

Ca-P Formation on Poly-L-lactide/ β -Tricalcium Phosphate Composite Scaffold in Simulated Body Fluid

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Abstract. Poly-L-lactide/ β -tricalcium phosphate (PLLA/ β -TCP) porous scaffold fabricated by freeze shrinking/particulate leaching was studied. The scaffold was immersed into simulated body fluid (SBF) for 1, 2, 3 and 4 weeks and analyzed by the SEM, XRD and FT-IR spectroscopy. The ability of inducing Ca-P formation was compared among the scaffolds with different content of β -TCP. SEM shows a typical feature of apatite precipitation. Diffraction peak of new crystal structure was detected by x-ray diffraction (XRD). IR Spectrum in which absorption bands arise from newly formed groups of carbonate apatite can be seen. At the same testing point, higher density of Ca-P crystal can be observed by SEM in scaffold with high content of β -TCP than in low group. Until 3 weeks, Ca-P individual crystal started on the wall of inner pore of pure PLLA. Porous PLLA/ β -TCP composite scaffolds also indicate good ability of Ca-P formation in vitro, the ability of which to form apatite was enhanced by addition of each other that has different degradable mechanism.

Introduction

Kokubo proposed that the bone-bonding ability of bioactive ceramics and bioceramics/polymer composites in vivo can be predicted by Ca-P layer formation in SBF [1]. Since then, bone bioactivities of various types of materials, including HA, α -TCP [2], A-W[3], BCP[4] have been evaluated by apatite formation in SBF. However, it has been reported that β -TCP exhibit a poor ability of inducing Ca-P formation in vitro [5,6] and that PLLA has the ordinary ability to form apatite in vitro [7,8]. β -TCP and biodegradable PLLA have been widely used in medicine because of their excellent biocompatible and biodegradable. Combining PLLA and β -TCP to synthesis porous PLLA/ β -TCP scaffold, thus taking the advantages of each other and overcoming the defects of each, has attracted extensive research interests.

In this study, a new method is used to prepare porous PLLA/ β -TCP scaffold. The effects of different content of β -TCP in the composite on the formation of apatite had been investigated.

Materials and method

Preparation of PLLA/ β -TCP scaffold. β -TCP powder was prepared in our laboratory. PLLA ($\eta=7.0$ g/ml) used in this study was provided by Chengdu organic chemicals co. LTD. Chinese academy of sciences. PLLA/ β -TCP scaffolds were prepared by a new method namely freeze

shrinking/particulate leaching technique. A known amount of PLLA was dissolved in the mixture solvent of chloroform and acetone, followed by adding β -TCP particles and sieved ammonium bicarbonate powders (200–400 μ m) into mixture solution under continuous stirring. Then, the resulting slurries were maintained in a refrigerator at -10°C - 0°C 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°C to leach out porogen and the de-ionized water was changed every 2 hours, then matrix was vacuum dried. The scaffolds were cut into cylinders with a diameter of 5 mm and a height of 10 mm for immersion experiments. For examining the effect of β -TCP contents, the β -TCP ratio was changed from 35 to 65 wt%. For the control experiments, pure PLLA without β -TCP were fabricated also by the same method.

In vitro experiment. SBF used for the in vitro degradation experiment was prepared carefully according to the procedure of Tadashi Kokubo[1]. In the SBF degradation experiment, scaffolds were put in an abrade beaker 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 refresh 30ml SBF to ensure the stable microenvironment of SBF. After 1, 2, 3, 4 weeks immersion, the specimens were removed from the SBF and washed gently with distilled water and then dried at room temperature.

Characterization. The surface morphologies of specimens were examined using SEM (JSM-5900LV, Japan) after gold coating. The Ca-P precipitation crystal structures were identified using X-ray diffraction (X'Pert pro MRD, Philips). The XRD measurement was to be performed in the range of 3 – 70° in 2θ at a rate of $2^{\circ}/\text{min}$. FTIR spectra were obtained with a NEXUS 670 FTIR spectrometer. A small amount of powder was scratched from the surface of an incubated scaffold, and then it was milled with KBr and pressed into a transparent film for IR analysis.

