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Fabrication and characterization of porous calcium polyphosphate scaffolds

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Porous calcium polyphosphate (CPP) scaffolds with different polymerization degree and crystalline phases were prepared, and then analyzed by scanning electron microscopy (SEM), Thermmogravimetry (TG) and X-ray diffraction (XRD). Number average polymerization degree was calculated by analyzing the calcining process of raw material $Ca(H_2PO_4)_2$, as a polycondensation reaction. Amorphous CPP were prepared by the quenching from the melt of $Ca(H_2PO_4)_2$ after calcining, and CPP with different polymerization degree was prepared by controlling the calcining time. Meanwhile, CPP with the same polymerization degree was prepared to amorphous or different crystalline phases CPP which was made from crystallization of amorphous CPP. *In vitro* degradation studies using 0.1 M of tris-buffered solution were performed to assess the effect of polymerization degree or crystalline phases on mechanical properties and weight loss of the samples. With the increase of polymerization degree, the weight loss during the degradation decreased, contrarily the strength of CPP increased. The degradation velocity of amorphous CPP, α-CPP, β-CPP and γ-CPP with the same polymerization degree decreased in turn at the same period. The full weight loss period of CPP can be controlled between 17 days and more than 1 year. The results of this study suggest that CPP ceramics have potential applications for bone tissue engineering.

1. Introduction

The capacity of the human body to regenerate bone components that are lost, damaged or diseased is limited in several situations, so synthetic biomaterials were developed to overcome the limited availability of natural bone graft materials for hard tissue repair and replacement [1]. Calcium phosphate materials have some outstanding properties, namely similarity in composition to bone mineral and osteoconductivity [2]. Products in bone graft market based on hydroxyapatite and tricalcium phosphate have been used clinically. However, the success of these materials is limited mainly due to low toughness, low elasticity, low ability to be resorbable and lack of osteogenic peoperties. To overcome the low mechanical properties several silicate glasses and glass ceramics have been developed and products such as Bioglass®, Bioverit® and Ceravital® have been available commercially for a long time. Nevertheless, all these materials still show a lack of both ostergenic properties and complete biodegradability after implantation. Biodegradable calcium polyphosphate ceramics, as a kind of inorganic polymeric biomaterials, have drawn attention due to their dissolution property in biological environments, their similar chemical elements with bones, and their diversity in chemical composition [3,

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4]. Yong-Moo Lee [5] has proved that not only osteoblastic cell proliferation and differentiation on the surface of CPP matrices *in virto* but also bone formation by ectopic implantation of these cell-matrix constructs in athymic mice *in vivo*. Pillar and his group [6] investigate that CPP materials seems to promote rapid bone ingrowth and can be tailored to degrade at a given rate *in vivo* to some degree through appropriate selection of the starting particle size. By the experiments of Tarek [7], results indicate that human gingival fibroblasts can attach and spreading on the CPP. Porter [8] compared the degaradation of amorphous CPP and crystalline CPP, however, the comparison experiments of CPP with different crystalline phases did not carry out.

Our interest in this material results from prior studies about effect of polymerization degree and crystalline phases on material performance. Because of a linear regular tetrahedral polymeric structure based of [PO_3^-] groups, some properties of CPP can be controlled by changing the polymerization degree. The number average polymerization degree can be calculated by the water loss amount during the polymerization reaction. Different polymerization degree of CPP ceramics can result in different degradation velocity. Meanwhile, by controlled sintering temperature and time, α-CPP, β-CPP and γ-CPP can be got respectively with different degradation velocity. By controlled polymeric degree and crystallization, CPP ceramics as scaffold with different degradation velocity can be obtained for bone tissue engineering.

