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Fabrication and *in vitro* degradation behaviour of TCP/CS composite bioceramics.

Bin Xu[1*#], Hanyuan Zhang[1#], Weijun Fang[2]

[1]Department of Sports Medicine and Arthroscopic Surgery, the First Affiliated Hospital of Anhui Medical University, Hefei, PR China

[2]College of Basic Medicine, Anhui Medical University, Hefei, PR China

[#]These authors contributed equally to this work

Abstract

$Ca_3(PO_4)_2$ (TCP) bio-ceramic materials are known as the ideal bone repair materials because they possess good biocompatibility and biodegradability. In this study, β-TCP bio-ceramic materials were prepared by wet process through controlled process conditions. $CaSiO_3$ and β-TCP bioceramics were ground and mixed to prepare the TCP/$CaSiO_3$ (TCP/CS) bioceramic embryonic bodies. TCP/CS porous composites were obtained by sintering at 800°C. TCP/CS bioceramics possessed the best strength after sintering for 2 h; the sample strength was about 122 MPa, which is approximately consistent with the strength of human bone. The degradation behaviour of porous TCP/CS bioceramics were investigated by static soaking and dynamic dissolution experiments in physiological saline to control the degradation rate of TCP/CS. The degradability of TCP/CS samples reached 10% within 12 days *in vitro*, suggesting an excellent *in vitro* bioactivity and biodegradability of TCP/CS composite bioceramics. The TCP/CS composite bioceramics might become potential biomaterials as good mechanical strength, bioactive and biodegradable for bone tissue repair applications.

Keywords: Tricalcium phosphate, Calcium silicate, Biocompatibility, Biodegradability, Bioactivity.

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Introduction

In these two decades, bone tissue engineering scaffold materials have been successfully applied in reconstruction as the carrier materials for bone tissue (including alveolar bone) due to controllable biodegradability, appropriate three-dimensional structure with connected porosity, good mechanical strength and acceptable biocompatibility [1,2]. Porous calcium phosphate TCP ($Ca_3(PO_4)_2$) bioceramic contains good biodegradability, biocompatibility and biological safety. It has been widely investigated by scientists and engineers all over the world as a potential bone tissue engineering scaffold material [3-12].

A major problem hindering the clinical application of porous TCP ceramics clinical is the difficulty to coordinate the degradation rate and the growth rate of newly formed bone [3,4,7]. TCP has two types of crystal forms, alpha phase and beta phase, which have different water solubility and *in vivo* degradation rate. The degradation rate of α-TCP is too high and cannot be applied clinically [6,7]. Moreover, the bone formation of β-TCP material is slow, and the integration of bone is not perfect [8,9]. Recent studies have shown that calcium silicate powder or ceramic has good biological activity and induced deposition of bone like hydroxyapatite layer *in vitro* [10-12]. Silicon has been considered as a medium for promoting new bone formation. The formation of hydroxyl apatite is very helpful for the promotion of bone conduction and bone regeneration in biomaterials, and it can promote the formation of chemical bonding between hard/soft tissues and materials, indicating that calcium silicate is a potential and promising bioactive material having great clinical applications. Previous studies also showed that $CaSiO_3$ (CS) possesses a good ability to induce the deposition of hydroxyapatite, acceptable biocompatibility and bone binding/repair properties [13-15]. Recently, calcium silicate ceramics have been studied as bioceramics for new bone regeneration [16].

In this study, porous α/β dual-phase composite TCP bioceramics was prepared using α-TCP and β-TCP powder materials respectively obtained by initial wet process, then TCP/CS bioceramics was prepared by a certain proportion of TCP bioceramics and CS powder. The *in vitro* degradation behaviours of porous TCP/CS bioceramics were investigated in physiological saline to control degradation rate of TCP bioceramics. This study provided a fundamental research for the potential clinical application of TCP/CS as one of the most important tissue engineering scaffold materials.

