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Incorporation of chitosan-alginate complex into injectable calcium phosphate cement system as a bone graft material

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ABSTRACT

Calcium phosphate brushite type of cements have been used to replace bone graft materials because of their biocompatibility and other attractive features. Especially, injectability of cement allows easy handling of minimally invasive surgical techniques. New calcium phosphate cement (CPC) system, brushite based cement incorporated into polyelectrolyte complex, was developed in this study. Chitosan-alginate complex produced by an interaction between a cationic polymer (chitosan) and an anionic polymer (alginate) was loaded in the cement. This improved the functional properties and biocompatibility of the final cement. We optimized the liquid/solid (L/S) ratio of the cement components and investigated the compressive strength, setting time, pH change of CPC0 (with only citric acid) and CPC0.5, 1, and 1.5 (0.5, 1, and 1.5 v/v % chitosan-alginate complex in citric acid solution, respectively). The L/S ratio did not affect structural formation, while the addition of polymer complex showed new formation of macro-pores within CPC. However, a lower L/S ratio and higher amount of added polymer complex shortened the setting time and improved the compressive strength. The appropriate conditions for the injectable bone substitute were CPC1 with an L/S ratio of 0.45. To investigate the effect of the chitosan-alginate complex on CPC system in physiological conditions, CPC0 and CPC1 were implanted in a rabbit femoral head defect model for 1 and 3 months. Micro-computed tomography revealed improved bone formation in CPC1 compared to CPC0 3 months after implantation. Histological analysis revealed newly formed bone tissues around the peripheral sides of CPC0 and CPC1. The results indicate the potential value of the CPC system containing polymer complex as an injectable bone substitute. The study of the CPC-polymer complex system incorporating drugs or cells can be further developed into a controlled release system for faster bone regeneration.

1. Introduction

Calcium phosphate cements (CPCs) are popular as injectable bone substitute materials to reconstruct bone defects. CPCs consist of one or more calcium orthophosphate powders, which form a paste when mixed in an aqueous solution [1]. The paste is self-setting and hardens after being implanted in the body. These properties are advantageous for minimally invasive surgical methods in clinical use. In addition, CPCs can effectively fill irregularly shaped bone defects. There are two possible final products for the CPC reaction, depending on the starting materials and their pH. One is brushite (dicalcium phosphate dihydrate, DCPD) and the other is apatite, such as hydroxyapatite (HA) and calcium-deficient hydroxyapatite (CDHA) [2,3]. The final products are similar to the inorganic part of bone [4].

Although CPCs appear highly promising for bone regeneration, some critical issues remain unresolved that have hampered the clinical use [3]. Self-setting CPCs generally have a dense microstructure. The lack of a porous structure is a substantial disadvantage, since the slow degradation behavior by osteoclast-dependent resorption over time decreases the potential of tissue in-growth [4,5]. Another main challenge is that they have poor mechanical properties in general, in terms of toughness, brittleness and reliability [3]. An approach that is getting more attention in the CPC field is to combine polymers into the CPC formulation, either as a second powder phase or dissolved in the aqueous phase. This may be an excellent option to improve CPC performance, enhance properties that are relevant for the clinical use of these

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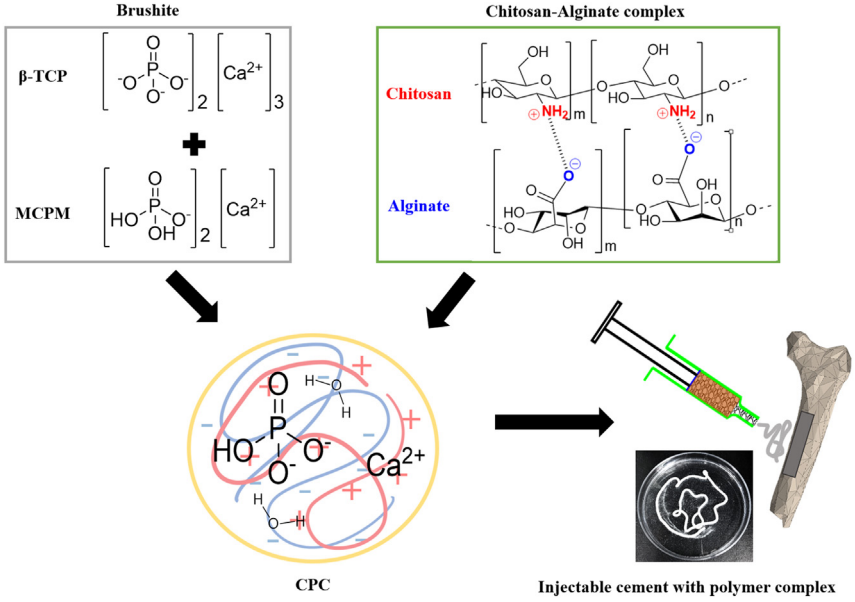

Fig. 1. Schematic representation of the molecular interaction of chitosan-alginate complex and CPC design.

materials, and improve the final performance in terms of resorption rate and cell response [6].

