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Effect of PEG amount in amorphous calcium phosphate on its crystallized products

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Abstract Biphasic α-tricalcium phosphate/β-tricalcium phosphate (α/β-TCP) with a designed phase ratio is thought to have controllable dissolution–reprecipitation behavior that is significant in the repair and regeneration of bone. Amorphous calcium phosphate (ACP) was selected as a precursor to prepare biphasic α/β-TCP. The influence of polyethylene glycol (PEG) content in ACP on its crystallization, or on the phase ratio of the resulting biphasic TCP, was investigated. ACP was synthesized by the reaction of $Ca(NO_3)_2$ with $(NH_4)_2HPO_4$ using PEG as an additive. Depending on the amount of PEG addition, resulting ACP could be crystallized to α-TCP, β-TCP or biphasic α/β-TCP after heat-treatment at 800°C, showing that PEG addition is a critical factor to tailor the phase ratio of biphasic α/β-TCP. One reason for the influence of PEG is that ACP with different PEG content could have two types of unit structures that tend to form α-TCP and β-TCP after crystallization.

1 Introduction

Tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) is highly biodegradable in body fluids compared with hydroxyapatite (HA; $Ca_5(PO_4)_3OH$) [1, 2]. TCP can form different polymorphs with different biodegradation behaviors. β-TCP (low-temperature phase) commonly dissolves into calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) in body fluids; these ions have an osteoconductive role [3] and the dissolved area permits ingrowth of new bone [4]. α-TCP (high-temperature phase [5]) usually hydrolyzes to form HA or octacalcium phosphate (OCP) through a rapid dissolution–reprecipitation process [6]. This process results in hardening of bone cements and modifying of surface morphology or microstructure of porous biocomposites [3]. The two TCP polymorphs can therefore contribute to the repair and regeneration of bone.

Optimization and maximization of the properties of calcium phosphate can be achieved by forming biphasic calcium phosphates such as biphasic β-TCP/HA [7]. The latter has been demonstrated to have much better biodegradability and osteoconductivity than single-phase HA or β-TCP [8]. Optimal combination of α-TCP and β-TCP could have controllable dissolution–reprecipitation behavior, which could result in a desired ion-release process, biodegradation rate and morphologic variation to promote bone formation. There are some reports on biphasic α/β-TCP materials with a fixed phase ratio, and in-vivo results showed that biphasic α/β-TCP has a similar biodegradation rate with α-TCP, as well as a good ability to promote bone growth [5, 9].

A mechanical mix of α-TCP and β-TCP is the simplest method to prepare biphasic calcium phosphate, but the two phases in the mixture are not dispersed homogenously, thus reducing biological performance. Incomplete phase transformation is another method. Biphasic α/β-TCP is thought to be readily obtainable by calcining β-TCP powders at a temperature near the phase transformation temperature (1,400°C) of β-TCP to α-TCP [10–12]. Calcination or

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sintering at such a high temperature leads to large particle size and broad size distribution, and it is difficult to control the phase ratio of biphasic α/β-TCP.

Amorphous calcium phosphate (ACP) has attracted growing attention due to its applications in biomaterials. Its synthetic techniques [13], characterizations [14] and modeling [15] have been reported. Our previous work showed that ACP stabilized by polyethylene glycol (PEG) was a good precursor that could be crystallized to form nano-sized biphasic α/β-TCP particles after heat-treatment [16].

The phase ratio of biphasic α/β-TCP demonstrates strong dependence on ACP formation conditions or heat-treatment temperature for crystallization. For example, β-TCP content in biphasic α/β-TCP powders increased with increasing pH and aging time during ACP precipitation in solutions [8], and also with increasing temperature of heat-treatment (800–900°C) [17]. It is important to understand the influence of preparation conditions which could guarantee obtaining biphasic α/β-TCP powders not only with a designed phase ratio, but also with reproducibility and reliability.

This study focused on the influence of PEG addition during ACP precipitation, the relation of content of PEG addition with ACP formation and the influence of PEG content on the phase ratio of the ACP-derived α/β-TCP biphasic particles.

