*Key Engineering Materials Vols. 330-332 (2007) pp. 483-486*
*online at http://www.scientific.net*


# A Study of Bone-Like Apatite Formation on β-TCP/PLLA Scaffold in Static and Dynamic Simulated Body Fluid

Yunqing Kang[a],Guangfu Yin[*b],Kefeng Wang[c],Lin Luo[d],Li Liao[e],Yadong Yao[f]

College of Materials Science and Engineering, Sichuan University, Chengdu, China

[a]yunqingkang@163.com,[*b]nic0700@scu.edu.cn, [c]fencal@163.com, [d]guitarboy6933@sohu.com

**Keywords:** Poly(L-lactic acid), β-tricalcium phosphate, Simulated body fluid, Apatite, Scaffold

**Abstract.** The ability of apatite to form on the surface of biomaterials in simulated body fluid (SBF) has been widely used to predict the bone-bonding ability of bioceramic and bioceramic/polymer composites in vivo. Porous β-tricalcium phosphate/poly(L-lactic acid) (β-TCP/PLLA) composite scaffold was synthesized by new method. The ability of inducing calcium phosphate (Ca-P) formation was compared in static simulated body fluid(sSBF) and dynamic simulated body fluid (dSBF). The Ca-P morphology and crystal structures were identified using SEM, X-ray diffraction and Fourier transform infrared (FT-IR) spectroscopy. The results showed that the typical features of bone-like apatite formation on the surface and the inner pore wall of β-TCP/PLLA. Ca-P formation on scaffold surfaces in dSBF occurred slower than in sSBF and was more difficult with increasing flow rate of dSBF. The ability of apatite to form on β-TCP/PLLA was enhanced by effect of each other that has different degradable mechanism. Porous β-TCP/PLLA composite scaffold indicates good ability of Ca-P formation in vitro.

## Introduction

The precipitation of bioactive calcium phosphate in simulated body fluid (SBF) has attracted extensive research interests [1,2].Through examining the ability of apatite to form on surfaces of biomaterials in SBF, the bone-bonding ability of a material can be often evaluated. Many researchers have evaluated the bone bioactivities of various types of materials by apatite formation in SBF [3,4]. However, it has been reported that β-TCP which have a good ability of osteointegration did not have apatite formation on their surfaces in SBF [5,6]. PLLA authorized by FDA was reported to have no flake-like mineral formed inside porous PLLA without bioglass [7]. Synthesized porous β-TCP/PLLA scaffold, taking the advantages of each other and overcoming the defects of each, is paid more attention to and demonstrates potential in bone tissue engineering.

In this study, a new method is used to prepare porous β-TCP/PLLA composite scaffold. In vitro experiments were conducted in static and dynamic simulate body fluid. The purpose of the study was to further evaluate the bioactivity of β-TCP/PLLA by investigating the formation of apatite on the β-TCP/PLLA surfaces in SBF.

## Materials and methods

**The preparation of porous β-TCP/PLLA scaffold.** β-TCP powder with an average particle size of 1.41μm(measured by a Rise LS 2008 Particle Analyzer)was prepared in our laboratory. PLLA (η=7.0g/nl) used in this study was provided by Chengdu organic chemicals co. LTD.. Porous β-TCP/PLLA composite scaffold were prepared by a new method named frozen

shrinking/particulate leaching technique. A known amount of PLLA was dissolved in the mixture solvent of chloroform and acetone, β-TCP and sieved ammonium bicarbonate powders (200–400μm) were added into the mixture solution under continuous stirring. The ratio of β-TCP to PLLA was 65:35, referring to the approximately ratio of inorganic and organic compound in nature bone. Then, the resulting slurries were maintained in a refrigerator at -10℃-0℃ to induce the volume shrinkage completely and taken out after 1 week. Until the volatile organic solvent was removed thoroughly in the air, the matrix was put into de-ionized water at 80℃ to leach out porogen, then matrix was vacuum dried. This technique yielded highly porous β-TCP/PLLA scaffold of desired porosity.

