J Mater Sci: Mater Med (2011) 22:839–844
DOI 10.1007/s10856-011-4280-0

Preparation and characterization of a novel porous titanium scaffold with 3D hierarchical porous structures

Yuejun Chen · Bo Feng · Yaping Zhu · Jie Weng · Jianxin Wang · Xiong Lu

Received: 17 May 2010 / Accepted: 6 March 2011 / Published online: 23 March 2011

Abstract This study aims to prepare a novel porous titanium (Ti) scaffold in order to improve the biocompatibility of the metallic implants. Porous Ti was produced by a Liquid foaming method and subsequent chemical treatments. It was found that the scaffold had three-dimensionally hierarchical porous structures with pore size ranging from nanometer to micrometer scale, and it also had activated surface. Mechanical test results showed that the scaffold also has sufficient compressive strength to meet the requirements of implantation. Protein adsorption results indicated that the novel scaffolds significantly enhanced the protein adsorption.

1 Introduction

With excellent mechanical properties, biocompatibility and good corrosion resistance, Ti and Ti alloy have already become the first-selected material for medical use such as artificial joints, bone trauma products and so on [1–5]. However, a large number of clinical studies found that as a result of the mismatch of elastic modulus between autogenous bone and Ti, the load can not be delivered to the adjacent bone tissue from the implants, which is called "stress shielding" [6]. This has become the biggest obstacle to constraint the application of Ti implant. Therefore, it is necessary to reduce the elastic modulus of Ti to increase its bio-mechanical compatibility and to modify its surface to enhance the biological activity.

Medical porous Ti was developed due to its ability to allow biological tissue growing into the pores to improve the mechanical fixation, which requires sufficient porosity, pore size and necessary mechanical properties. The structure of pores in biomaterial scaffolds plays a critical role in bone formation in vitro and in vivo. For most of these scaffolds, 3D interconnected and hierarchical porous structure is desirable in order to optimize the activity of the surface and to promote tissue regeneration and reconstruction [7–16]. Liquid foaming method was commonly used in the preparation of porous ceramics, however, report on the preparation of porous metal by this method has rarely been seen. In this paper, porous Ti scaffolds with 3D hierarchical porous structure and activated surface was produced by Liquid foaming method, and the purpose of our study was to obtain a more biocompatible implant surface.

2 Materials and methods

2.1 Liquid foaming

Commercial pure Ti powders with average diameter of 38 μm were supplied by Bangzheng Titanium Co. Ltd., Shangxi, China. Elemental composition of Ti powders was shown in Table 1. Carboxymethyl cellulose sodium (CMC), Sodium hexametaphosphate (SHMP) and hydrogen peroxide (H_2O_2) were used as the binder, dispersant and foaming agent, respectively. All the reagents were of analytical grade. Ti powders were dispersed into H_2O_2 solution containing CMC and SHMP. Then, the mixture

Y. Chen (✉) · B. Feng · Y. Zhu · J. Weng · J. Wang · X. Lu
Key Laboratory of Advanced Technologies of Materials (Ministry of Education), Southwest Jiaotong University, Chengdu, Sichuan, People's Republic of China
e-mail: yuejun_chen@sina.com

Y. Chen · B. Feng · Y. Zhu · J. Weng · J. Wang · X. Lu
School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan, People's Republic of China

Table 1 Elemental composition of Ti powders

Elemental	C	Ti	H	O	N	Si	Cl	Al	Fe
Content (%)	0.023	99.3	0.011	0.19	0.017	0.024	0.084	0.41	0.096

was stirred at room temperature for 1 h to form homogeneous Ti slurry. Subsequently, the obtained slurry was poured into molds, dried in 40–60°C for 3 h, and sintered at 1300°C for 3 h in the vacuum sintering furnace.

2.2 Chemical treatment

The obtained Ti scaffolds were subsequently immersed in a mixed acid of 48% (volume ratio) H_2SO_4 and 18% HCL for 30 min and in 6 M aqueous NaOH solution at 70°C for 6 h. After acid-alkali treatment, the samples were washed with distilled water and dried at 40°C for 24 h in an air atmosphere. The preparation process of porous Ti scaffolds was shown in Fig. 1.

