J Mater Sci: Mater Med (2008) 19:1291–1295
DOI 10.1007/s10856-007-3235-y

Effect of polymerization degree of calcium polyphosphate on its microstructure and in vitro degradation performance

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Received: 29 October 2006 / Accepted: 5 July 2007 / Published online: 23 August 2007

Abstract Preparation, characterization and in vitro study of a series of calcium polyphosphate (CPP) with different polymerization degree were reported. A series of CPP with different polymerization degree were prepared by controlling calcining time. Average polymerization degree was analyzed by liquid state ^{31}P nuclear magnetic resonance (NMR). The microstructure was observed by scanning electric microscope (SEM). X-ray diffraction (XRD) analysis was used to demonstrate that polymerization degree would not affect the crystal system and space group of CPP. The results showed that polymerization degree increased with the increase of calcining time. Degradation studies were performed during 32 days in physiological saline solution (aqueous solution, 0.9 wt.%NaCl) to assess the effect of polymerization degree on the degradation velocity of the samples. It was also shown that the degradation velocity of CPP (polymerization degree = 13) doubles than another two samples (polymerization degree = 9,19). The results in the present study may be able to provide some fundamental data for controlling CPP degradation.

Introduction

Calcium phosphate has drawn increasing attention due to its good biocompatibility and the same element with bone. The commercial products of this material include hydroxyapatite (HA), tricalcium phosphates (TCP), tetracalcium phosphate, and biphasic Ca–P (BCP), which is a mixture of HA and TCP. However, these calcium phosphates have some limits such as low toughness, low absorption and lack of osteogentic properties. Moreover, these materials cannot be completely biodegradated after implantation [1, 2].

Recently, calcium polyphosphate (CPP), a kind of inorganic polymers, has draw attention due to its good biocompatibility and degradability in biological environments [3–8]. In vivo and in vitro, the porous and dense CPP ceramics showed different biodegradation velocity [7–9]. CPP fibers degraded totally within 16 weeks in rat muscle [10]. Porous CPP ceramics sintered at different temperature (585–900 °C) also greatly demonstrated different degradation velocity [11]. Our previous studies have confirmed that the degradation velocity of different crystalline phases CPP was different [12].

The variation in degradation velocity leads to the idea that might be possible to control biodegradability of CPP, so that degradation velocity could be adjusted to meet clinical requirements. Unfortunately, at present there are no reports about the effect of polymerization degree on the degradation velocity of CPP, except for some primary studies by Qiu [12]. The lack of polymerization degree study not only probably inhibits the research and development of controllably degradable CPP, but also further inhibits the understanding of CPP degradation mechanism in vivo.

The object of this study is to investigate the effect of polymerization degree on the microstructure and degradation of CPP. The polymerization degree of CPP were investigated by liquid state ^{31}P NMR. The variation of polymerization degree would affect the microstructure, and further affect the degradation velocity of CPP. By

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controlled polymerization degree, CPP ceramics as scaffold with different degradation velocity can be obtained for bone substitutes.

Material and methods

Calcium phosphate monobasic monohydrate ($Ca(H_2PO_4)_2 \cdot H_2O$) powder was synthesized by solution method as described elsewhere [5]. The prepared powders were placed into a 100 mL cubic, and were calcined at 500 °C for 0, 3, 5,7 and 10 h under atmospheric conditions through the following reaction:

$$nCa(H_2PO_4)_2 \cdot H_2O = [Ca(PO_3)_2]_n + 3nH_2O$$

Then calcined aggregates were heated to 1200 °C with a heating rate 10 °C/min, resulting in powder melting, and held for 1 h. Then the melt promptly was poured into distilled water to get amorphous samples. The amorphous was sintered at 800 °C and it dwelled at the set sintering temperature for 3 h. The sintered samples were cooled down to room temperature. The aggregates were grounded into fine granules.

