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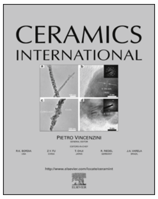

Synthesis of β-TCP and CPP containing biphasic calcium phosphates by a robust technique

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ABSTRACT

Biphasic calcium phosphate (BCP) compositions consisting of β-tricalcium phosphate (β-TCP) and calcium pyrophosphate (CPP) are potential biodegradable ceramics for bone regeneration. The present work demonstrates the formation of such dense ceramics by first preparing the precursors of nano-sized, amorphous, and equiaxed calcium pyrophosphate particles, and then sintering the precursors at 900 °C to transform them into desired BCP. However, if the complex of calcium tripolyphosphate was used, only CPP could be generated. It was also observed that the incorporation of Mg^{2+} had several effects on the resulting products including: (1) promoting the generation of meso-porous precipitates; (2) favoring the formation of β-TCP instead of CPP; (3) reducing the grain size and increasing the density of the sintered ceramics, and (4) enhancing the negative electric charge of the BCP surface. Thus, the as-prepared BCP ceramics can serve as potential bone substitute materials in orthopedic applications.

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1. Introduction

The motivation behind this paper is derived from our long-standing effort in developing: (1) non-conventional biphasic calcium phosphate (BCP) compositions as bone substitutes, and (2) robust synthesis of such bioceramics. From a historical viewpoint, conventional BCP compositions have been developed as composite materials consisting of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and β-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$). As Daculsi et al. argued in their seminal paper, optimized combination of HA and β-TCP can result in controlled biodegradation [1]. However, the combination of HA and β-TCP is not necessarily the only choice for developing such materials. As we reported previously, BCP materials containing HA and β-rhenanite (β-NaCa-PO4), an alkali calcium orthophosphate, can result in even higher expression levels of osteogenic markers as compared to those containing HA and β-TCP [2]. It is important to note that Na^+ is present in both bone mineral and body plasma, and therefore the presence of Na^+ in the BCP can affect its biodegradability and osteogenicity. In addition, Li et al. reported yet another strategy to create BCP by combining α-and β-TCPs [3]. Interestingly, all of the aforementioned avenues have used the orthophosphates. In comparison, its lesser-known counterpart, the calcium pyrophosphate (CPP, $Ca_2P_2O_7$) remains relatively unexplored. In the following we give a brief review/critique of the previous attempts to incorporate CPP into BCP.

Lin et al. reported that addition of $Na_4P_2O_7.10H_20$ results in efficient sintering of β-CPP powders to high density, and β-CPP is more resorbable than TCP since it has a Ca/P ratio equal to 1 [4]. However, when evaluated in animal models, the sintered samples also contain $NaCa(PO_3)_3$, macropores, as well as hydrolytic enzymes such as alkaline phosphatase (ALP) [4]. These factors can all contribute to the resorption of β-CPP *in vivo*. On the other hand, Banerjee et al. reported the formation of BCPs consisting of β-TCP and β-CPP [5]. Their results of *in vitro* tests show that the BCP material containing β-CPP gains less weight and exhibits less extent of apatite deposition as compared to that containing β-TCP.

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This indicates that β-CPP *in vitro* may in fact be more stable than β-TCP, probably due to the absence of hydrolytic enzymes. Therefore, the results as discussed in the above are inconclusive with regards the role of CPP in BCP. Nevertheless, in a more recent and comprehensive study, Grover et al. reported that incorporation of CPP in brushite-based cement stimulates bone growth *in vivo* due to the presence of ALP [6]. This finding seems to indicate that there is merit in pursuing the synthesis of BCP incorporating CPP.

From the viewpoint of synthesis processing, BCP composed of β-TCP and CPP can be formed by different methods. For example, Banerjee et al. reported a method in which TCP-CPP precursors are first prepared using a chemical technique *via* adding phosphoric acid to 0.1 M calcium nitrate with a Ca/P ratio of 1.25, and then the precursors are calcinated at 800 °C to obtain desired BCP ceramic compositions [5]. Mitsionis et al. used a sol-gel method with the assistance of citric acid and 900 °C calcination to produce BCP [7]. Wagner et al. used a microwave sintering method to produce BCP from brushite (DCPD, $CaHPO_4 \cdot 2H_2O$) [8]. Among all these methods, the CPP phase is formed mainly due to the thermal conversion of calcium phosphate precursors, and the resulting products from either brushite or monetite (DCPA, $CaHPO_4$) are mostly micro-sized plate-like particles. For sintering highly dense ceramics, however, it is preferable to use powders consisting of reactive, nano-sized equiaxed particles to obtain highly dense products [9,10]. For example, Safronova et al. prepared amorphous hydrous CPP as precursors for the preparation of crystallized pyrophosphate ceramics [11]. They also used $CaCl_2$ to react with $Na_4P_2O_7 \cdot 10H_2O$ under ball milling to produce an amorphous precursor [10]. The resulting product contains CPP, $NaCaPO_4$ and $Ca_5(PO_4)_3Cl$ after sintering at 900 °C due to the presence of Na^{2+} and Cl^- in the process. In our previous work, we have shown that CPP can be easily synthesized as amorphous nano-sized equiaxed particles *via* thermal treatment of calcium sodium tripolyphosphate [12].