2.3 Characterization

Total porosity (P) was measured by gravimetry according to the following equation

$$P = 1 - \frac{\rho_{\text{scaffold}}}{\rho_{\text{material}}}$$

where ρ_{material} is the density of the material of which the scaffold is fabricated and ρ_{scaffold} is the apparent density of porous titanium measured by dividing the weight by the volume of the samples. ρ_{scaffold} was determined by measuring the physical dimensions and mass of the samples.

Scanning electron microscopy (SEM) (Quanta 200, FEI, The Netherlands) equipped with an Everhart–Thornley SE-detector and energy dispersive spectrometer (EDS) was used to examine the morphology and elemental composition of the porous Ti scaffolds after sintering. The samples were coated with a gold film before SEM observation at an accelerating voltage of 20 kV.

The X-ray diffraction patterns were obtained using a thin film X-ray diffractometer (TF-XRD) (X'pert pro-MPD, PANalytical, The Netherlands). The TF-XRD measurements were performed on a stage using a Cu-Kα (wavelength = 1.54056 Å) X-ray source and the angle 2θ was varied from 20° to 50° at a step rate of 0.03° per second. Database of PDF2004 was used in X-ray diffraction analysis and the monocrystalline silicon was used as an internal standard to calibrate the diffraction angle.

Compression tests were performed using an Instron mechanical testing machine (Instron5567, Instron Ltd., USA) with a loading rate of 1 mm/min at room temperature. Six cylindrical samples with diameters of 7 mm and the length of 14 mm were tested to obtain the average value. Young's modulus could be calculated as the slope of the straight-line portion of the stress–strain curve.

2.4 Protein adsorption

Bovine serum albumin (BSA) is the most abundant protein in serum, and it has an important role in bio-mineralization and cell response. Bovine serum albumin with the purity of more than 98% (Amresco-0332, Amresco Ltd., USA) was selected for protein adsorption experiment. In order to maintain a balance of PH value, protein solution was prepared in phosphate buffer saline (PBS). The PBS was prepared by dissolving 8 g NaCl, 0.2 g KCl, 0.24 g KH_2PO_4 and 1.44 g Na_2HPO_4 in 0.9 L distilled water, adding HCl to adjust pH and topping up to 1 l. Porous Ti scaffolds before (PT) and after chemical treatment (SPT) were immerged in BSA/PBS solution (1 mg/ml, pH = 7.4) at 37°C. One milliliter of BSA solution was taken out after 0.5, 1, 2, 4, 8, 16 and 24 h, respectively. According to Lambert–Beer law, the absorbance of a solution is directly proportional to its concentration at a given wavelength of light. The absorbance of BSA solution at different time points was measured at 280 nm wavelength by UV spectrophotometer (UV-2550, Shimadzu, Japan). The concentration was obtained using a standard curve from known concentrations of BSA solutions, and the curves of adsorption quantity-time were plotted. Three samples were measured per time point to get the average value.

3 Results and discussion

3.1 Porous structure

Porous Ti scaffold prepared by liquid foaming method is shown in Fig. 2a. The scaffold has interconnected pore structure and high porosity of 76%. Figure 2b shows the