2 Materials and methods

PEG (molecular weight, 10000; Sinopharm Chemical Reagent Company Limited) was dissolved in an aqueous solution of 0.1 M $Ca(NO_3)_2$ (500 ml; Sinopharm). The amount of PEG addition was designed to produce the ethylene glycol/calcium (EG/Ca) ratios of 1:8, 1:4, 1:1, 4:1, 8:1, 12:1 to 16:1. An aqueous solution of 0.15 M $(NH_4)_2HPO_4$ (Huzhou Hushi Chemical Reagent Company Limited) was added drop-by-drop into $Ca(NO_3)_2$ solution with PEG to realize a Ca/P ratio of 1.5. During the precipitation reaction, the basicity (pH 10) was maintained by adding NH_4OH (Hangzhou Changzheng Chemical Reagent Company Limited). After addition of $(NH_4)_2HPO_4$ solution, the reaction was allowed to proceed for 30 min. The reaction vessel was situated in an ice-water bath until the reaction ended. When the precipitation reaction was complete, the resulting precipitates were filtrated, washed, frozen and freeze-dried in a lyophilizer (FD-1A-50, Boyikang Company) for 48 h. Freeze-dried powders were heat-treated for crystallization in a furnace at 800°C for 3 h at a heating rate of 10°C/min.

Phases of freeze-dried powders and crystallized powders were determined in an X-ray diffractometer (Rigakud/Max-C) at a step of 0.02° at a speed of 2°/min. To measure relative amounts of the two TCP phases in the biphasic powders, a calibration curve based on X-ray diffraction (XRD) peak intensity was established by five mixtures of α-TCP and β-TCP powders with different relative contents. Morphology of freeze-dried powders with different PEG content was observed in a transmission electron microscope (TEM, JEOL 1200). Thermal evolution of freeze-dried powders was measured by differential thermal analysis (DTA) and thermal gravimetry (TG) (SDT Q600, TA).

3 Results

XRD patterns of freeze-dried precipitates with different EG/Ca molar ratios during precipitation (Fig. 1) showed that PEG amount in the precipitation reaction had a clear influence on the resulting phase of the freeze-dried precipitates. When EG/Ca was $\leq$1:4, the precipitates contained a crystalline phase: apatite. When the ratio was $\geq$1:1, the precipitates were amorphous, indicating that ACP had been formed. The center of the broadened peak gradually shifted from 32° toward 31° with increasing EG/Ca molar ratio.

DTA curves (Fig. 2) showed a similar thermal evolution for all ACP samples with different EG/Ca molar ratios during precipitation. The following were noted in the measured temperature range: an endothermic peak in 65–120°C due to removal of free and adsorbed water; an exothermic peak in 165–275°C due to removal or pyrolysis of PEG; and an exothermic peak at about 650°C for ACP crystallization. The changes in water removal range were similar for all ACP samples in TG curves (Fig. 2), but the changes for removal or pyrolysis of PEG range had a clear

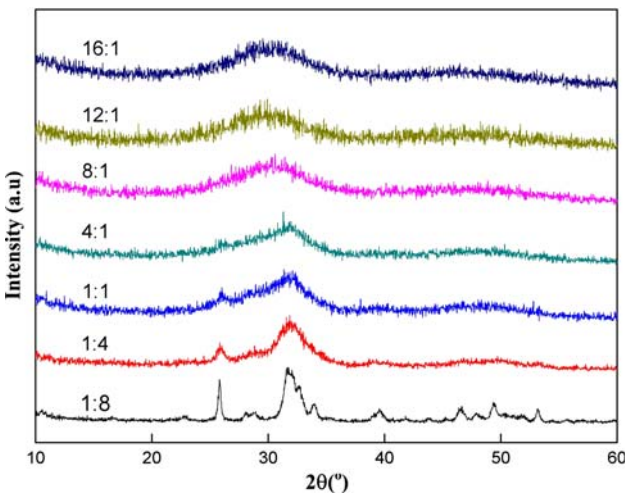

Fig. 1 X-ray diffraction patterns of freeze-dried precipitates with different EG/Ca molar ratios during precipitation

