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A novel injectable, cohesive and toughened Si-HPMC (silanized-hydroxypropyl methylcellulose) composite calcium phosphate cement for bone substitution

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ABSTRACT

This study reports on the incorporation of the self-setting polysaccharide derivative hydrogel (silanized-hydroxypropyl methylcellulose, Si-HPMC) into the formulation of calcium phosphate cements (CPCs) to develop a novel injectable material for bone substitution. The effects of Si-HPMC on the handling properties (injectability, cohesion and setting time) and mechanical properties (Young's modulus, fracture toughness, flexural and compressive strength) of CPCs were systematically studied. It was found that Si-HPMC could endow composite CPC pastes with an appealing rheological behavior at the early stage of setting, promoting its application in open bone cavities. Moreover, Si-HPMC gave the composite CPC good injectability and cohesion, and reduced the setting time. Si-HPMC increased the porosity of CPCs after hardening, especially the macroporosity as a result of entrapped air bubbles; however, it improved, rather than compromised, the mechanical properties of composite CPCs, which demonstrates a strong toughening and strengthening effect. In view of the above, the Si-HPMC composite CPC may be particularly promising as bone substitute material for clinic application.

1. Introduction

The applications of calcium phosphate cements (CPCs) have been increasing in different fields, such as orthopedic surgery, dentistry, maxillofacial surgery and reconstructive surgery [1–3], since they were first proposed by LeGeros et al. [4] and Brown and Chow [5] in the early 1980s. CPCs are commonly considered as good candidates for bone substitution because of their good biocompatibility, bioactivity and bone-replacement ability (osteoconductivity) [6,7]. These materials are obtained by mixing one or several reactive calcium phosphate powders with an aqueous solution to form a paste that hardens in vivo through a low-temperature setting reaction [8,9]. The products formed in this setting reaction have many similarities with the mineral phase of the natural bone.

A major advantage of CPCs compared to traditional calcium phosphate bioceramics is that they are usually injectable and can self-set in vivo in the bone cavity without machining. This property is important in clinical applications that involve intricate bone cavities or narrow defect sites, and favors the development of minimally invasive surgical techniques [10,11]. As bone-filling cements, CPCs have to be distinguished from the classic bone "cement" commonly used to fix joint prosthesis, namely polymethyl methacrylate, which is not degradable and often causes necrosis of the surrounding tissue due to either the high curing temperatures or the effects of leaching of methyl methacrylate monomer [12,13].

Although CPCs appear highly promising for bone regeneration and have already been commercialized [14,15], it is generally accepted that there are still some crucial issues that need to be addressed to satisfy clinical requirements and the needs of surgeons [16,17]. Specifically, CPCs without any additives normally demonstrate poor injectability due to liquid–solid phase separation (so-called filter-pressing) [18,19]. Moreover, extraosseous cement leakage has been reported to be a major complication in both acrylic and calcium phosphate cement applications relevant to vertebroplasty or kyphoplasty procedures [20]. In most cases, the purely inorganic CPC pastes tend to disintegrate upon early contact with blood or biological fluids due to their weak cohesion [21]. The release of calcium phosphate particles into the blood

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stream might cause some risks, such as pulmonary embolism and cardiovascular deterioration, by stimulating blood coagulation [22]. Besides the poor handling properties, another challenge facing CPCs is that their mechanical properties are much different from those of the cortical or even the cancellous bone, not only in terms of strength, but especially in terms of toughness, ductility and fatigue resistance [23], preventing them from being applied in load-bearing places. In addition, due to their high brittleness, any overload may cause cracking of the material and the release of debris in the body.

Numerous studies have been dedicated to improving the aforementioned performances of CPCs for broader clinic applications. Previous studies have shown that CPC performances can be tailored by changing different factors, including cement composition, particle size, the liquid-to-powder (L/P) ratio [24–27] and preparation processing (such as compaction) [28,29]. In addition, many organic or inorganic additives [21,30], such as citric acid [31–33], chitosan [34,35], cellulose ethers [36,37], gelatin [38], collagen [39], sodium alginate [40], hyaluronic acid [41], polymer fibers [23,42–44] and their admixtures, have been added either in the powder phase or in the liquid phase to improve the handling and mechanical properties of CPCs.

Among these approaches, incorporating polymers into the formulation of CPCs is attracting much attention because it has been considered as a useful strategy to overcome the intrinsic limitations of inorganic CPCs [45]. The cellulose ethers, such as methylcellulose and hydroxypropyl methylcellulose, are particularly interesting as polymer candidates due to their good biocompatibility and rheological properties [2,46]. Even small amounts (a few weight per cent) of cellulose ethers can not only dramatically improve the injectability and cohesion of CPC pastes [2,45], but also increase the fracture toughness of hardened CPC products [36,37]. However, cellulose ethers generally tend to delay the setting time of CPCs. A long setting time could cause problems, especially in some traumatology cases, because of the inability of the cement to support stresses during this time period [26]. On the other hand, despite good cohesion, composite CPC pastes (especially with high L/P ratios) generally tend to flow away (in other words, they cannot maintain their shape and/or position) from the place where they are implanted at the initial stage, which restricts their application to only closed bone defects (cavities).