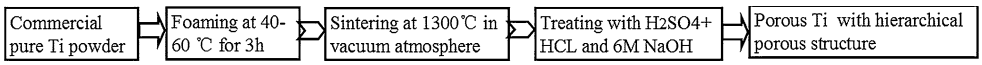

Fig. 1 Processing steps for fabricating porous Ti scaffold

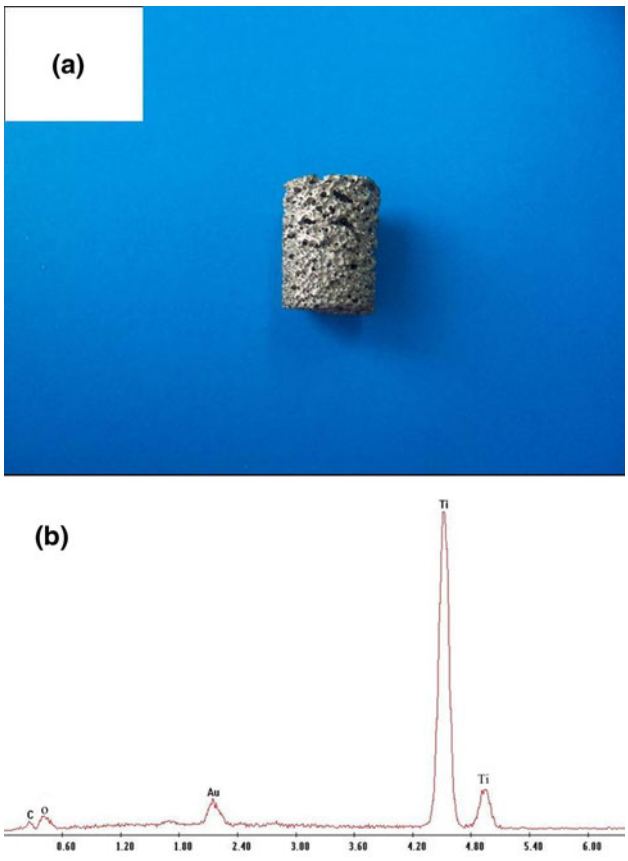

Fig. 2 Porous Ti scaffold with 76% porosity prepared by liquid foaming method and EDS results of the samples

EDS results. It can be seen that nearly all the vesicants disappeared after sintering, which will ensure the safety of porous Ti after been implanted. Compared with other techniques, the liquid foaming process is more attractive because it is easy to operate, non pressure-treated and do not need other complex equipment. Moreover, this method can also prepare porous Ti with high porosity and 3D interconnected pore structure.

In order to obtain hierarchical porous structure, chemical methods are used to treat the porous Ti. Scanning electron microscopy observations of porous Ti after chemical treatment are presented in Fig. 3. There are three kinds of pores in this Ti scaffolds: macropores with pore size larger than 100 μm (Fig. 3a), micropores about 10 μm (Fig. 3b) and network-like nanopores (Fig. 3c–d). Macropores and micropores were obtained after sintering, while nanopores were produced by chemical treatment. In addition, chemical treatment also changed the relative density of the material from 0.24 to 0.19. Because of nutrients supply and vascularization, pore size has been shown to affect the progression of osteogenesis. Macropores in the range of 100–400 μm can promote the ingrowth of bone and blood vessels, while micropores are favorable for nutrients transmission and osteochondral formation before osteogenesis. As for nanopores, they are conducive to the adhesion of cells because of its increased surface area energy. Vassilis and others studies [17] have also shown that the minimum requirement for pore size is considered to be $\sim$100 μm due to cell size, migration requirements and transport. However, pore sizes above 300 μm are recommended, due to the enhanced new bone formation and the formation of capillaries. Gradients in pore sizes are recommended on the formation of multiple tissues and tissue interfaces. Porous Ti scaffolds with 3D hierarchical porous structures prepared in this study are expected to meet the requirements of specificity of bone repair needs.

3.2 XRD results

As can be seen from Fig. 3c–d, a lot of white network-like sediments were generated on the surface of Ti scaffold after sintering and chemical treatment. X-ray analysis results (see in Fig. 4) show that the main ingredients of the sediments are Sodium titanate ($Na_2Ti_5O_{11}$) and Rutile (TiO_2). Some chemical and thermal treatments have been developed and tested in order to modify the composition and morphology of surface layers, thereby lead to a moderate bioactive behavior. In this work, we have chosen acid-alkali approach to treat the porous Ti scaffolds, for it can not only get graded porous structure, but also activate the surface of porous Ti. The erosion of hydrochloric acid and sulfuric acid further increase the roughness and surface area, while alkali treatment generate porous oxide layer in the surface of materials. Usually, the formation of bone-like apatite on the surface is regarded as a sign of better biological activity of materials. Some studies [18] have also showed that TiO_2 layers on the surface can promote the deposition of bone-like apatite by ion-exchange with body fluids. In addition, the oxide layer formed on the surface of Ti scaffold in physiological environments could limit ion release, which ensures bio-safety.

3.3 Mechanical properties

The mechanical test result, which is shown in Fig. 5, indicates that the compressive strength and Young's modulus of the porous Ti scaffold were 23.6 ± 3.4 MPa and 2.1 ± 0.5 GPa, respectively. Bone regeneration requires materials to be capable of providing mechanical and chemical signals in order to promote biomineralization. Traditional inert biomaterials that illicit a minimal immune response were considered ideal for implants, however, these materials often do not match the mechanical needs of the implant site, or are subject to fibrous encapsulation over time, leading to implant failure[19–21]. It can be observed that the compressive strength of porous