**In vitro degradation experiments.** SBF used for the in vitro degradation experiment was prepared carefully according to the procedure of Tadashi Kokubo[8]. Each liter of SBF was prepared by dissolving reagent-grade chemicals in de-ionized water and buffered to pH 7.4 at 37°C with tris(hydoxylmethyl) aminomethane and 1mol HCl, which had ion concentrations that were nearly the same with those of human blood plasma [8]. In sSBF, scaffolds were put in an abrade bottle filled with 150ml SBF. The SBF temperature was kept at 37°C using a water bath with shaking. Every other day, 30 ml SBF was replaced by fresh 30ml SBF to ensure the stable microenvironment of SBF. At the same time, to simulate the dynamic environment in body, SBF was circulated by a peristaltic pump at 2ml/100ml•min flow rate based on equivalent flow rate of bone fluid in bone muscle. A customized dynamic system as shown in Fig.1 was used in this study. The effect of flow rate of the SBF in sample chamber on the precipitation formation was also investigated. After 7,14,21,28 days, the specimens were removed from the SBF and washed gently with distilled water and then dried at room temperature.

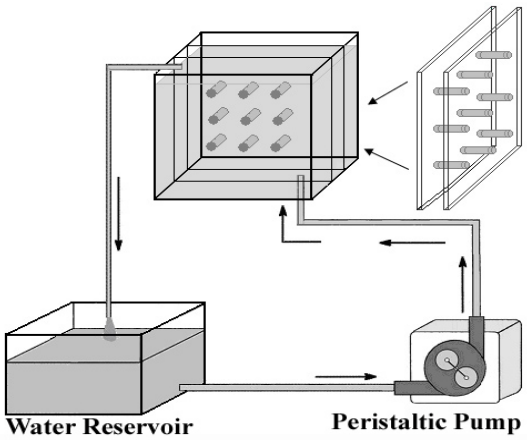


Fig.1 Schematic showing the apparatus for studying the degradation of scaffolds under fluid flow condition.

**Characterization.** The surface morphologies of specimens after experiments were examined using SEM (JSM-5900LV, Japan) after gold coating. The Ca-P precipitation crystal structures on the surface of scaffolds were identified using X-ray diffraction (X´Pert pro MRD,Philips). FTIR spectra were obtained with a NEXUS 670 FTIR spectrometer.

## Results and discussion

SEM indicated that typical morphology of precipitation which is similar on the other bioceramic surfaces was formed [6]. Fig.2a indicated that there were spherical crystals on the wall of inner pore after 14 days immersion in the sSBF. The spherical crystals were only individual granules which were regarded as early stage of Ca-P precipitation. After 21 days, a large number of tiny flake-like crystals which were similar with petal were observed from Fig.2b.With increasing immersion, the petal-like granules grew, and eventually the apatite layer were so high dense that it heavenly piled up on the surface after 28 days immersion as shown in Fig.2c.However, after immersion in the dSBF for 14 days, Fig.2d showed that there was not Ca-P formation on the wall of inner pore. Until after 21days, typical precipitation morphology can be observed from Fig.2e, but the Ca-P layer did not cover the surface of inner pore wall, which did not still happen after 28 days as shown in Fig.2f.

Apatite formations were analyzed based on classical crystallization theories of thermodynamics and kinetics [9]. In sSBF, the $Ca^{2+}$, $PO_4^{3-}$ from the scaffolds diffused difficultly into sSBF solution

and accumulated around the surfaces so that the concentration of Ca and P increased to threshold of nucleation and formed crystal nuclei. PLLA surface is hydrolyzed by water molecules in solution, converting ester bonds in the polymer into surface carboxylic acid groups. These groups become carboxylate anions in the solution (pH7.4) and therefore provide a negatively charged substrate surface which chelates calcium ions to stimulate surface nucleation. The mineral crystal growth was followed by the supplement of fresh SBF. In dSBF case, the $Ca^{2+}$, $PO_4^{3-}$ can diffuse easily into solution and be brought out of the sample chamber by the flow of SBF. Therefore, on the surface of specimens, the concentration of nucleation was hard to achieve threshold. But in the inner pore, the dissolved ion was taken away difficultly and easy to accumulate high $Ca^{2+}$, $PO_4^{3-}$ concentration to nucleate. Therefore, spherical apatite individual crystal was observed on the wall of inner pore until after 21days as shown in Fig.2e. When flow rate of the dSBF increased to 10ml/100ml•min, a few small individual crystals were observed on the inner walls until after 28 days (not shown).