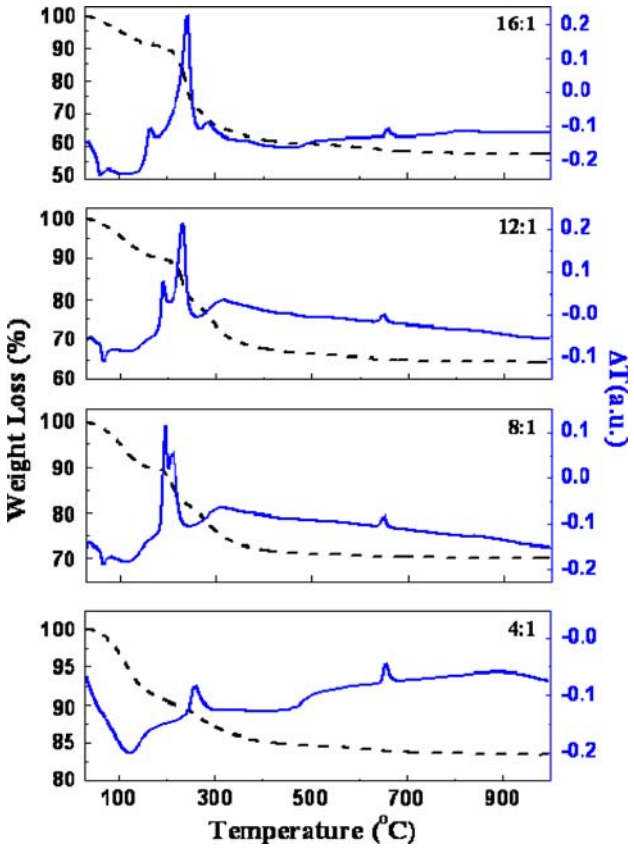

Fig. 2 Thermal gravimetry and differential thermal analysis curves of ACP prepared with different EG/Ca molar ratios

difference: weight loss increased with increasing EG/Ca molar ratio during ACP precipitation. In the relatively wide temperature range of 312–650°C (before crystallization), the gradual weight loss was due to the combustion of residual organic PEG or C. Based on the above analysis, the contents of water and PEG for different samples could be estimated (Table 1). Water content in ACP was in a narrow change range of 7.6–9.7 wt%, while PEG content in ACP varied in a large range of 8.4–33.2%. TEM observation of ACP samples (Fig. 3) revealed hollow particles with smooth surfaces; particles became more agglomerated and denser as PEG content increased. After heat-treatment at 800°C for 3 h, XRD patterns (Fig. 4) of ACPs showed that they were transformed to TCP crystalline phases with different polymorphs. PEG content in the ACP could be tailored to the phase ratio of biphasic TCP (Table 1).

4 Discussion

ACP is usually formed as a metastable phase when calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) in aqueous solution react to precipitate [18]. ACP is postulated to be built by $Ca_9(PO_4)_6$ clusters as aggregated spherical particles with inter-cluster spaces filled with water, which has the role of binder or stabilizer [19]. Aggregated particles readily dissolve and crystallize to form apatite, a thermodynamically stable phase, because the binding effect of water is not strong.

Freeze-dryed precipitates become ACP only if EG/Ca molar ratio $\geq$1:1 during precipitation (Fig. 1). PEG is thought to react with Ca^{2+} in the solution [20], substitute for water as a binder to form stable aggregates of $Ca_9(PO_4)_6$ clusters, and cover the aggregates (Fig. 3) to avoid their dissolution. The minimum amount of PEG for ACP formation is estimated to be 2.1 wt% or 5.2 vol% in dehydrated ACP based on TG data (Table 1). This implies that the long-chain PEG molecules have a key function in stabilizing ACP structure.

The shift of the center of the broadened peak from 32° toward 31° with increasing EG/Ca molar ratio (Fig. 1) implies a structural difference in the resulting ACP. It is reported that there are two types of ACPs: one is similar to the basic structure of β-TCP, with a broadened peak centered at $\sim$31°; another is similar to the basic structure of apatite, with a broadened peak centered at $\sim$32° [21]. PEG content during ACP precipitation could therefore affect the unit structures of ACP.

Increase of PEG amount in ACP promotes the joining of these hollow particles, causing heavy agglomeration of ACP particles (Fig. 3). The cavity in the particles could result from removal of absorbed water during freeze-drying of the prepared ACP precipitates.

ACP crystallized to single-phase TCP or to biphasic α/β-TCP after heat-treatment at 800°C (Fig. 4). With

Table 1 ACPs prepared with different EG/Ca ratios and the phase ratio of their crystallized products based on TG and XRD results

ACP sample	EG/Ca molar ratio during ACP precipitation	Content in ACP (wt%)		α-TCP (wt%)	β-TCP (wt%)
		Water	PEG		
ACP	1:1	8.5	2.1	100	0
ACP-A	4:1	7.6	8.4	100	0
ACP-B	8:1	9.7	20.2	67	33
ACP-C	12:1	8.9	26.3	33	67
ACP-D	16:1	8.6	33.2	0	100