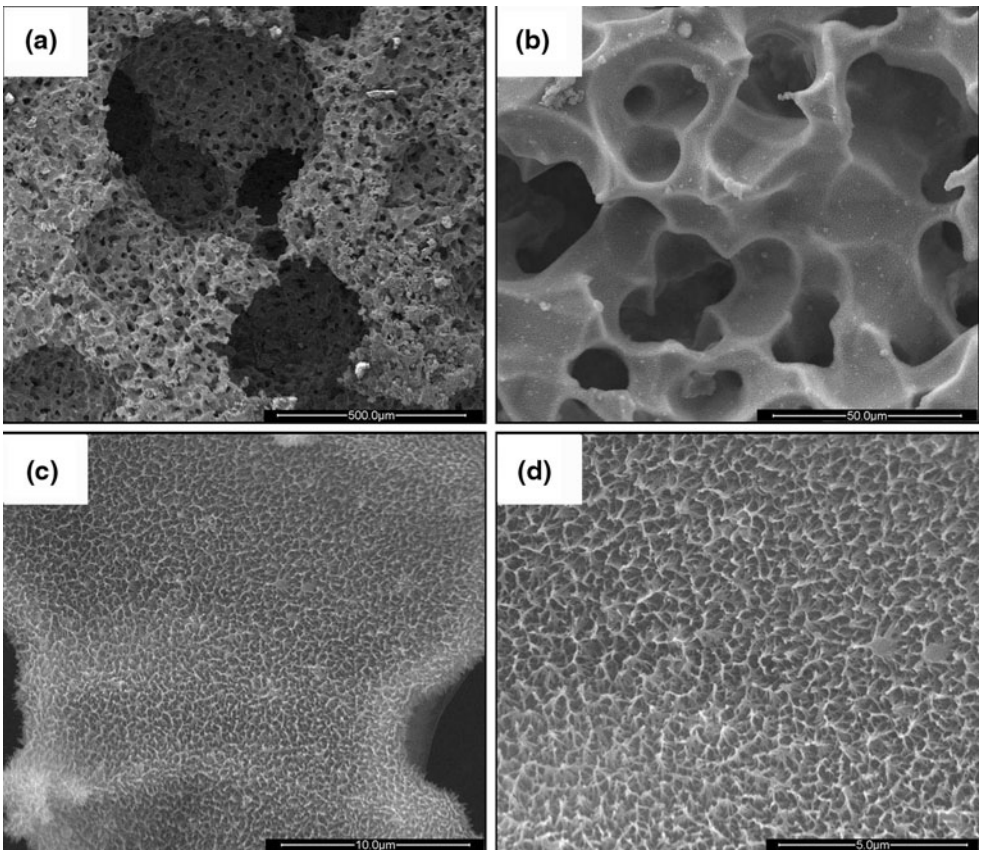

Fig. 3 Three kinds of pores in the Ti scaffold with hierarchical porous structure after sintering plus chemical treatment. **a** macropores, **b** micropores, **c** nanopores and **d** higher amplification pictures of (**c**)

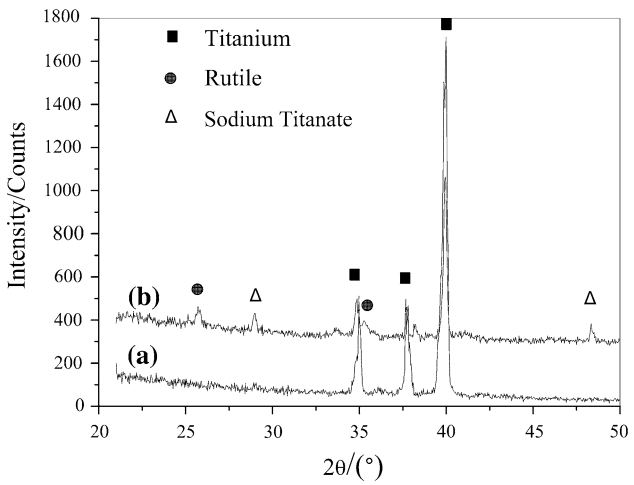

Fig. 4 XRD patterns of porous Ti scaffolds **a** before and **b** after chemical treatment