Results and discussion

SEM showed that the typical features of Ca-P precipitation on the surface and the internal pore wall of PLLA/ β -TCP. Ca-P layer can be observed after 14 days. Apatite precipitation started at individual granules (Fig.1c) and the granules gradually grew together to form a dense layer. The quantity of Ca-P precipitation on the surface of low β -TCP amount (35wt%)(Fig.1c,e) in PLLA/ β -TCP was less than that of high β -TCP amount(65wt%)(Fig.1d,f) at the same immersion time. SEM images further revealed that each Ca-P granule consisted of a large number of tiny flake-like crystals. With immersion time increasing, the individual crystals became spherical and petal-like cluster crystals, on the surface of which new flake-like crystals grew. Ca-P precipitations with similar morphology have been reported in other literatures [2]. The porous PLLA scaffolds were also subjected to the in vitro immersion. SEM indicated that the typical Ca-P precipitation was not found until after 21 days. The individual crystal can be seen only on the wall of inner pore (Fig.1b).

Apatite formations in SBF were analyzed based on classical crystallization theories of thermodynamics and kinetics. In SBF, the Ca^{2+} , PO_4^{3-} dissolved from the surface of scaffolds diffused difficultly into SBF solution and assembled around the surfaces so that the concentration of Ca and P increased to threshold of nucleation and formed crystal nuclei. More while, the PLLA surface is hydrolyzed by water molecules in solution, converting ester bonds in the polymer into

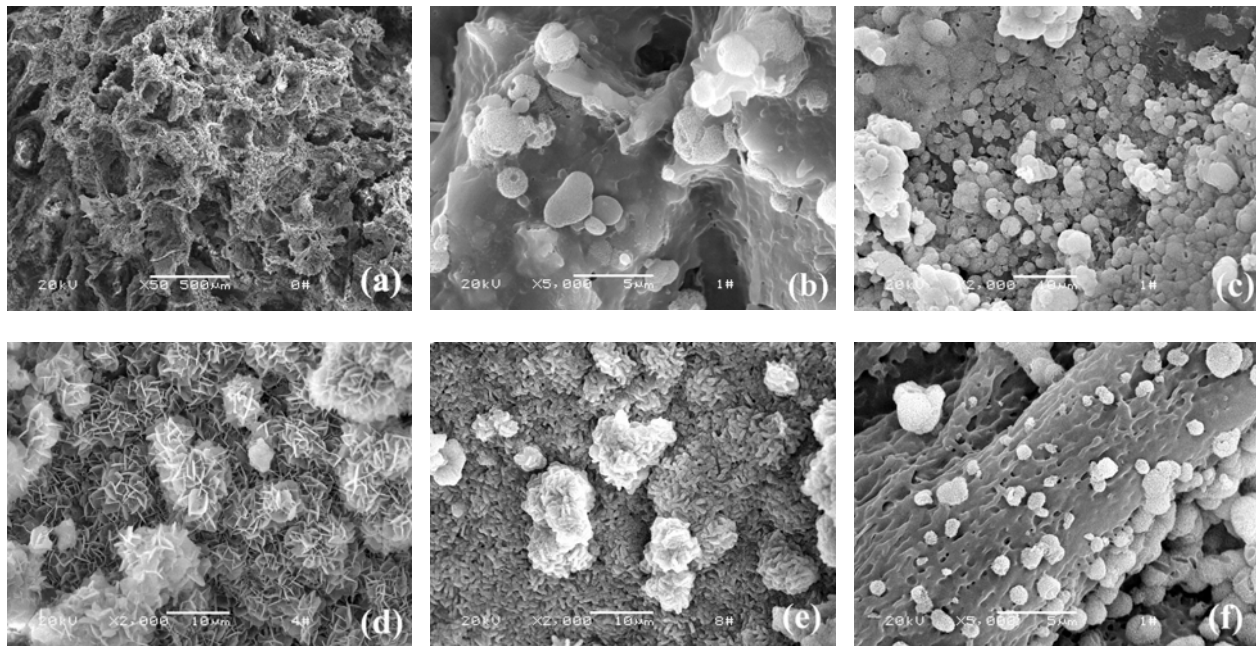


Figure1 SEM micrographs of the original pore (a), scaffold with low content of β -TCP and high content of β -TCP incubated for 14 days (b, c) and for 21 days (d, e), and the pure PLLA scaffold incubated for 21 days (f).

surface carboxylic acid groups. These groups become carboxylate anions at the solution pH (7.4) and therefore provide a negatively charged substrate surface. The negatively charged surface chelates calcium ions in solution. The binding of these soluble ionic precursors to mineral formation stimulates surface nucleation. The mineral crystal growth was followed by the supplement of fresh SBF. Therefore, the speed of Ca-P formation was slower in pure PLLA.