For liquid state ^{31}P NMR measurements, the glasses were dissolved in aqueous solution of 0.25 mol/L disodium ethylenediaminetetracetate dihydrate (Na_2–EDTA) adjusted to pH = 10 (±1) [13–17]. This approach prevents the hydrolysis process by binding Na^{2+} ions to polyphosphate chains to form soluble sodium polyphosphate chains. Molar proportion of Ca^{2+} ions to EDTA was constant for all samples and equal to 1:2.2. Multiple NMR analyses were conducted for all samples at 300 K on a Bruker AV300 NMR spectrometer with ^{31}P probe operating at 121.49 MHz. Two hundred scans were collected for each sample with a 10.275 μs pulse duration width and a 6.00 μs pulse separation to obtain spectra, being referenced to an 85% solution of H_3PO_4.

The sintered CPP powders were analyzed for XRD experiments performed on the X'Pert Pro MPD X-ray diffractometer (Philips, Netherlands). The voltage and anode current were 40 KV and 40 mA, respectively. The Cu Kα = 0.15405 nm and continuous scanning mode with 0.02 step size and 0.5 s of set time were used in all XRD experiments for collecting the data of samples.

A JEOL scanning electron microscope (JSM-5900LV) was used to characterize the microstuctures of the samples. The samples were examined using an accelerating voltage of +15 keV on samples ground and polished down to a 1 μm finishing using diamond paste.

For in vitro degradation, 0.2 g CPP was immersed in physiological saline (0.9% NaCl). The ratio of solid to liquid was 1:10. At the end of each period of immersion time (0.5 h, 1 h, 2 h, 4 h, 8 h, 16 h, 32 h, 4 d, 8 d, 16 d and 32 d), the samples were separated from the solution by filtration, washed in deionized water and dried to constant weight.

A relative weight loss of samples was calculated from the following equation:

$$\text{Weight loss } (\%) = [(W_0 - W_t)/W_0)] \times 100,$$

where, W_0 and W_t were the initial weight and the weight after interval time, t, respectively.

The PO_4^{3-} concentration in the degradation solution was measured by the phospho–vanado–molybdate method [18]. Nitric acid (0.1 μL, 1 M) was added [19] to corresponding degradation solution to break down poly- and meta-phosphate groups, which would not be detected by the methods [20], into orthophosphate groups.

Results

The liquid state ^{31}P NMR spectra of CPP was shown in Fig. 1a–e, respectively. Q^0 corresponded to orthophosphate groups having no bridging oxygen, whereas Q^1 described an end phosphate group with only one oxygen bridging to a neighboring tetrahedral, and Q^2 described an internal phosphate with two oxygen bridging to two neighboring tetrahedral, which was indicative of a long chain polyphosphate structure in CPP [15]. Based on previous ^{31}P-NMR analysis of sodium and zinc polyphosphate glasses [14], comparative average polymerization degree was calculated by integrating standardized delimited areas under the respective peaks for the ortho groups (Q^0), end groups (Q^1) and internal groups (Q^2):

$$PD = \frac{Q^0 + Q^1 + Q^2}{Q^0 + \frac{1}{2}Q^1}$$

As shown in Fig. 2, the polymerization degree increased with the increase of calcining time. Only a little increase of polymerization degree happened between 0 h and 3 h. However, with the increase of calcining time from 3 h to 10 h, polymerization degree increased linearly with the calcining time.

Figure 3 was the XRD patterns of CPP with different polymerization degree. From the patterns, it was noted that there was no difference among the four patterns. The three strongest d-spacings compared to PDF 77-1953 (PDF2 2004 Version) was shown in Table 1, and this analysis was used to ensure desired crystalline phase for the initial powder, *β*-CPP [11]. The soft ware of MID JADE was used to refine the crystal cell parameters, and the results showed only small changes in the crystal cell parameters (datas not shown).

