Microwave hydrothermal synthesis of calcium phosphates using inorganic condensed phosphate salts as precursors

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Abstract

As a potential phosphate source, inorganic soluble condensed phosphate salts represent a viable alternative to both orthophosphate and organic condensed phosphate salts. The present work demonstrates a simple but versatile microwave hydrothermal method to prepare calcium phosphates using pyrophosphate and tripolyphosphate salts. After 5-minute microwave heating, white fine powders are precipitated from calcium and condensed phosphate containing solution. The as-synthesized precipitates are crystallized calcium pyrophosphate hydrate whiskers and calcium tripolyphosphate hydrate porous microspheres. After calcination, both ceramics are converted into calcium pyrophosphate. *In vitro* study reveals both precipitates and calcined powders are cytocompatible compared to microwave hydrothermally synthesized hydroxyapatite. With all of these attributes, it is expected different inorganic soluble condensed phosphate salts together with microwave can be used to generate a series of calcium phosphates with unique features.

Key Words

Bioceramics; Powder technology; Microwave; Inorganic condensed phosphate

1. Introduction

Microwave-assisted synthesis of calcium phosphates (CaPs) in liquid phase has attracted many research interests [1]. A variety of CaPs with different morphologies, including rods, whiskers, spheres and mesoporous particles, have been prepared [2, 3]. It is noticed in the synthesis process, the crystal phase and morphology of as-prepared ceramics are strongly influenced by the reactants. For example, Wang et al. prepared hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) powders of rod-like or prism-like nature using microwave-assisted hydrothermal system with orthophosphate salt as precursor [4]. Meanwhile, Zhu et al. reported the microwave-assisted hydrothermal production of mesoporous amorphous calcium phosphate (ACP) microspheres with adenosine 5'-triphosphate (ATP) or adenosine 5'--diphosphate (ADP) as the phosphorus source [5, 6]. Therefore it is reasonable to speculate that the replacement of regular orthophosphate salts using soluble condensed phosphate salts can induce significant changes to the as-synthesized CaPs.

In nature, condensed phosphate salts can be divided into two groups, organic and inorganic. To the best of our knowledge, only organic condensed phosphate salts have been used together with microwave energy to synthesize CaPs. Reports on the application of soluble inorganic condensed phosphate salts in microwave-assisted hydrothermally synthesizing CaPs are limited. However, it is noted that inorganic condensed phosphate salts have been applied to generate CaPs in other systems. For example, free calcium ions can react with inorganic condensed phosphate salts to form amorphous porous complex once contacted [7, 8]. After that, with thermal or chemical treatment, the complex can be converted into a series of CaPs, such as calcium pyrophosphate, HA, monetite etc. [7, 9]. Hence, the purpose of present work is to evaluate whether the usage of inorganic condensed phosphate salts as phosphate source can result in CaPs with unique crystal phase or morphology to regular orthophosphate one.

2. Material and Methods

Two inorganic condensed phosphate salts, $\text{Na}_4\text{P}_2\text{O}_7$ and $\text{Na}_5\text{P}_3\text{O}_{10}$, were used. Typically, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was first dissolved in H_2O , followed by adding $\text{Na}_4\text{P}_2\text{O}_7$ or $\text{Na}_5\text{P}_3\text{O}_{10}$ separately to make a Ca/P molar ratio of 1.5. In order to have settled solution, the pH was adjusted to 4.5 by 1M hydrochloric acid. Solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and NaH_2PO_4 with Ca/P at 1.5 was used as control. Beaker with solution was placed into a 720 W household microwave oven for 5 min heating at maximum

power. After that, the solution was successively filtered. The collected precipitates were washed with deionized water and dried at 80 °C overnight.

Precipitates were firstly characterized by X-ray diffraction (XRD, Rigaku) to determine the material phases. The chemical bands were analyzed using Fourier-transformed infrared spectroscopy (FTIR, Digilab). The thermal transition of precipitates was measured using a thermogravimetry differential scanning calorimetry analyzer (TGA, Tacompany). The powders, calcined at 1000 °C, were also characterized using XRD and SEM. The surface morphologies were studied using a scanning electron microscope (SEM, Zeiss). Osteoblast cells (MC3T3-E1) were used to study the cytocompatibility of precipitates before and after calcination. Wells containing 1000 mg/ml powders in 500µl culture medium were incubated 48 hours prior to the cell seeding. After that, MC3T3-E1 cells were seeded on to wells at a density of 10,000 cells/well. Immediately after seeding, powder extracts were added to the wells. The medium was changed every other day. Cell density was measured using MTT after 1 and 7 days.