Materials and Methods

Preparation of TCP/CS nanocomposite bioceramics

Based on Ca $(OH)_2$-H_3PO_4 system, calcium hydroxide suspension was added drop wise into phosphoric acid solution with stirring at room temperature, the reaction proceeded for 5 hours followed by filtering and drying at 85°C to prepare TCP powder.

Porous TCP ceramics was prepared by stearic acid particle filling. The high purity spherical particles of stearic acid were divided into different mesh with sample sieve, the stearic acid particles with appropriate particle size and TCP powder were mixed, and Polyvinyl Alcohol (PVA) solution was added as binder. After molding, stearic acid was molten at 80°C then dried to obtain the porous body.

The porous body was placed in a sintering furnace, heated up to 300°C, and heat was preserved for 1 h in order to remove residual moisture, PVA, stearic acid and other residues. The porous body was then heated up to 800°C for 1 h and to 1000°C and 1200°C for 3 h, cooled down to room temperature to obtain β-TCP and α-TCP bioceramics, respectively.

The prepared α-TCP and β-TCP bioceramics were ground and mixed with CS powder according to different mass ratios. The powder mixtures were calcined at 800°C for 2 h to yield TCP/CS nanocomposite powder. Polyethylene Glycol (PEG) 350-400 μm was used as pore forming agent, TCP/CS powder and PEG particles were homogeneously mixed according to a mass ratio of 4:6, PVA solution (5% wt) was added as the binder to obtain biscuits. The biscuits were sintered at 800°C to prepare porous α-TCP/β-TCP dual phase TCP/CS composite bioceramics.

Composition and structure analysis

Ca and P contents in synthesized TCP powder were determined by potassium permanganate method and quinoline phosphomolybdate gravimetric method, respectively. In the experiments of *in vitro* degradation of physiological saline, Ca^{2+} concentration was measured with PW-2404 (Philips, Holland) atomic absorption spectrophotometer. The crystal phase composition of samples was analysed by MAX 2550V type (Rigaku Co., Japan) X-ray diffraction analysis. The morphology changes of the samples before and after degradation were observed with JSM-6700F (JEOL, Japan) Scanning Electron Microscope (SEM). The mechanical properties of TCP/CS bioceramics were measured with AG-IS type Shimadzu Universal Testing Machines.

In vitro static dissolution experiment

The various porous TCP/CS bioceramics (shape: column; length: 15 mm; diameter: 5 mm) were immersed into saline filled in sealed jar, the normal saline was prepared according to a solid/liquid ratio (g/ml) of 1/100. The system was loaded in a water bath thermostat to maintain a temperature of 37°C. The soaking liquid was taken after different time periods for determination of Ca^{2+} concentration.

In vitro dynamic degradation experiment

The various porous TCP/CS bioceramics (shape: column; length: 15 mm; diameter: 5 mm) were immersed into saline filled in sealed jar, the normal saline was prepared according to a solid/liquid ratio (g/ml) of 1/100. The saline was controlled to flow through the porous TCP/CS bioceramics with a fixed circulation flowing volume by a peristaltic pump, the temperature of circulation system was at 37°C. The circulation liquid was taken for determination of Ca^{2+} concentration. The degradation rates of TCP/CS bioceramics were calculated according to Equation 1.

$$R_D\,(\%)=m_o\text{-}m_1/m_o \times 100\% \rightarrow (1)$$

Where R_D is rate of degradation, m_0 is the mass of sample before soaking and m_1 is the mass of sample after soaking.

Results and Discussion

The linear shrinkage, relative density and bending strength of TCP/CS bioceramics were listed in Table 1. It can been seen that linear shrinkage and relative density improved with an increase of sintering time from 0.5 h to 2 h. The relative density reached 95.36% after 2 h, but remained constant when the time was longer than 2 h. The sintering experiments of TCP/CS bioceramics indicated that the sample possessed the best strength after sintering for 2 h; the sample strength was about 122 MPa, which is approximately consistent with the strength of human bone. TCP/CS bioceramics prepared in this paper is obviously better than that reported in literatures, whose relative density was only around 92% after sintering for 2 h at 800°C, the mechanical strength was less than 50 MPa.