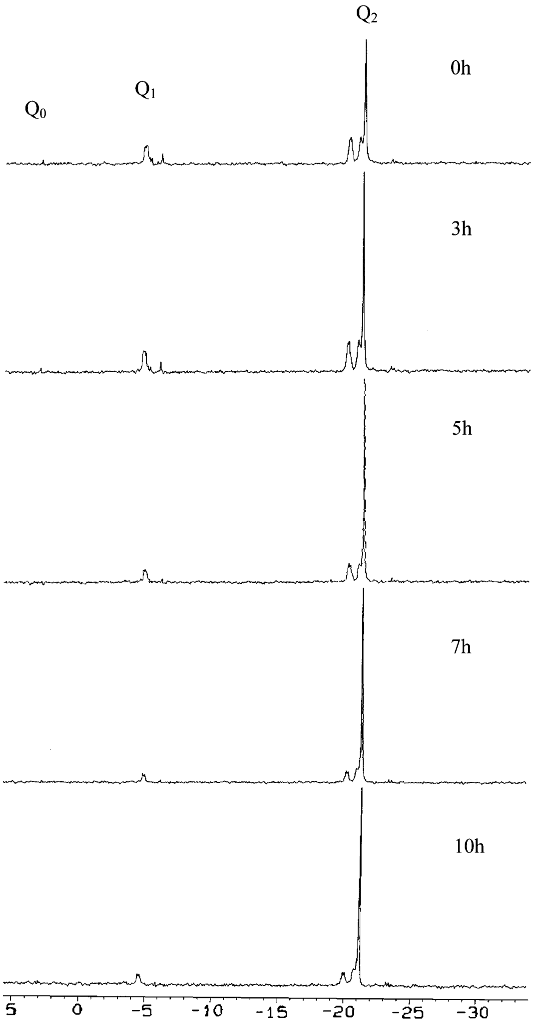

Fig. 1 Liquid state ^{31}P spectra of CPP calcining for 0, 3, 5, 7, 10 h

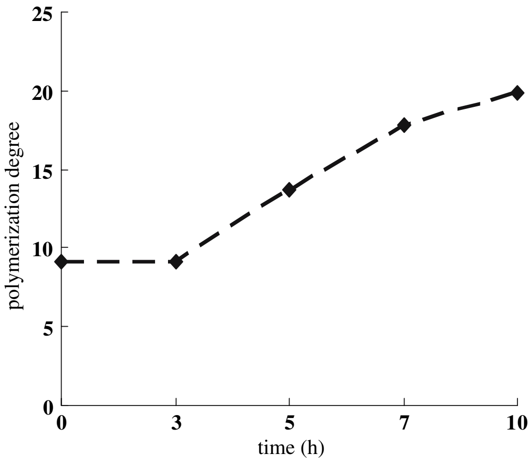

Fig. 2 Change of polymerization degree against calcining time

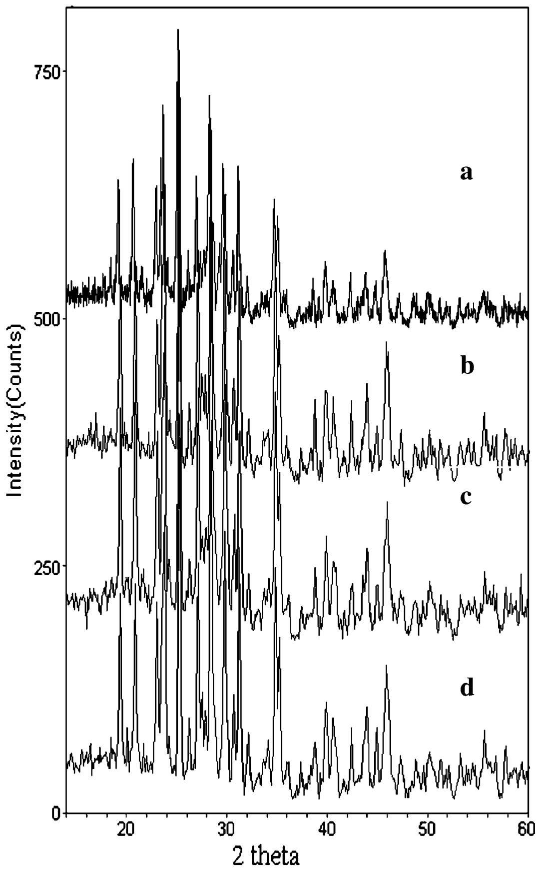

Fig. 3 XRD patterns of CPP different polymerization degree (polymerization degree = 9, 13, 17, 19 for a, b, c, d)

The morphology of CPP with different polymerization degree showed different microstructure (Fig. 4). It was obvious in Fig. 4a that the grain size is larger than others, and the densification was well. When polymerization degree was larger than 13, grain size became small and the grain boundaries were visible (Fig. 4b–c). As shown In Fig. 4d, the grain boundaries became thinner.