The X-ray diffraction of Ca-P precipitates revealing the new phase were found on the surfaces of both low β -TCP amount group and high amount group immersed in SBF. Fig.2 shown that OCP (near 4.61°) were found on the surfaces of scaffolds after 3 weeks, while the peak intensity at near 31° was enhanced after 4 weeks and the position of HA peak had subtle drift. This indicated that with time increasing, the bone-like phase formed on the scaffolds [10].

In order to confirm further the composite of the mineral formed on the inner pore surfaces of the scaffold, Fourier transfer IR was performed to attain more information about the Ca-P layer. The spectrum of PLLA/ β -TCP sample is presented in Fig.3. Tiny absorption band at 3502cm^{-1} - 3572cm^{-1} was attributed to the OH groups from bone-like apatite, while one pronounced absorption bands at 864cm^{-1} arise from the ν_2 vibration mode of carbonate. The peaks at 1508 - 1621cm^{-1} are indicative of the ν_3 vibration of carbonate. These results suggested that the crystal particles formed on PLLA/ β -TCP in SBF incubation were carbonated apatite, which was similar in composition and structure to the natural apatite in human hard tissues.

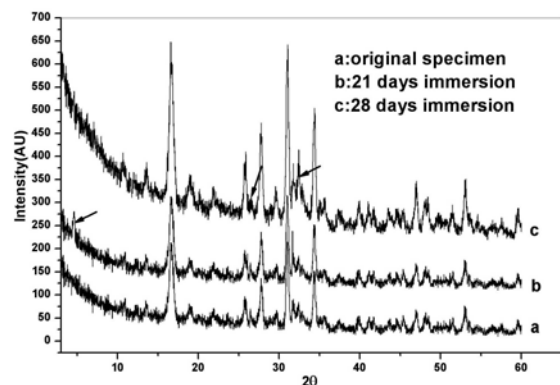


Figure2 XRD spectra of specimens from (a) original scaffold, (b) incubated for 21 days and (c) incubated for 28 days in SBF.

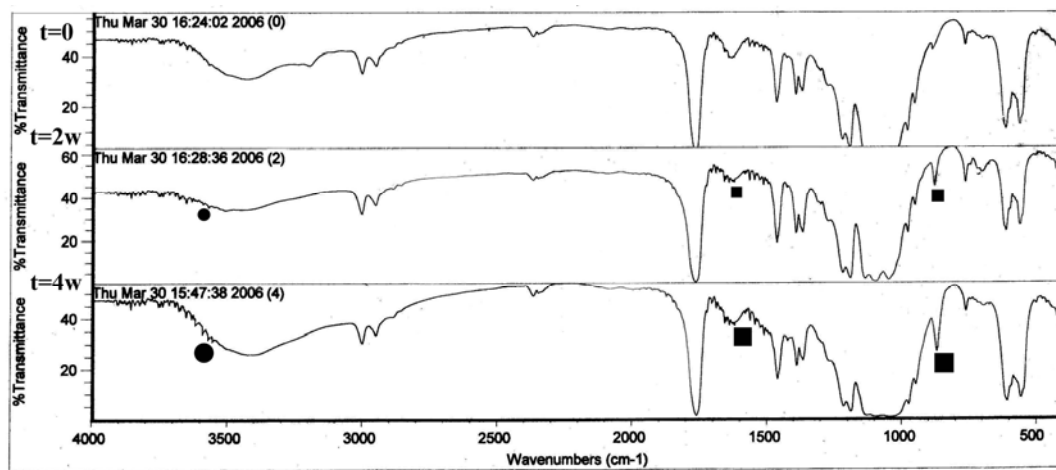


Fig. 3. IR spectrum of PLLA/ β -TCP composite biomaterial after 2, 4 weeks of testing in SBF: carbonate peaks (■) and subtle hydroxyl stretch (●).

Conclusions

Typical Ca-P precipitation morphology, the diffraction peak of new phase and Characteristic absorption bands confirm the formation of new bone-like apatite during the immersion process using SBF. By comparison, Ca-P precipitation phenomena easily occurring in PLLA/ β -TCP scaffolds with high amount of β -TCP can be determined, the reason of which is that the inducing of β -TCP can enhance the ability of Ca-P formation because of difference degradation mechanism of β -TCP and PLLA. Study shows the PLLA/ β -TCP composite scaffolds also indicate good ability of Ca-P formation in vitro as well as other bioceramics reported in the literature.

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