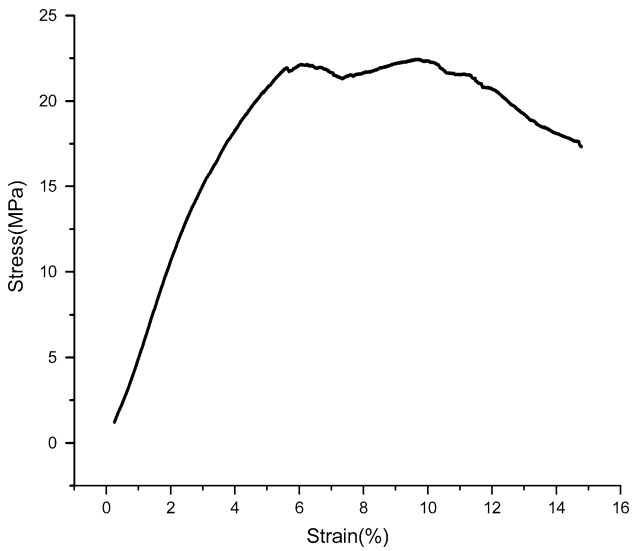

Fig. 5 Stress–strain curve of porous Ti scaffold tested in compression

Ti scaffolds is higher than other types of scaffolds, such as hydroxyapatite (HA) and poly (L-lactide-*co*-D,L-lactide) scaffolds [22], and this value for the porous Ti scaffold is very similar to that of the cancellous bone [23].This is a very relevant result in terms of load transfer to bone since it should minimize the stress shielding effect.

3.4 Protein adsorption

The curves of BSA protein concentration over time are shown in Fig. 6. Two samples have the same trend. In the

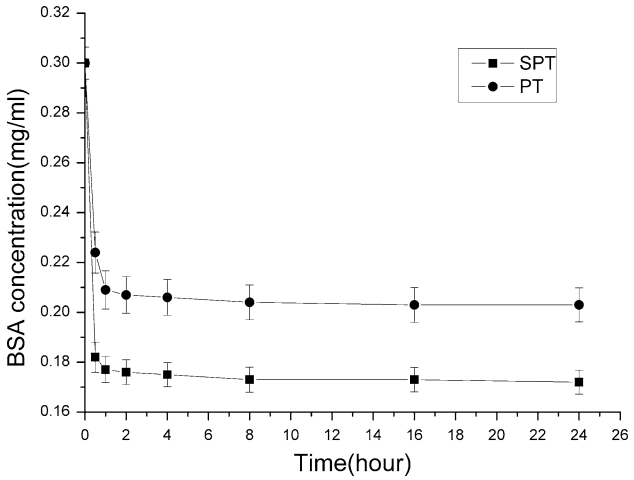

Fig. 6 BSA concentrations with time when the scaffolds immersed in BSA solution. (*PT* before chemical treatment; *SPT* after chemical treatment)

initial, the protein concentration declines rapidly, and then drops down until it reaches dynamic equilibrium gradually. In addition, the protein adsorption of SPT samples is higher than that of PT samples.

Possible reasons are as follows. Protein adsorption is a complex process which is related with surface chemical composition, morphology, wettability, surface group and so on. When the acid-alkali treated samples immersed in BSA solution, ion-exchange began between Sodium Titanate and water. Na^+ was replaced by H_3O^+, soon after a large number of Ti–OH groups formed on the surface of the samples. Then, the negatively charged Ti–OH groups adsorbed the positively charged ions of proteins through electrostatic in order to achieve the adsorption of proteins on the surface of materials.

Furthermore, porous Ti scaffold after chemical treatment provide more total surface area for protein adsorption due to network-like nanopores exposed on the surfaces of pore walls. Others studies [24–26] have shown that high capacity of protein adsorption is an important property of a scaffold in application. The more the protein is adsorbed in a scaffold, the more cells can attach to it and survive during the initial culture period.

4 Conclusions

Porous Ti scaffold with 3D hierarchical porous structure and sufficient compressive strength was successfully prepared by Liquid foaming method followed by acid-alkali treatment. Chemical treatment can not only get nano-hole mesh, but also activate the surface of Ti scaffold. The novel porous Ti scaffold with morphological and mechanical properties is potentially to be used to meet the requirement of bone repair.

Acknowledgments This research was supported by the Chinese FANEDD (200554) and the National Key Project of Scientific and Technical Supporting Programs Funded by the Chinese MSTC (2006BAI16B01).