Table 1. *Effect of the sintering time on linear shrinkage, relative density and bending strength of the samples.*

Sintering time (h)	0.5	1	2	5
Linear shrinkage (%)	21.34	23.67	25.84	25.92
Relative density (%)	90.54	93.28	95.36	95.41
Bending strength (MPa)	94.51	103.48	122.21	122.56

This study showed that the basic requirement of high-strength bioceramic is high density, because only high density can guarantee the strength of the bioceramics. Compared with general ceramics, the TCP/CS nano composite bioceramics have some advantages such as large surface area, good sintering activity, fine particles and so on. Therefore, high strength TCP/CS composite bioceramic materials can be prepared at lower sintering temperature. However, the relative density of prepared TCP/CS composite powder even under 800°C and sintering for 5 h can only reached about 95%. The reason was attributed to the poor sintering property of CS component, which is difficult to achieve complete densification. Further increasing the sintering temperature and

time might improve the sintering density of samples, but that will make β-TCP phase transfer to α-TCP phase, thus properties of TCP/CS bioceramic degradation will change.

Figure 1 show that the prepared porous TCP/CS bioceramics have rich micro porous structure, the size of pore and its distribution can be controlled through the porogen particle size and dosage of PEG. These pores are mainly used to combine with osteoinductive Bone Morphological Protein (BMP), and it also helpful for new bone growth.

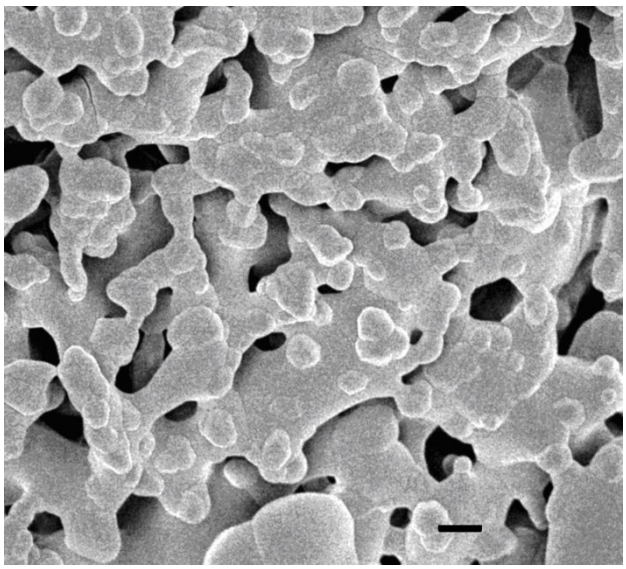

Figure 1. *SEM image of TCP/CS composite bioceramic, scale bar=100 nm.*

According to the XRD standard of TCP and CS, the purity of the TCP/CS bioceramic composite is high, and is in accordance with the requirements (Figure 2).

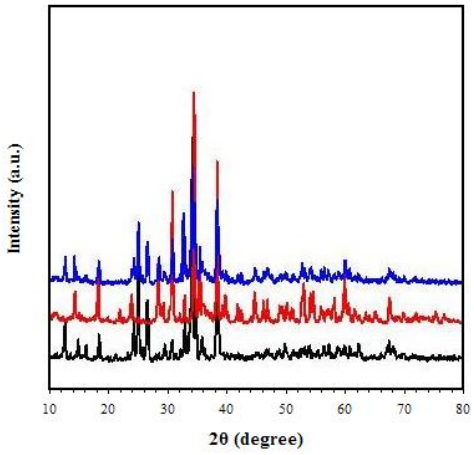

Figure 2. *XRD patterns of the CS standard (bottom), TCP (middle) and TCP/CS (top) bioceramic composite sintered at 800°C for 2 h.*

The solubility property of porous TCP/CS bioceramics in physiological saline solution was estimated based on the solubility of prepared TCP and CS bioceramics. The three ceramics samples were soaked in physiological saline respectively; the change of Ca^{2+} concentration in solution was acquired and shown in Figure 3. The degradation rate of TCP/CS bioceramics in saline is higher than TCP but lower than CS, suggesting that the method for regulation of degradation rate of porous bioceramics is feasible.