2. Materials and methods

Homogeneous amorphous CPP powders were produced according to a procedure described followed. Calcium phosphate monobasic monohydrate(SIGMA), $Ca(H_2PO_4)_2{\cdot}H_2O$ was calcined at 500°C for 1, 5, 10 h to yield CPP with prospective different polymerization degree. The resulting powder was heated to 1100°C with a heating rate 15°C/min under atmospheric conditions, resulting in powder melting, and held for 1 h. The melt promptly was quenched in distilled water to avoid crystallization upon cooling. The amorphous frit was milled and screened to yield powder in a size range of <25 μm.

Porous calcium polyphosphate (CPP) ceramics were fabricated by mixing the stearic acid(300–355 μm) as the porogen with the amorphous powders, sintered at 550°C, 875°C, 1000°C for 1 h to obtain porous α-CPP, β-CPP and γ-CPP scaffold respectively. For evaluation degradability and the mechanical intensity change during the degradation, granules and blocks measuring $\Phi 10\times 20$ mm of sintered CPP ceramic were prepared (Fig. 1a). The same procedure of the tests was repeated 6 runs for each specimen.

Thermmogravimetry (TG) was conducted using a TG92 Staram in a carbon crucible and in an inert(argon) atmosphere. Sample weights were approximately 50 mg and the heating rate was 5°C/min.

A JEOL scanning electron microscope (JSM-5900LV) was used to characterize the microstuctures of porous scaffolds and the connection of sintered grains. The samples were examined at the magnification of 50× and 800× using an accelerating voltage of +15 keV on samples ground and polished down to a 1 μm finishing using diamond paste.

X-ray diffraction (XRD) analysis was used to identify and quantify the crystalline phases that precipitated from the amorphous powders. For X-ray diffraction measurements, samples were ground to a fine powder and analyzed using a PHILPS 3 KW diffractometer, using flat plate geometry and Cu K_α radiation. Data were collected using a scintillation counter and a graphite diffracted beam monochramator, from 10° to 70°(2θ), with a step size of 0.02° and a counting time of 5 s per step. Quantitative phase analysis was performed by the Rietveld method using General Structure Analysis Software(GSAS, Los Alamos National Laboratory). Diffiraction peaks were labeled and compared to McIntosh and Jablonski [9] to insure the crystalline phase was detected α-CPP, β-CPP and γ-CPP sintered at 550, 875, 1000°C respectively.

The degradation tests were performed according to ISO 10993–14-‘Biological evaluation of medical devices—Part 14: Identification and quantification of degradation products from ceramics’. Granules with a size between 355 and 415 μm were used as obtained by automated controlled sieving technique. *In vitro* degradation studies were performed to evaluate the dissolution properties of CPP. Samples were places in 50 mL of 0.1 M Tris[hydroxymethyl] aminomethane-HCl (Tris-HCl) solution (pH = 7.4) at 37°C, with a continual agitation speed of 120 rpm, using triplicate samples. At the end of each period of immersion time (0, 1, 2, 3, 4... days), CPP scaffold were separated from solution by filtration, washed in deionized water and dried to constant weight [10].