References

1. Brunette DM, Tengvall P, Textor M, Thomsen P. Titanium in medicine. Berlin: Springer; 2001.
2. Van Noort R. Titanium: the implant material of today. J Mater Sci. 1987;22:3801–11.
3. Wang K. The use of titanium for medical applications in the USA. Mater Sci Eng. 1996;213 A:134–7.
4. Head WC, Bauk DJ, Emerson RH. Titanium as the material of choice for cementless femoral components in total hip arthroplasty. Clin Orthop. 1995;311:85–90.
5. Takemoto M, Fujibayashi S, Neo M, Suzuki J, Kokubo T, Nakamura T. Mechanical properties and osteoconductivity of porous bioactive titanium. Biomaterials. 2005;26:6014–23.
6. Sul YT, Johansson CB, Petronis S, Krozer A, Jeong Y, Wennerberg A, Albrektsson T. Characteristics of the surface oxides on turned and electrochemically oxidized pure titanium implants up to dielectric breakdown: the oxide thickness, micropore configurations, surface roughness, crystal structure and chemical composition. Biomaterials. 2002;23:491–501.
7. Svehla M, Morberg P, Zicat B. Morphometric and mechanical evaluation of titanium implant integration: Comparison of five surface structures. J Biomed Mater Res. 2000;51:15–6.
8. Liu LS, Thompson AY, Heidaran HA. An osteoconductive collagen/hyaluronate matrix for bone regeneration. Biomaterials. 1999;20:1097–108.
9. Ayers RA, Simske SJ, Bateman TA, Petkus A, Sachdeva RL, Gyunter VE. Effect of nitinol implants porosity on cranial bone ingrowth and apposition after 6 weeks. J Biomed Mater Res. 1999;45:42–7.
10. Kujala S, Ryhanen J, Danilov A, Tuukkanen J. Effect of porosity on the osteointegration and bone ingrowth of a weight-bearing nickel-titanium bone graft substitute. Biomaterials. 2003;24:4691–7.
11. Fisher JP, Vehof JW, Dean D, Waerden JP, Holland TA, Mikos AG. Soft and hard tissue response to photocrosslinked poly (propylene fumarate) scaffolds in a rabbit model. J Biomed Mater Res. 2002;59:547–56.
12. Tsuruga E, Takita H, Itoh H, Wakisaka Y, Kuboki Y. Pore size of porous hydroxyapatite as the cell-substratum controls BMP-induced osteogenesis. J Biochem. 1997;121:317–24.
13. Gotz HE, Muller M, Emmel A, Holzwarth U, Erben RG, Stangl R. Effect of surface finish on the osseointegration of laser-treated titanium alloy implants. Biomaterials. 2004;25:4057–64.
14. Kuboki Y, Jin Q, Takita H. Geometry of carriers controlling phenotypic expression in BMP-inducedosteogenesis and chondrogenesis. J Bone Joint Surg Am. 2001;83:105–15.
15. Schwarz K, Epple M. Hierarchically structured polyglycolide-a biomaterial mimicking natural bone. Macromol Rapid Commun. 1998;19:613–7.
16. Thelen S, Barthelat F, Brinson LC. Mechanics considerations for microporous titanium as an orthopedic implant material. J Biomed Mater Res. 2004;69A:601–10.
17. Vassilis K, David K. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26:5474–91.

18. Kokubo T, Miyaji F, Kim HM, Nakamura TJ. Spontaneous formation of bone like apatite layer on chemically treated titanium metals. J Am Ceram Soc. 1996;79:1127–9.
19. Gotman I. Characteristics of metals used in implants. J Endourol. 1997;11:383–9.
20. Elbert SE, Hubbell JA. Functional biomaterials: design of novel biomaterials. Annu Rev Mater Res. 2001;31:183–201.
21. Wen CE, Mabuchi M, Yamada Y. Processing of biocompatible porous Ti and Mg. Scr Mater. 2001;45:1147–53.
22. Lin AS, Barrows TH, Cartmell SH, Guldberg RE. Microarchitectural and mechanical characterization of oriented porous polymer scaffolds. Biomaterials. 2003;24:481–9.
23. Ontanon M, Aparicio C, Ginebra MP, Planell JA. Biological materials. Oxford: Pergamon Press; 2000.
24. Wang XJ, Song GJ, Lou T. Fabrication and characterization of nano composite scaffold of poly (L-lactic acid)/hydroxyapatite. J Mater Sci Mater Med. 2009;21(1):183–8.
25. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. J Biomed Mater Res. 2000;51(3):475–83.
26. Woo KM, Seo J, Zhang R, Ma PX. Suppression of apoptosis by enhanced protein on polymer/hydroxyapatite composite scaffolds. Biomaterials. 2007;28:2622–30.