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Novel bioactive composite bone cements based on the β-tricalcium phosphate–monocalcium phosphate monohydrate composite cement system

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Abstract

Bioactive composite bone cements were obtained by incorporation of tricalcium silicate (Ca_3SiO_5, C_3S) into a brushite bone cement composed of β-tricalcium phosphate [β-$Ca_3(PO_4)_2$, β-TCP] and monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2{\cdot}H_2O$, MCPM], and the properties of the new cements were studied and compared with pure brushite cement. The results indicated that the injectability, setting time and short- and long-term mechanical strength of the material are higher than those of pure brushite cement, and the compressive strength of the TCP/MCPM/C_3S composite paste increased with increasing aging time. Moreover, the TCP/MCPM/C_3S specimens showed significantly improved in vitro bioactivity in simulated body fluid and similar degradability in phosphate-buffered saline as compared with brushite cement. Additionally, the reacted TCP/MCPM/C_3S paste possesses the ability to stimulate osteoblast proliferation and promote osteoblastic differentiation of the bone marrow stromal cells. The results indicated that the TCP/MCPM/C_3S cements may be used as a bioactive material for bone regeneration, and might have significant clinical advantage over the traditional β-TCP/MCPM brushite cement.

Keywords: Calcium phosphate cement; Brushite; In vitro test; Cytotoxicity; Silicate

1. Introduction

Materials with self-setting properties have been exploited to augment human bone tissues [1–4], and resorbable calcium phosphate cements based on brushite have raised interests in recent years [5–8]. These kinds of cement are usually presented as a solid phase and a liquid phase. These two components are mixed together in order to form a workable paste that would set into a hard material. The self-setting occurs through an acid–base reaction between a basic calcium phosphate, usually β-tricalcium phosphate (β-TCP), and an acidic phosphate such as orthophosphoric acid or monocalcium phosphate monohydrate (MCPM) [5–9]. In clinical applications, the brushite cements can be used in the form of blocks or as a self-setting paste, which could provide scaffolds for bone regeneration and be gradually replaced by tissue [6,7,10]. However, this cement system still has some inherent drawbacks that need to be dealt with. Due to the extremely rapid setting reaction, the brushite cement shows a rather short setting time that makes it difficult to work with, which also results in high porosity and consequently weak mechanical strength [11,12]. In addition, as soluble acidic phosphates are used as sources of phosphate ions, the brushite cement usually will cause a rapid decrease of pH in vivo immediately after implantation. This phenomenon can have an adverse impact on the biocompatibility of the material [13,14]. Other studies also showed that the calcium phosphate cement is less bioactive as compared

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with some silicate-containing bioactive materials such as A-W glass-ceramic and Bioglass [15,16]. Therefore, incorporation of bioactive materials has been investigated to improve the bioactivity of the cement materials [17,18].

Tricalcium silicate (Ca_3SiO_5) is one of the main components of Portland cement. Once mixed with water, Ca_3SiO_5 will react with water to create calcium–silicate–hydrate (C–S–H). The polymerization and solidification of the C–S–H network contribute to the self-setting properties and increased mechanical strength of the tricalcium silicate paste after aging. Recent studies have shown that the tricalcium silicate cements are bioactive and can induce bone-like apatite formation in simulated body fluid [19,20], which is partly attributed to the release of silicate ions. The silicate ions are also known to be an effective additive to improve the bioactivity and dissolution rate of hydroxyapatite [21–23]. Furthermore, these materials have the potential to activate bone-related gene expression and stimulate cell proliferation [19,20,24]. It has also been proven that the hydration product of tricalcium silicate cement is degradable in simulated body fluid (SBF) [20]. However, this material shows a rather low self-setting rate, and the setting time of the cement paste is too long (>60 min), which may not be suitable for orthopaedic applications [20].

In this paper, considering the advantages and disadvantages of the brushite and Ca_3SiO_5 cements, composite cements were designed and prepared by mixing the β-TCP/MCPM brushite cement system with bioactive Ca_3SiO_5. The setting time, workability, mechanical properties, in vitro bioactivity and cytotoxicity of the β-TCP/MCPM/C_3S composite cements were evaluated and compared with those of the brushite cement.

2. Materials and methods

2.1. Material preparation and characterization

The brushite cement used in this study was prepared by mixing two phosphate powders in the presence of water. The starting powders were β-TCP (synthesized in-house) and monocalcium phosphate monohydrate (Sinopham Chemical Reagent Co., Ltd). The weight ratio of the two components (β-TCP:MCPM) is 3:2, and they react in the presence of water to form precipitated brushite (dicalcium phosphate dehydrate) according to he reaction presented below [8,25].

$$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O$$

Tricalcium silicate (Ca_3SiO_5) powders were prepared by the sol–gel method, as previously described [19], and they were ground and sieved through a 300-mesh sieve (50 μm). To prepare the brushite/Ca_3SiO_5 composite cements, Ca_3SiO_5 powders (0–40 wt.%) were uniformly mixed with the brushite cement powders. To prepare paste samples, powders were mixed with deionized water using a solid/liquid (S/L) ratio of 2.0 g ml^{-1}.

The cement paste was prepared in a mortar, thoroughly kneaded with a spatula and poured into a cylindrical polytetrafluoroethylene mold. Air bubbles entrapped in the paste during mixing were allowed to escape by gently shaking the mold (6 mm diameter and 12 mm height). After demolding, the samples were placed in a 100% humidity water bath at 37 °C and kept for 24 h. The phase composition was characterized by X-ray diffraction (XRD; Geigerflex, Rigaku Co., Japan) using monochromated Cu K_α radiation, and the 2θ range was from 10° to 60° at a scanning speed of 10° min^{-1}. The cross-section of the samples was observed by scanning electron microscopy (SEM) using a scanning electron microscope (JSM-6700F, JEOL, Tokyo, Japan) equipped with an energy dispersive X-ray (EDX) detector (INCA Energy, Oxiford Instruments, UK).

2.2. Setting times and injectability

The setting times of the composite pastes with 0%, 10%, 20%, 30%, and 40% content of Ca_3SiO_5 were measured with the Vicat needle according to ISO9597-1989E. To test the initial setting time of the paste, a needle weighing 280 g and of 1.13 mm diameter is lowered into a specimen of fresh cement paste and the penetration depth is recorded. The cement paste is kept in a standard frustum 40 mm in height. The initial setting time is when the needle penetration is 35 ± 1.0 mm. The final setting time is defined as the time necessary so that the heavy needle (350 g, Ø 2.0 mm) no longer leaves a visible print on the surface of the paste. The measurement was conducted on five specimens from a group of the same composition, and then the average value was calculated.

The injectability of the composite paste was evaluated by extruding a certain amount of paste through a disposable syringe by hand according to a modified method described previously [19,26,27]. The syringes have a capacity of 2.5 ml, with an opening nozzle diameter of 2.0 mm. Six grams of as-prepared paste was added into the syringe and, after storage in a water bath at 37 °C for 2 min, the paste was gently extruded from the syringe by hand until it could no longer be continued. Then the weight of the paste expelled from the syringe was measured and the injectability was calculated using Eq. (1) [27]. Each test was repeated at least three times and the average value was calculated.

$$\text{Inj}\% = \frac{\text{Paste weight expelled from the syringe}}{\text{Total paste weight before injecting}} \quad (1)$$

2.3. Mechanical test of the cement paste

For the compressive mechanical testing, the as-prepared slurries were poured into a cylindrical polytetrafluoroethyl-

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