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

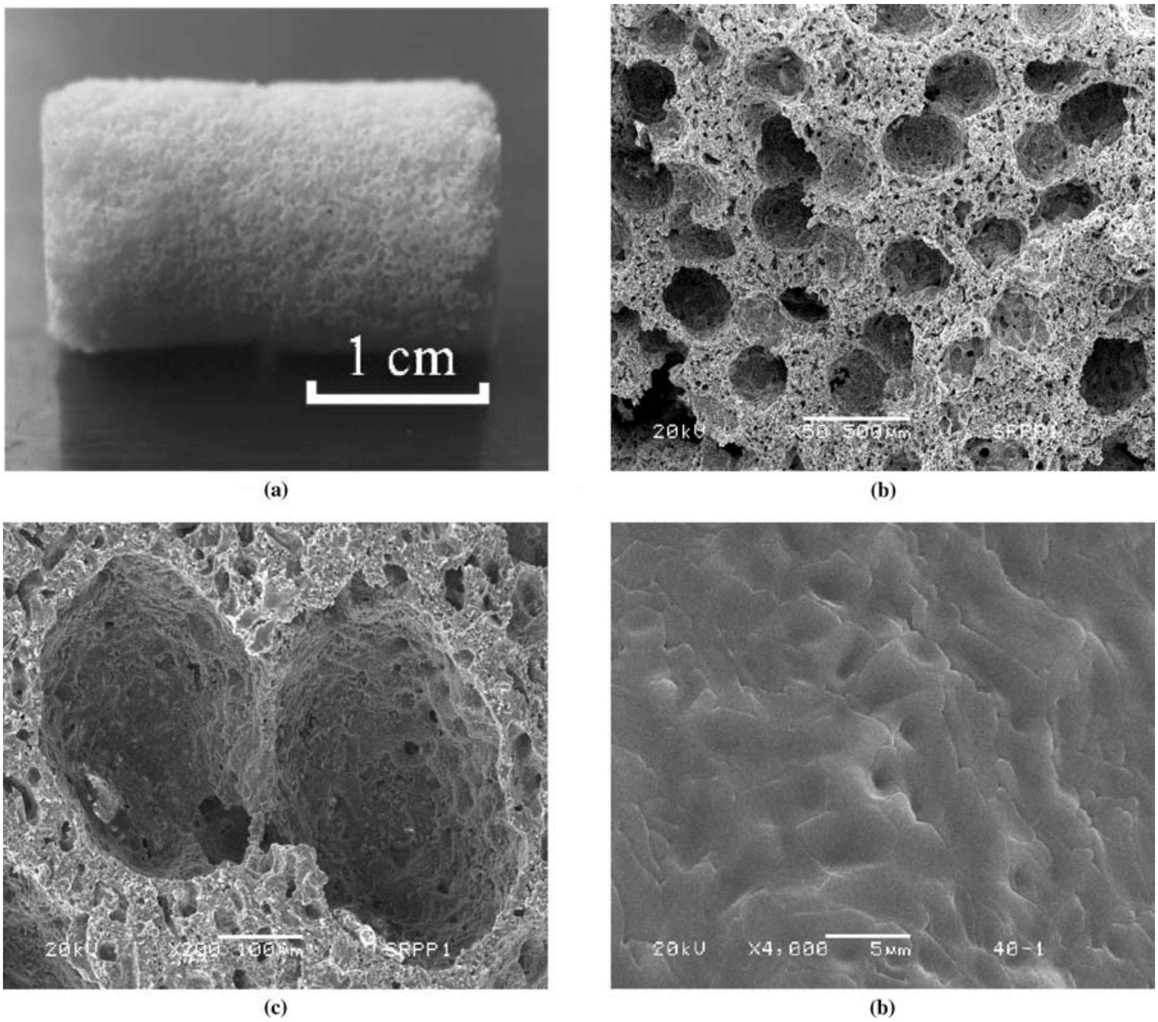
$$\text{Weight loss }(\%) = [(W_0 - W_t)/W_0] \times 100,$$

where, W_0 and W_t stand for initial weight and weight after a specific immersion time, respectively.

Statistical analysis was performed with SPSS11.0. Data was analyzed by one-way ANOVA and Duncan’s Multiple Range Test was also adapted for the post-hoc test. Corresponding p-values were considered significant at values less than 0.05.

3. Results and discussion

Fig. 1b is scanning electron microgragh (SEM) of the transect of the sintered structures. The expected structure with its three-dimensional interconnected porosity is evident. From the SEM pictures, it is observed that the pores of porous CPP are homogeneous and about 200–300 μm, which are mostly interconnected. Meanwhile it is mea-



(a) (b)

(c) (b)

Figure 1 Porous CPP scaffold. (a), SEM of the fracture zone of porous CPP scaffolds (50×) (b), Open porosity connection with small porosity (800×) (c), Fused individual grains make up the characteristic micro-roughness of the surface(4000×) (d).

sured in the experiment that the scaffolds full of about 70 vol% porosity. Fig. 1c shows the connection of two porosity with much small porosity at higher magnification. At the magnifications of 4000 times, the fused grain structures of the CPP material make up the characteristic micro-roughness of the surface (see Fig. 1d).

