.5 mm/min. The DTS value of the cement specimens was calculated from the relationship $DTS = 2P/\pi bw$, where P is the peak load in Newton, b is the diameter (mm) and w is the thickness (mm) of the specimen. The maximal compression load at failure was obtained from the recorded load–deflection curves. At least six specimens from each group were tested.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the significant differences between the means in the DTS or setting time data. In the event of a significant difference between test groups, it necessitated testing all the possible differences *via* multiple comparisons that were characterized by considering any significant differences between all possible pairs of groups. Scheffe's multiple comparison test was used to determine the significance of the standard deviations in the measurement data of

ID	Title	Pages
2323	Immersion behavior of gelatin-containing calcium phosphate cement	10