Thus we hypothesize that a simple modification of the technique used in our above work can lead to a new and robust method for synthesis of BCP consisting of CPP and β-TCP. Indeed, a thorough literature search did not reveal any previous work along these lines. Hence is the choice of topic for this study. An additional effort in this study was to evaluate the potential effects of Mg^{2+} as additive on the synthesis process. There are several reasons behind the choice of Mg^{2+}. First, Mg^{2+} is the fourth abundant ion in human body and most of it is present in bones. Second, Mg^{2+} is known to increase the temperature of β to α transformation in TCP, and thus tends to raise the temperature for effective sintering of β-TCP [13]. Third, Mg^{2+} tends to help retain the amorphous phase of calcium phosphates [14]. Finally, Mg^{2+} tends to help stabilize β-TCP as opposed to β-CPP [15].

2. Material and methods

All the chemicals used were purchased from Aladdin (Shanghai, China). Initially, 110.98 g $CaCl_2$ was dissolved in 1 L water (H_2O), followed by adding 1.329 g of $Na_4P_2O_7$ (SPP), or 1.226 g $Na_5P_3O_{10}$ (STPP) respectively into solution with continued magnetic stirring for 30 min (Table 1). The nominal Ca/P molar ratio of all reactants was set to 10/1 to ensure no sodium calcium tripolyphosphate or sodium calcium pyrophosphate formed. The precipitates were filtered and collected, followed by drying at 60 °C in an oven overnight. To study the effects of Mg^{2+} incorporation, 50% of Ca^{2+} was replaced with Mg^{2+} in SPP and STPP groups. These precipitates were characterized using x-ray diffraction (XRD, D/MAX2500, Rigaku, Japan), Fourier Transform Infrared Spectroscopy (FTIR, PROTÉGÉ 460, Nicolet, USA) and scanning electron microscopy (SEM, SUPRA 55, Zeiss, Germany) equipped with energy dispersive X-ray spectroscopy (EDS). The chemical stability of the precipitates was studied *in vitro* by soaking the material samples in simulated body fluid (SBF) for up to 3 weeks and subsequently examining them using XRD, as reported before [16].

The precipitates were ground into powder and then pressed into pellets using a mold of 1.2 cm diameter and at a pressure of 4 MPa. The as-prepared pellets were loaded into a furnace for sintering. The temperature was raised to 900 °C with a rate of 3.5 °C/min and held for 4 h before cooling in air flow. The thermal decomposition of the precipitates was measured using a thermogravimetric analyzer (TGA, SDT Q600, TA Company, USA). The resulting materials were characterized using XRD and SEM. The cytocompatibility of either the precipitates, or the calcined materials was studied using MC3T3-E1 osteoblastic cells, respectively. For the precipitates, the powder was incubated with the cell culture medium (1000 mg/mL powder in 500 μL medium) for 48 h prior to use. Subsequently, MC3T3-E1 cells were seeded to the bottom surface of each cell culture well at the density of 10,000 cells/well. Immediately after seeding the cells, the medium pre-incubated with the powder was extracted and added to the wells. For the calcined pellets, MC3T3-E1 cells were seeded directly onto the surface of each pellet at a density of 10,000 cells/pellet, which was placed in the cell culture well with 500 μL of the fresh cell culture medium. Afterwards the cells in either the pre-incubated or the fresh medium were further incubated for up to 7 days with the respective medium refreshed every other day. At day 1 and 7, the cultured cells were measured for cell density using MTT assay. Precipitates from the solution of 110.98 g/L $CaCl_2$ and 1.56 g/L $NaH_2PO_4 \cdot 2H_2O$, and corresponding pellets calcined at 900 °C were obtained and studied in the same way as described above, and then used as controls in this study.

3. Results

The XRD patterns of the as-precipitated powders are shown in Fig. 1a. All the powders displayed the characteristics of amorphous phase except for some small but unidentified peaks. The presence of condensed phosphates as well as the incorporation of Mg^{2+} appeared to have no effect on the diffraction patterns of the precipitates. After incubation in SBF for 3 weeks, the powders mainly preserved their amorphous phases (Fig. 1b). However, in the case of Ca-PP, the pattern of DCPD (PDF#09-0077) was observed. After calcination at 900 °C (Fig. 1c), the precipitates of Ca-PP and Ca-TPP were converted into β-CPP (PDF#09-0346) and a mixture of β-CPP

Table 1
Compositions of 1 L aqueous mixture to prepare precipitates.

Group	$CaCl_2$ (g)	$MgCl_2 \cdot 6H_2O$ (g)	Phosphate Source (g)		(Ca+Mg)/P	Ca/Mg
Ca-PP	110.98	–	$Na_4P_2O_7$	1.329	10	–
Ca-TPP	110.98	–	$Na_5P_3O_{10}$	1.226	10	–
Ca–Mg-PP	55.49	50.83	$Na_4P_2O_7$	1.329	10	1
Ca–Mg-TPP	55.49	50.83	$Na_5P_3O_{10}$	1.226	10	1

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