3. Results and Discussion

All precipitates are white fine powders. As shown in Fig.1a, the precipitates from Ca-P are HA as expected (PDF #09-0432). In previous work we have shown that a calcium orthophosphate solution under microwave irradiation can only generate HA due to the thermal driving force unless stabilizer added [10]. It is known condensed phosphates can easily react with Ca^{2+} to produce amorphous complex in aqueous solution. However, due to HCl addition and microwave-assisted HCl evaporation, these complexes follow a precipitation-dissolution-precipitation process in response to pH change, and the end product is found to be crystallized. The precipitates from Ca-P2 are mainly crystallized calcium pyrophosphate hydrate (CPPH, $\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$, PDF #41-0488). However, the material phase of precipitates from Ca-P3 is unidentified. In FTIR (Fig.1b), all precipitates showed peaks at 1010 cm^{-1} , referred to phosphate. Absorption peaks from water were shown in 1656 and 3448 cm^{-1} for all precipitates. In addition, both Ca-P2 and Ca-P3 precipitates show various P-O bands at wavelengths of 766 , 918 , 1093 , 1162 , and 1238 cm^{-1} , attributed to the presence of condensed phosphate bands (Fig.1b). Therefore, it is suggested Ca-P3 precipitates should be calcium condensed phosphate. After calcination, both Ca-P2 and Ca-P3 become dehydrated crystallized CPP ($\text{Ca}_2\text{P}_2\text{O}_7$, PDF #09-0346) (Fig. 1c). In the TGA experiment (Fig.1d), Ca-P3 presents a sudden weight change between 100 and $200\text{ }^\circ\text{C}$,

possibly caused by the loss of absorbed water in Ca-P3. After that, both Ca-P2 and Ca-P3 show similar weight change behavior with loss of crystal water. In our previous work we have shown amorphous complex of Ca^{2+} and $\text{P}_3\text{O}_{10}^{5-}$ can be thermally transformed into CPP [7]. Therefore, based on XRD, FTIR and TGA, it is speculated the precipitates from Ca-P3 are thermally unstable calcium tripolyphosphate hydrate ($\text{Ca}_5(\text{P}_3\text{O}_{10})_2 \cdot n\text{H}_2\text{O}$, CTPH). Meanwhile, based on the water loss observed in TGA, n is calculated to be 3.87.

The representative SEM images of as-prepared Ca-P, Ca-P2 and Ca-P3 precipitates are presented in Figures 2(a) to (c). For Ca-P, it shows plate-like HA crystals together with some microspheres. We have reported amorphous calcium phosphate (ACP) microspheres can be formed when microwave irradiation is applied [10]. Due to the water-boiling, these ACP microspheres are immediately transformed into HA, though sphere-like structure can be partially preserved. For Ca-P2 the precipitates are whiskers with a length of a few microns, and a width of sub-microns. CPPH particles with different morphology have been reported. Vasant et al. have generated CPPH nanoparticles in the range from 4 nm to 40 nm [11]. Groves et al. synthesized elongated CPPH crystals, 45–190 μm long, with considerable variation in length/width ratio [12]. However, it is the first time reporting such crystal structure of CPPH. These CPPH whiskers can be potentially used in constructing matrix as reinforcement additives. Meanwhile, the precipitates from Ca-P3 are plate-like particles assembled microspheres. The EDS study indicates the P/Ca molar ratio is in the range of 1.2-1.25, matching the speculated CTPH chemical formula. The morphology of precipitates after calcination is shown in Figures 2(d) to (f). In Ca-P solid-state diffusion in crystalline solids is seen, similar to reported calcined CaP [13]. Ca-P2 shows bonded long rods and Ca-P3 becomes irregular agglomerates of plate-like particles.

Fig.3a shows both Ca-P2 and Ca-P3 are cytocompatible. In addition, the extracts from Ca-P2 have the best cell proliferation after 7 days ($p < 0.05$). CPP based ceramics have been reported to be potential bone repairing materials with higher biodegradation than HA *in vivo* and possibility to intensify osteoblast differentiation via releasing Ca^{2+} and PO_4^{3-} [14, 15]. Therefore our finding is in agreement with these reports. On the other hand, precipitates from Ca-P3 are also comparable to Ca-P1, possibly attributed to the presences of Ca, P, O in its composition. While for calcined powders, no significant difference was observed (Fig.3b). Possibly due to the calcination, the degradation rate of

materials was limited.

4. Conclusions

Soluble inorganic condensed phosphate salts together with microwave can be used to prepare biocompatible calcium condensed phosphates with unique morphologies. Depending on the condensed phosphate source used, CPPH whiskers as well as CTPH microspheres can be generated after 5 min microwave heating. The potential applications of these materials as well as microwave hydrothermal synthesis of CaPs using other soluble inorganic condensed phosphate salts will be investigated in future.

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Figures

Figure 1. (a) XRD of precipitates, “*” refers to unidentified pattern; (b) FTIR of precipitates; (c) TGA of Ca-P2 and Ca-P3; (d) XRD of calcined powders

Figure 2. Representative SEM images of precipitates: (a) Ca-P; (b) Ca-P2; (c) Ca-P3; and calcined powders: (d) Ca-P; (e) Ca-P2; (f) Ca-P3

Figure 3. *In vitro* study of 7 days osteoblast cell culture using (a) precipitates and (b) calcined powders

Tables

Table 1. Compositions of aqueous mixture used in microwave hydrothermal experiment

Sample	CaCl ₂ .2H ₂ O (g)	Phosphate source (g)		H ₂ O (mL)	Ca/P
Ca-P	0.294	NaH ₂ PO ₄	0.16	200	1.5
Ca-P2	0.294	Na ₄ P ₂ O ₇	0.177	200	1.5
Ca-P3	0.294	Na ₅ P ₃ O ₁₀	0.1635	200	1.5