The rate of weight loss was shown in Fig. 5a. It appeared indicatively an incessant increase of weight loss upon degradation time expanding within relatively short degradation period (32 days) in every CPP sample. As shown in Fig. 5b, the phosphorus content increased with the degradation time, which was consistent with the weight loss (Fig. 5a).

Discussion

Hench postulated the definition of the third generation of biomaterials, both bioactive and biodegradable materials [21]. At present, one of the main tasks is to search and develop the controllably biodegradable scaffold for biomaterials science and engineering. The degradation velocity of CPP in vivo and in vitro can be tailored through variation of sintered temperature and particles size or others factors, especially polymerization degree [11, 12,

Table 1 Comparison of the three strongest d-spacings in the XRD data of experiment dates and standard dates

Samples	d-spacing
PDF77#-1953	3.71, 3.51, 3.13
3 h	3.72, 3.51, 3.13
5 h	3.72, 3.51, 3.13
7 h	3.71, 3.51, 3.13
10 h	3.74, 3.53, 3.14

19]. So, basic research about polymerization degree of CPP is urgently needed for adjusting the degradation velocity and other properties.

One objective of this study was to analyse the polymerization degree of CPP prepared by different processing. The peaks of Q^n (Fig. 1) were similar to the spectra of ZnPP [15] and CPP [16, 17] investigated by liquid state ^{31}P NMR. It is a polycondensation reaction from $Ca(H_2PO_4)_2$ to CPP. With the increase of calcining time, the loss of water from two MCP molecular increased, resulted in the increase of polymerization degree (Fig. 2).

β-CPP crystal structure was constructed based on the following data [23]: monoclinic system; space group: P21/c; lattice parameter: a = 0.69600 nm; b = 0.77114 nm; c = 1.6994 nm; α(°) = 90.0000; *β*(°) = 90.4400; *γ*(°) = 90.0000. The crystal cell of *β*-CPP was composed of two elemental units consisting of a circular chain connected by eight PO^{4-} tetrahedrons. The polymerization of $[Ca(PO_3)_2]_n$ probably resulted in the connection of two circle chain units through O atoms at the two circle chains. And the polymerization degree changes did not affect the crystal system and space group (Fig. 3), which was indicated by Guo [24]. Meanwhile, Guo [24] also indicated that

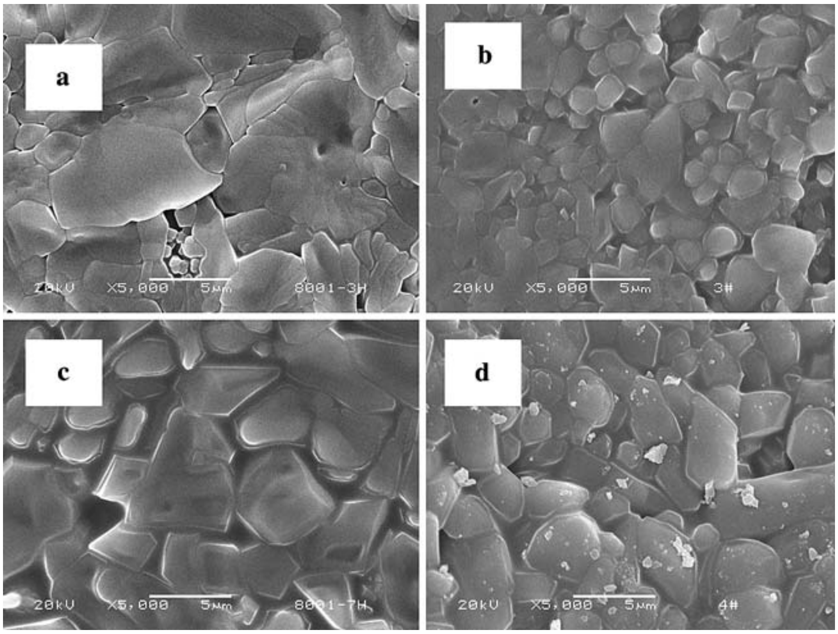

Fig. 4 Micrographs of CPP with different polymerization degree obtained by SEM (polymerization degree = 9, 13, 17, 19 for a, b, c, d)