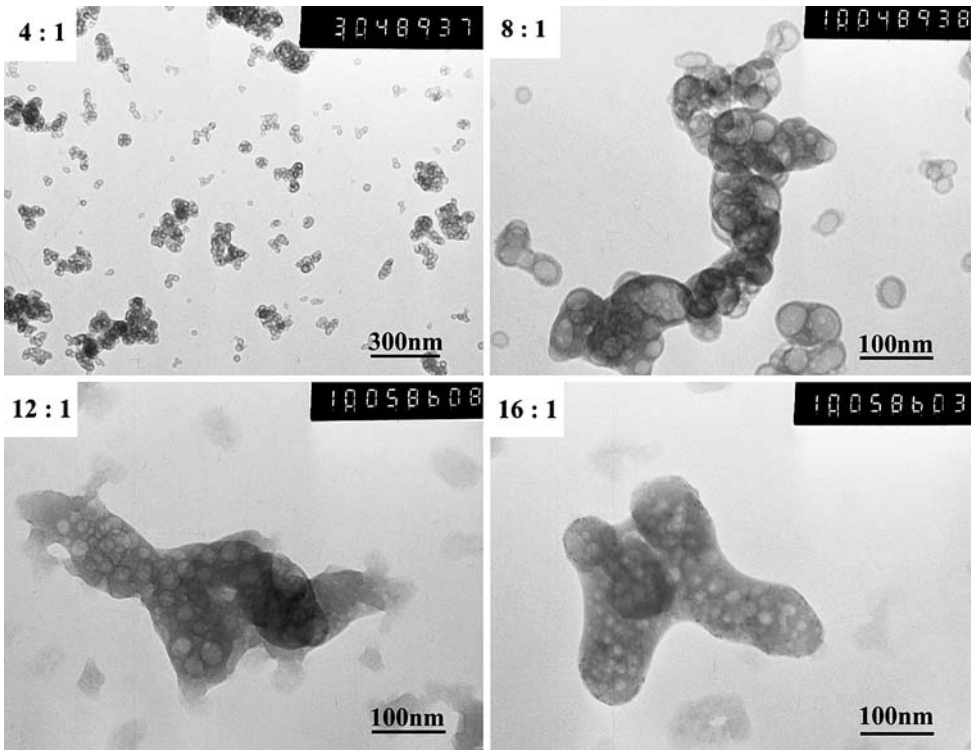

Fig. 3 Transmission electron micrographs of ACP prepared with different EG/Ca molar ratios

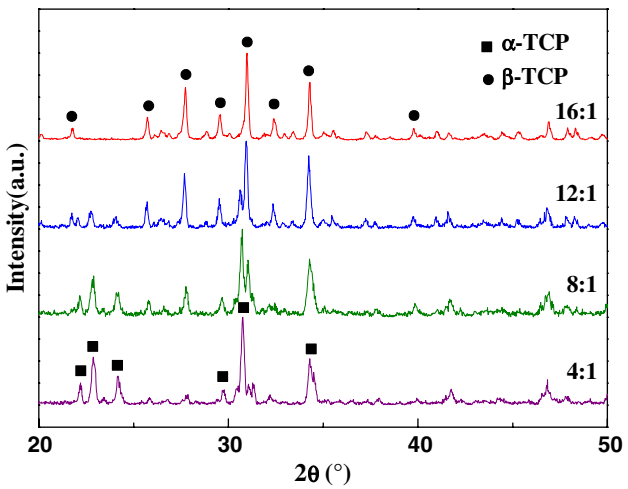

Fig. 4 X-ray diffraction patterns of heat-treated (800°C) ACP prepared with different EG/Ca molar ratios

increasing PEG content in ACP, TCP phase of the resulting crystallized precuts changed from single-phase α-TCP, biphasic α/β-TCP (33 wt% β-TCP), α/β-TCP (67 wt% β-TCP) to single-phase β-TCP (see Table 1).

The changing tendency of phase ratio with PEG content in ACP (Table 1) was in good agreement with the shift of the center of the broadened peak of the corresponding ACP (Fig. 1). ACP with the center of the broadened peak from 32° crystallized to pure α-TCP; ACP with a broadened peak centered at ~31° crystallized to pure β-TCP. This demonstrates that ACP crystallization to α-TCP or β-TCP is strongly related to the unit structure of ACP [22–24]. ACP containing the two types of ACPs stated above transforms to a biphasic α/β-TCP, and the phase ratio of the biphasic TCP depends on the relative content of two ACP phases, which may be characterized by the center of the broadened XRD peak between 31° and 32°.

5 Conclusion

ACP can be obtained through PEG addition during precipitation of calcium phosphate. ACP with different PEG content has differences in its unit structure, which leads to crystallization of α-TCP, biphasic α/β-TCP or β-TCP after heat-treatment. Also, PEG amount in ACP is a key factor to tailor the phase ratio of biphasic α/β-TCP after its crystallization.

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