As an inorganic polymer, the polymerization degree of calcium polyphosphate has great effect on the property of scaffolds. Gomez [11] studied on the dehydroxylation mechanisms of polyphosphate glassed, and described the structure of calcium polyphosphate. In our study, the polymerization degree was calculated by analyzing the process of polymeric reaction and TG. The process from $Ca(H_2PO_4)_2$ to CPP, as a polycondensation reaction, has been researched to evaluate the polymerization degree. With the increase of calcining time, the released water produced from the two $Ca(H_2PO_4)_2$ molecular increased. Therefore CPP with different polymerization degree can prepared by controlling the calcining time. From the TG curve (see Fig. 2), there are three reactions from the reactant, crystalline hydrate calcium dihydrogen phosphate (SIGMA), to generate CPP, as follows.

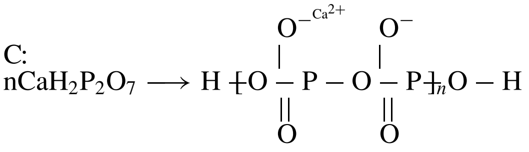
A:

$$Ca(H_2PO_4)_2 \cdot xH_2O \longrightarrow Ca(H_2PO_4)_2 + xH_2O$$

B:

$$Ca(H_2PO_4)_2 \longrightarrow CaH_2P_2O_7 + H_2O$$

C:



$$nCaH_2P_2O_7 \longrightarrow H\left[O-\underset{\displaystyle O}{\overset{\displaystyle O^{-}\,{}^{Ca^{2+}}}{\underset{\|}{\overset{|}{P}}}}-O-\underset{\displaystyle O}{\overset{\displaystyle O^{-}}{\underset{\|}{\overset{|}{P}}}}\right]_n O-H$$

$$+ (n-1)H_2O$$

Total reaction:

$$nCa(H_2PO_4)_2{\cdot}xH_2O \longrightarrow H\left[Ca(PO_3)_2\right]O-H + (2n+nx-1)H_2O$$

Step A means the crystalline hydrate calcium dihydrogen phosphate losses its crystalline water; step B means the calcium dihydrogen phosphate dehydrates inside the molecule, then the product is calcium pyrophosphate ($CaH_2P_2O_7$); step C means the calcium pyrophosphate dehydrates outside the molecule, then the polyesterification reaction happens and the polymer chain of CPP is generated. From above, with the increasing of water release during the reaction, the polymerization degree will increase too.

From the reaction equation and analysis, the polymerization degree can be calculated as follows. Assuming m_0 represents the weight of reactant, crystalline hydrate calcium dihydrogen phosphate; m represents the weight of production, CPP. From the step A of TG curve, the weight loss of water is 7.0671%. So m′, the weight of anhydrous $Ca(H_2PO_4)_2$, equals m×(1–7.0671%). From the step B and C, the polymerization degree can be calculated according to the reaction equation, simplified as below:

$$n = \frac{1}{14!\acute{A}\frac{m}{m_0} - 11} \qquad (1)$$

In the Equation 1, m_0 and m can be weighted respectively. According to the experiment data, the polymerization degree can be calculated as 60, 97 and 120 after calcining crystalline hydrate calcium dihydrogen phosphate for 1, 5, 10 h. From the results, we can see that with the increasing of dwell time, the polymerization degree increased too.

Fig. 3 shows the effect of polymerization degree on the compression strength of blocks without porosity. With the increase of polymerization degree, the molecular structure is more tighter and the strength of CPP is increasing. The compressive strength of blocks (polymerization degree = 100, 120) in Fig. 3 show the statistically significant difference ($p < 0.05$) compared to the blocks (polymerization degree = 100, 120). Meanwhile, the compressive strength of blocks (polymerization degree = 120) is twice more than it of blocks (polymerization degree = 60). As a result, polymerization degree has great effect on the mechanical performance of CPP.