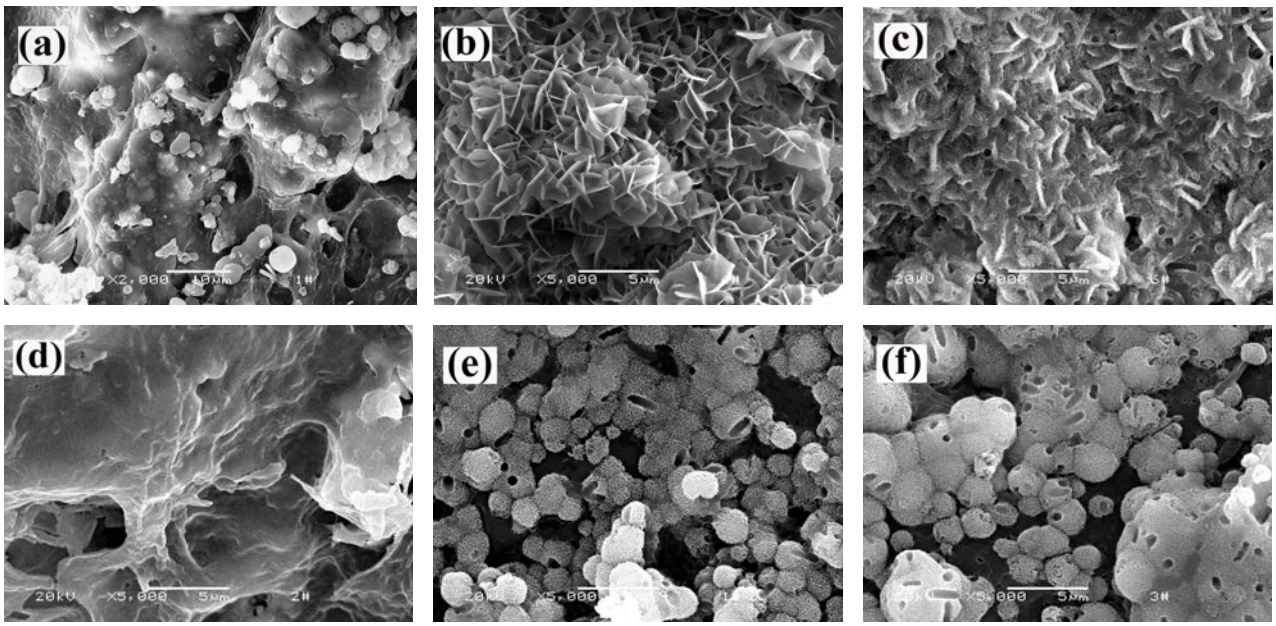


Fig.2 SEM micrographs of scaffolds after immersion in static SBF and dynamic SBF: 14days (a, d); 21days (b, e); 28days (c, f).

Figure 3 revealed the X-ray diffraction patterns of Ca-P precipitates on the surface of scaffolds immersed in sSBF and dSBF. We performed XRD pattern and carefully scanned the scaffold at low rate from 3-60°, because the OCP phase has a unique peak near 5°. It can be seem from Fig.3 that the OCP peak is found at near 4.61° and the diffraction peak position of a new crystal phase is so close to the known position of HA(near 31°). The peak position has subtle space gap near the position of HA and the diffraction peak intensity is enhanced compared to the original specimen. Andre-Frei[10] study showed that the spectrum of carbonated-HA and HA has no significant difference but only the peak position has subtle drift. Therefore, it can be inferred that bone-like apatite was formed on β-TCP/PLLA in SBF.

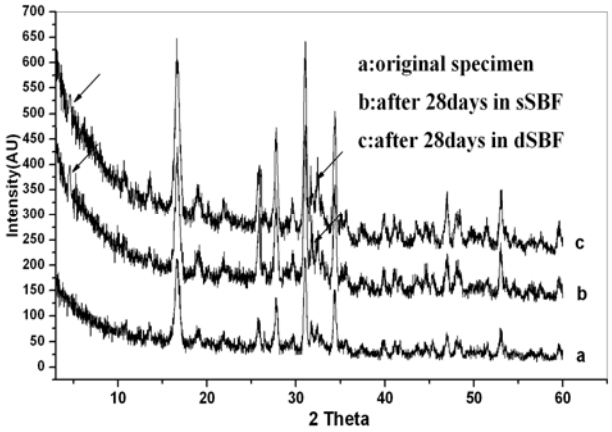


Fig.3 X-ray diffraction spectrum of specimens in sSBF and dSBF after 28days.