In this study, a polymer complex was incorporated into the brushite cement system to improve CPC properties. Chitosan-alginate complex has previously been applied to a different type of cement such as hydroxyapatite. [7] Nevertheless, as best of our knowledge, this study is the first attempt to apply it to brushite-based cement system with a polymer complex. Several compositions have been proposed for brushite cements, most of them containing beta-tricalcium phosphate (β-TCP) and an acidic component, monocalcium phosphate monohydrate (MCPM) or phosphoric acid [8,9]. In this study, MCPM and β-TCP were selected as a solid phase, and a chitosan-alginate polymer dissolved in aqueous solution was used as a source of liquid phase. The negatively charged surface of alginate can create a polyelectrolytes complex by reaction with positively charged chitosan in the liquid phase (Fig. 1) [10]. *In situ* electrostatic attraction from the chitosan-alginate polymer enhances heterogeneous agglomeration into the formed CPC matrix [11].

The objective of this study is to investigate the potential of incorporation system of chitosan-alginate complex and brushite cement as an injectable bone substitute. CPC containing polymer complex was optimized by characterization of morphology, chemical, and mechanical properties depending on the mass ratio of liquid/solid (L/S) and on polymer complex amount. The final CPC systems were implanted in a rabbit defect model for 1 and 3 months (See Table 1). The effect of polymer complex contents in the CPC system on biological degradation and bone regeneration *in vivo* was determined using micro-computed tomography (micro-CT) and histological analysis.

Table 1
Distribution of the animals per group and time points.

Follow-up time	Condition	Number (n)	Implant size
1 month	CPC 0	3	6 × 10 mm cylinder
	CPC 1	3	
3 months	CPC 0	3	
	CPC 1	3	

2. Materials and methods

2.1. Preparation of CPC containing polymer complex

β-TCP (Innobone. Co., Ltd., South Korea) powder was mixed with MCPM (Duksan Chemicals, South Korea) to produce a cementing reaction with 60% and 40% mass fraction, respectively. This solid mixture was mixed with 0.25 M citric acid (Samchun Chemical, South Korea) with different L/S mass ratios (0.4, 0.45, and 0.5). Such ratios were chosen in order to produce pastes that exhibited workable consistencies and were convenient for clinical use. The corresponding cement product was labeled as CPC0 (without polymer complex content in liquid phase). Biodegradable polymers were added into liquid phase to generate the polyelectrolyte complex.

Chitosan (Sigma-Aldrich, USA) and alginate acid sodium salt from brown algae (Sigma-Aldrich, USA) were separately dissolved in 0.5 M citric acid and distilled water, respectively. Both solutions were prepared to a concentration of 0.5, 1, or 1.5 v/v %. Chitosan and alginate solution were mixed well at a ratio of 1:1. *In situ* electrostatic attraction from the oppositely charged polymer solution turned the solution into a gel matrix.

The chitosan-alginate complex solution containing and TCP/MCPM powder were mixed well using a spatula. The cement was quickly transferred to a mold. The CPC samples were placed in a water bath with 100% humidity and 37 °C until the setting steps are completed. The final CPC samples with chitosan-alginate were labeled as CPC0.5, CPC1 and CPC1.5 according to polymer solution's concentration.

2.2. Characterization of CPC

2.2.1. Structural and chemical analysis

The reaction products were examined by X-ray diffraction (XRD) using an Iniflex2 apparatus (Rigaku, Japan). Raw β-TCP, MCPM, and CPC0 with different mass ratio of L/S (0.4, 0.45, and 0.5) were characterized by 2θ ranging from 10° to 80° at a scanning speed of 2°/min. To compare the morphology after the addition of chitosan-alginate complex, the surfaces of CPC0 and CPC1 with L/S ratio of 0.45 were sputter-coated with platinum using a model 108 Auto device (Cressington Scientific Instruments, UK) and observed by scanning electron microscopy (SEM) using a JSM-6701F micropscope (JEOL, Japan).