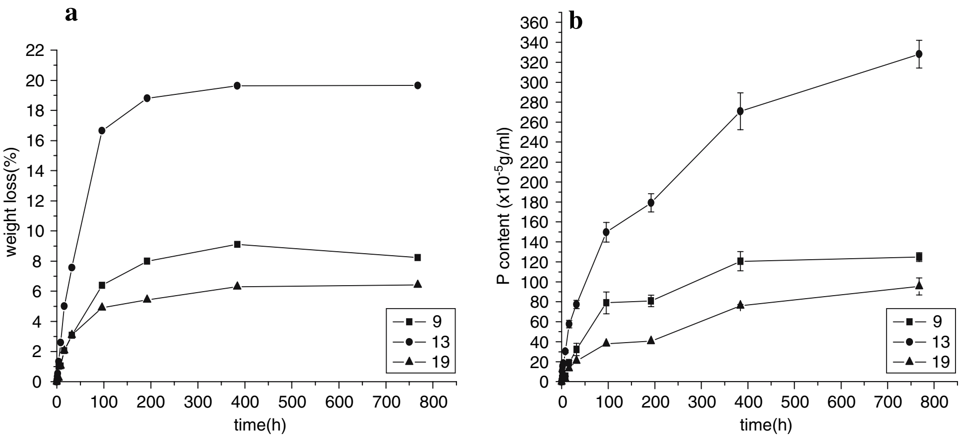

Fig. 5 (**a**) The rate of weight loss (%) and (**b**) P concent ($\times 10^{-5}$ g/mL) in the degradation solutions of CPP with polymerization degree = 9, 13, 19

the change of polymerization degree of CPP would produce a complex chain structure along the *C*-axis, which would probably result in the small change of crystal cell dimensions. We could draw a conclusion that the different polymerization degree of CPP would have the same space group and crystal system.

The effect of polymerization degree on the microstructure of CPP sintered at the same temperature may be interpreted as following. With the lower polymerization degree, the melting point of CPP would decrease. When sintered at 800 °C, CPP with lower polymerization degree easily was melted, which resulted in coarsening of grain, and the grain size were larger than others (Fig. 4a). With the increase of the polymerization degree, the melting point of CPP would increase too, and only the boundaries of the particles were molten, resulted in smaller grains and visible and thinner grain boundaries.

From the results of the degradation experiment, it could be seen that polymerization degree could affect degradation velocity of CPP (Fig. 5). However, it was observed that with the increase of polymerization degree, the degradation velocity was not decreased [12]. It was also indicated by Pilliar [19] that the grain boundary regions of CPP were the weekness points, where represents the regions of greater atomic or molecular disorder (higher energy zones), and they could easily be attacked by degradation solution. The observed cusping or grooving of grain boundaries grain boundaries in Fig. 4b could be attacked easily by the degradation solution, and resulted that the degradation velocity of CPP with polymerization degree = 13 was the fastest.

Conclusion

Results showed that polymerization degree of CPP was increased with the increase of calcining time. Sintered at 800 °C, crystal system and space group of CPP with different polymerization degree couldn't be changed. However, the variation in polymerization degree could affect the microstructure of CPP. The in vitro degradation velocity reported here suggested polymerization degree could affect CPP degradation velocity. The studies suggested the microstructure could be changed with the change of polymerization degree, and then affect the degradation velocity of CPP. Our study provided a potential method to develop controllable-degraded CPP bioceramics.

Acknowledgment The authors are grateful to the NSFC for providing financial support through project 50472091 (The research of molecular self-assemble nano RADA16-RGD/CPP compounds for bone repair). The author also would like to thank Fu Qingquan, who provided the liquid state ^{31}P NMR measurement. XRD and SEM were provided by the Center of Forecasting and Analysis of Sichuan University.

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