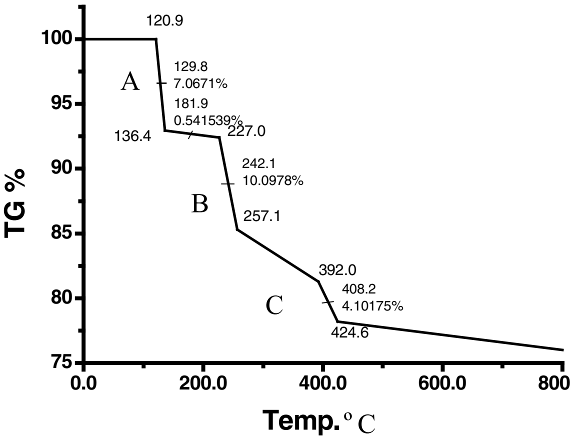


Figure 2 The TG curve of $Ca(H_2PO_4)_2$, heated at a linear rate of 10°C/min.

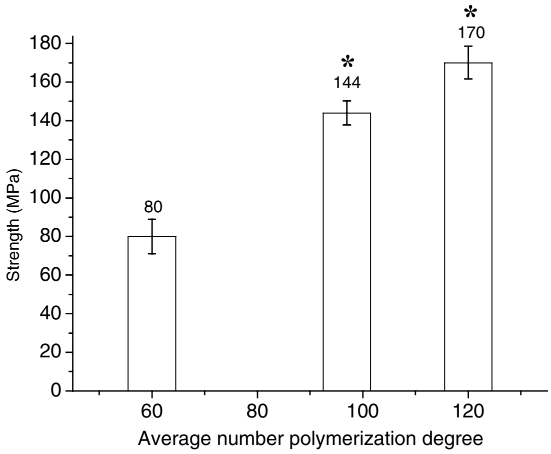


Figure 3 Strength of CPP with different polymerization degree. The columns show the statistically significant difference (*$p < 0.05$) compared to the lowest value.

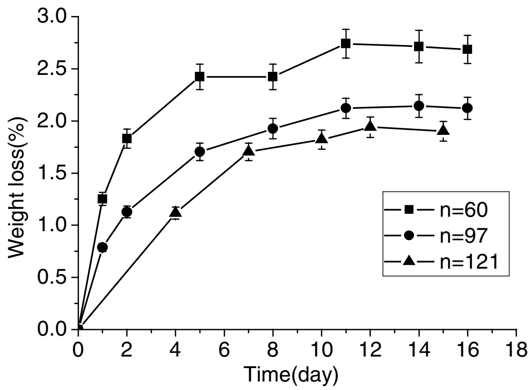


Figure 4 Weight loss curve of CPP with different polymerization degree during the degradation.

To evaluate the effect of polymerization degree on the degradation, the granules were soaked in aqueous solution for different periods occurring as a result of a hydrolytic degradation process. We can draw the conclusion from Fig. 4, that polymerization degree of CPP can effect the degradation velocity. With the increase of polymerization degree, the weight loss during the degradation decreased. The reason is presumably that with the increasing of polymerization degree, the molecular structure become more tighter and more difficult to destroyed by the hydrolyzation.

The XRD spectra obtained for the sample of the quenched frit did not reveal any diffraction peaks; therefore it was considered to be amorphous. In contrast, sharp diffraction peaks were observed in the spectra obtained for the sample sintered at 550°C, 875°C and 1000°C. The three strongest d-spacings were compared to those previ-

TABLE I Comparison of the three strongest d-spacings in the XRD data of experiment and standard

Crystalline phases	Experiment data (′)	Quoted data [9] (′)
Amorphous	–	–
γ-CPP	4.76, 3.50, 2.76	4.76, 3.49, 2.76
β-CPP	4.58, 3.74, 3.52	4.57, 3.72, 3.52
α-CPP	3.54, 3.15, 2.83	3.55, 3.15, 2.87

ously report [9] showed in Table I This analysis was used to ensure that the initial powder was amorphous and that the sintered porous structures were crystallized and of the desired crystalline phases.