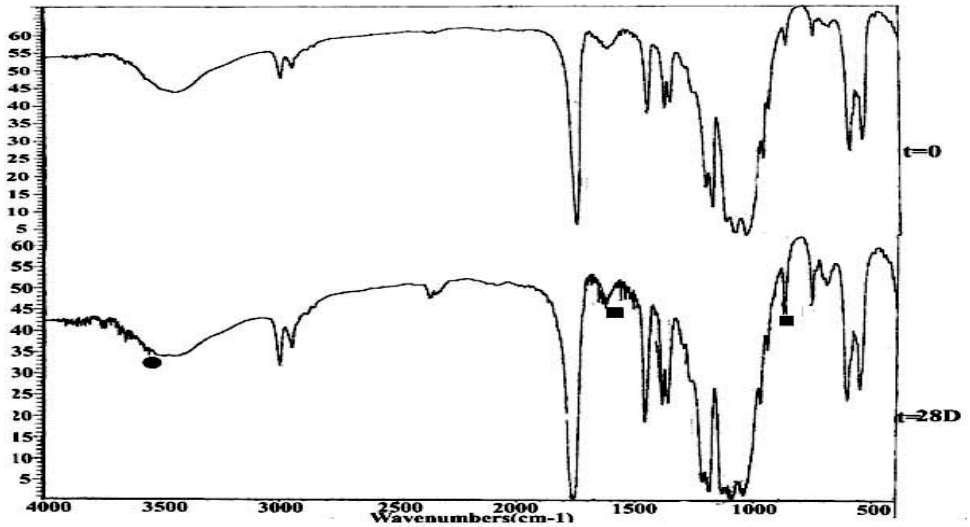


Fig.4 FTIR spectra displaying carbonate peaks (■) and hydroxyl stretch (●) in dSBF.

The FTIR analysis was performed to gain more information about the composition of the apatite. The peaks at 1508-1621 $cm^{-1}$ are indicative of the $\nu_3$ vibration of carbonate. The spectra of the formed particles has an absorption band at 864.9 $cm^{-1}$ corresponding to the $\nu_2$ vibration mode of carbonate. Tiny hydroxyl stretch is observed at 3570 $cm^{-1}$ in the spectrum of dSBF. These results suggest that the crystal particles formed on β-TCP/PLLA in SBF incubation are carbonated apatite which is similar in composition to the natural apatite.

## Conclusions

The study of Ca-P formation on β-TCP/PLLA scaffolds under two conditions generates the following results. (1). Apatite formation did not occur simultaneously in sSBF and in dSBF. Apatite formation occurred sluggishly in dSBF and was more difficult with increasing flow rate of dSBF. (2). Ca-P layer formation on β-TCP/PLLA is owed to the inducing of β-TCP in the composite scaffolds and the ability of apatite to form is enhanced by effect of each other which has different degradable mechanism. Study shows the porous β-TCP/PLLA scaffold indicates good ability of bone-like apatite formation in vitro as well as other bioceramics reported in the literature.

## References

[1] Kokubo T: Biomaterials Vol.12 (1991), p. 155.

[2] Kim HM,Miyaji F,Kokubl T and Nakamura T: J Biomed Mater Res Vol.32 (1996), p.409.

[3] Legeros RZ, Lin S and Rohanizadeh R: J Mater Sci Mater Med Vol.14 (2003), p. 201.

[4] Ohtsuki C, Kushitani H and Kokubo T: J Biomed Mater Res Vol.25 (1991), p. 1363.

[5] Ohtsuki C, Kokubo T, Neo M and Kotani S: Phos Res Bull Vol.1 (1991), p. 191.

[6] Renlong Xin, Yang Leng, Jiyong Chen and Qiyi Zhang: Biomaterials Vol.26 (2005), p. 6477

[7] Kai Zhang, Yunbing Wang and Marc A.Hillmyer: Biomaterials Vol.25 (2004), p.2489.

[8] Tadashi Kokubo, Hiroaki Takadama: Biomaterials Vol.27 (2006), p. 2907.

[9] Xiong Lu,Yang Leng:Key Eng.Mater Vol.192-195 (2001), p.47.

[10] V. Andre-Frei, B. Chevallay and I. Orly: Calcif. Tissue Int., Vol.66 (2000), p. 204.