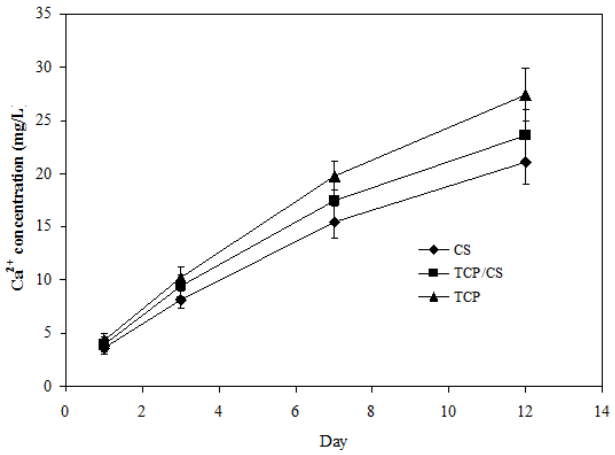

Figure 3. *Static soaking experiment of porous bioceramics in physiological saline.*

The skeleton of human or animal body is located within the tissue fluid, the body tissue fluid is about 13-15% wt of human body weight. The tissue fluid keeps constantly circulating to maintain a dynamic balance. As a result, the bioceramics implanted inside body will be in a dynamic equilibrium of the body fluid. In this study, the dynamic environment of porous TCP/CS bioceramics was simulated by dissolving the samples in a dynamic environment of organism, and the liquid membrane surface was continuously refreshed to ensure the significant power of dissolution. The data presented in Figure 4 shows that the results obtained from dynamic soaking experiment are similar with the results from static soaking experiment, but the dissolution rates in a dynamic environment are much larger than the rates in static conditions.

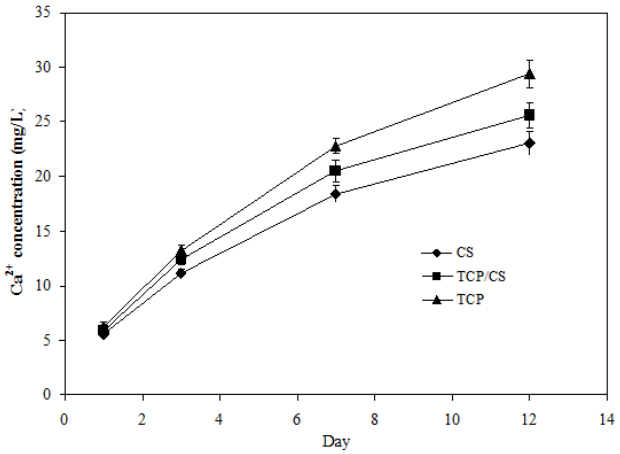

Figure 4. *Dynamic dissolution experiment of porous bioceramics in physiological saline.*

Conclusions

Porous TCP/CS bioceramics was prepared from CS and TCP ceramics by sintering at 800°C. TCP/CS bioceramics exhibited the best strength after sintering for 2 h, the sample strength was about 122 MPa, which is quite similar with the strength of human bone. The degradation behaviour of porous TCP/CS bioceramics were investigated by static soaking and dynamic dissolution experiments in physiological saline to control the degradation rate of TCP/CS. The degradability of TCP/CS samples reached 10% within 12 days *in vitro*, suggesting an excellent *in vitro* bioactivity and biodegradability of TCP/CS composite bioceramics. This study showed that CS and TCP ceramics could be used to prepare the biodegradable and bioactive TCP/CS composite bioceramic materials having good mechanical strength, which is expected to be used for hard tissues like bone repairing materials.

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*Correspondence to

Bin Xu

Department of Sports Medicine and Arthroscopic Surgery

The First Affiliated Hospital of Anhui Medical University

PR China