Fig. 5 shows the weight loss against immersion time for all studied CPP. From the Fig. 5, We can draw the conclusion that CPP scaffolds with different crystalline phases have different degradation velocity. The degradation velocity of amorpous CPP is the fastest, and theα-CPP is the slowest. Rapid degradation of amorphous CPP has reported by Grynpas *et al.* [11]. The same observations have been reported by Porter [8], who indicated that amorphous CPP was found to degrade four times faster than crystalline CPP. Furthermore, degradation velocity of CPP from fast to slow is amorphous CPP, α-CPP, β-CPP and γ-CPP in turn.

Time points of 0, 5, 10, and 15 days initially were chosen to assess the compressive strength's change of the CPP blocks and porous scaffold soaked in buffed solution (see Fig. 6). It was found that the compression strength decreased significantly over time for both sample sets. However, the samples with 70% porosity were more susceptible to degradation compared to the samples without porosity, perhaps because the preferential degradation of CPP occurred at the higher energy surface or interface regions of grains. This effect is ever more critical for porous structures with sinter neck regions that have grain boundaries traversing the neck zone. Meanwhile, analysis of variance of both samples shows that a rapid loss of strength within the beginning of aging followed by a slower loss subsequently.

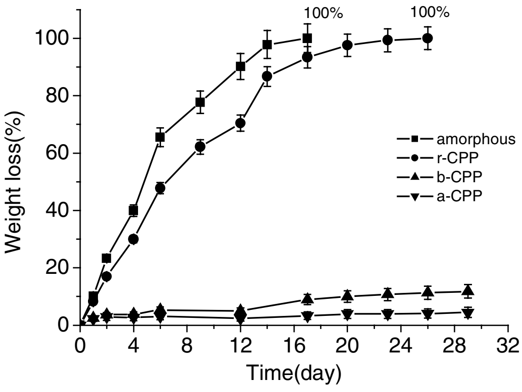


Figure 5 Weight loss curve of CPP with different crystalline phases (70% porosity, $n = 120$)

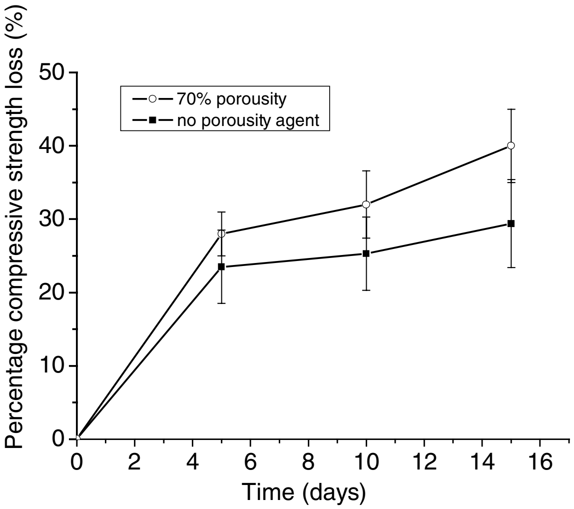


Figure 6 The average compressive strength loss during degradation ($n = 120$).

4. Conclusion

Results show that, by analysis of the polymerization process, the polymerization degree can be calculated. The polymerization degree mainly depends on the calcining time. However, the crystalline phase is mainly determined by the sintered temperature. The weight loss of CPP with different polymerization degrees or crystalline phases is different. With the increase of polymerization degree, the weight loss during the degradation decreased, contrarily the strength of CPP increased. The degradation velocity of amorphous CPP, α-CPP, β-CPP and γ-CPP of the same polymerization degree decreased in turn at the same period. The full weight loss period of CPP ceramics can be controlled between 17 days and 1 year. From the results above, CPP ceramics have potential applications for bone tissue engineering.

Acknowledgments

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