One aim of this study, therefore, is to develop novel injectable composite CPCs which not only have a general improvement in both injectability and cohesion but can also avoid prolonged setting time and can overcome the tendency of flowing away from the implant site, as found in many polymer composite CPCs. To this end, a self-crosslinkable polysaccharide derivative, namely silanized hydroxypropyl methylcellulose (Si-HPMC), which is a cellulose-based hydrogel that has already been proposed for biomedical applications [47,48], appears to be an attractive candidate. The rationales of using Si-HPMC are as follows: first, Si-HPMC hydrogel has been shown to be biocompatible and to be a potential scaffold for three-dimensional culture and differentiation of osteoblastic [49] and chondrocytic [50] cells in tissue engineering; moreover, due to its particular rheological properties and self-crosslinking, Si-HPMC has been mixed with biphasic calcium phosphate ceramic particles to prepare injectable bone substitutes [51,52], effectively suppressing the long-term flow of the latter. It is therefore expected that the addition of Si-HPMC into CPC may also restrain its flow from implant sites.

Strength, especially compressive strength, has been widely tested in studies of CPCs as a criterion (often the only criterion) evaluating their mechanical performance. However, this notion appears to be inadequate in many cases. On the one hand, since complex three-dimensional loads are often applied at the site of implant, other mechanical properties (e.g. bending (tensile) strength and shear strength, as well as the corresponding elastic modulus) also need to be taken into account. On the other hand, and more importantly, strength is not an intrinsic property, but depends both on the size of defects (e.g. pores) and on fracture toughness, which is a real limitation for CPCs. In fact, it is poor fracture toughness, describing the resistance of a material to crack propagation, and high brittleness which prevent CPCs from being used in load-bearing places. Unfortunately, however, unlike the abundant studies on strength, fracture toughness is sparsely reported in the literature [53]. Therefore, the other aim of the study is to systematically investigate, after hardening, the mechanical properties of Si-HPMC composite CPCs, including compressive strength, flexural strength, Young's modulus and fracture toughness, and to try to relate these properties to microstructural features (such as porosity, pore size, crystal size and morphology), which are the intrinsic factors determining the mechanical performance of materials but are often ignored.

In view of all the above-mentioned aims, in the present work Si-HPMC is incorporated into α-tricalcium phosphate (TCP)-based apatitic CPCs, and the effects of Si-HPMC on the handling properties (e.g. injectability, cohesion and setting time) and mechanical properties (e.g. Young's modulus, fracture toughness, flexural strength and compressive strength) of the composite CPCs are systemically investigated.

2. Materials and methods

2.1. Fabrication of the solid phase of CPCs

All of the reagents used in this study were of analytical grade and were used without any further purification. The α-TCP powder was synthesized by heating a mixture of dicalcium phosphate anhydrous ($CaHPO_4$; Alfa Aesar, Germany) and calcium carbonate ($CaCO_3$; VWR, BDH, Prolabo) with a molar ratio 2:1 at 1360 °C for 15 h and subsequently quenching it to room temperature. The phase purity of the synthesized α-TCP powder was checked by using X-ray diffraction (XRD, X'pert pro, PANalytical, Netherlands) and no other phase was found. To prepare the solid phase of CPCs, 2 wt.% of precipitated calcium-deficient hydroxyapatite (CDHA; Ca/P ratio: 1.6; BET specific surface area: $125\ m^2\ g^{-1}$; average crystal size: 23.1 nm [54]) was added to the α-TCP powder as a seed for subsequent crystallization of apatite and the powder mixture was milled in a Mortar Grinder (Retsch RM100, Germany) for 1 h to get a fine powder. The mean particle size of the fine powder mixture was 6 μm, as determined by laser diffraction granulometry (Beckman Coulter LS230, USA) after dispersion in ethanol in an ultrasonic bath.

2.2. Preparation of the liquid phases of CPCs

The liquid phases of Si-HPMC composite CPCs were prepared by mixing Si-HPMC solutions and NaH_2PO_4 solution. Si-HPMC powder, the synthetic procedure of which was detailed by Bourges et al. [55], was dissolved in NaOH solution to prepare Si-HPMC solutions of different concentrations (2, 3 and 4% w/v, 1% w/v = $10\ g\ l^{-1}$), using a method described by Fatimi et al. [56]. The final pH value of the Si-HPMC solutions was around 12.8.

Si-HPMC solutions are stable in strong basic media (pH > 12.1). When the pH decreases, the gelation of Si-HPMC takes place, and the Si-HPMC solution transforms into a hydrogel with a three-dimensional network of Si-HPMC chains [56–58]. The gelation principle of the Si-HPMC is illustrated in Fig. 1. In this study, a 30 wt.% NaH_2PO_4 solution was mixed with Si-HPMC solutions in a syringe for 20 s to initiate the gelation of the latter, forming the liquid phases of CPCs, with the final pH ranging from 7 to 8. During the above mixing process, Si-HPMC starts to gel, and the