A novel hydrophilic poly(lactide-*co*-glycolide)/lecithin hybrid microspheres sintered scaffold for bone repair

Xuetao Shi,[1,2]* Yingjun Wang,[1,3]* Li Ren,[1,3] Chen Lai,[1,3] Yihong Gong,[2] Dong-An Wang[2,3]*
[1]*School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, China*
[2]*Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637457, Republic of Singapore*
[3]*Key Laboratory of Specially Functional Materials and Advanced Manufacturing Technology, South China University of Technology, Ministry of Education, Guangzhou 510641, China*

Received 24 September 2008; revised 15 December 2008; accepted 17 December 2008
Published online 16 March 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.32423

Abstract: Novel 3-D porous scaffolds made of sintered poly(lacide-*co*-glycolide) (PLGA)/lecithin hybrid microspheres (PLGA/Lec-SMS) were developed and investigated. The addition of lecithin in PLGA bulk successfully managed the desired hydrophilic modification without sacrificing bulk properties. The outcomes were verified with infrared (ATR-FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and contact angle analyses. Specifically, this model of scaffold gained significant improvement in mechanical (mainly compressive) strength upon an optimization of lecithin fractions aligning with sintering conditions. Given a perspective of bone tissue engineering use, human fetal osteoblasts were seeded into a series of these PLGA/Lec-SMS scaffolds upon which key parameters of cytocompatibility and osteoconductivity (including cell viability, alkaline phosphatase activity, calcium secretion, and osteogenic genes expression) were assessed. Osteoblasts seeded on PLGA scaffolds with 5 wt % lecithin demonstrated high cell viability and alkaline phosphatase activity. Moreover, elevated lecithin also enhanced the expression of type I collagen. Taken together, these results suggest PLGA/Lec-SMS are promising scaffolds for bone repair. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 92A: 963–972, 2010

Key words: poly(lattice-*co*-glycolide); lecithin; scaffolds; bone repair

INTRODUCTION

Tissue engineering is a multidisciplinary science that utilizes basic principles of engineering and life sciences to create new tissues from their cellular components; and furthermore it provides an alternative approach to aid in tissue repair via creating a microenvironment that could induce cells residing in a scaffold to produce a desired extracellular matrix and regenerate tissue.[1–3] Bone tissue engineering always demands a scaffold to ideally possess both mechanical and biofunctional superiorities upon which the projected regeneration can be fulfilled with both instant and permanent efficacies.[4–6]

In this perspective, poly(lactic-*co*-glycolide) (PLGA)-based biodegradable polymeric materials have been extensively employed due to their excellent processibility and mechanical properties as well as the talents of controlled degradation.[7,8] As a synthetic polymer, PLGA lacks functional groups; and improvement of biocompatibility is also demanded. Many approaches have been carried out to enhance the bio-functionality of PLGA, including surface plasma treatment, surface entrapment, biomolecules blending, etc.[9–11] Blending PLGA with special natural biomolecules provides a simple and effective pathway for this purpose. Lecithin is a substance extracted from mammalian neuronal tissues or brain.[12] It is a typical amphiphilic phospholipid with good biocompatibility and capability of mixing with PLGA, poly(lactic acid) (PLA), or poly-ε-caprolactone (PCL) in their chloroform or dichloromethane

*These authors contributed equally to this work.

Correspondence to: Y. Wang; e-mail: imwangyj@scut.edu.cn or D.-A. Wang; e-mail: DAWang@ntu.edu.sg

Contract grant sponsor: China Scholarship Council (CSC) scholarship recipient; contract grant number: 2007U33046
Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 50572029
Contract grant sponsor: Key Programs of the Ministry of Education; contract grant number: 305012
Contract grant sponsor: The State Key Program of National Natural Science of China; contract grant number: 50732003
Contract grant sponsor: China Postdoctoral Science Foundation; contract grant number: 20070410822
Contract grant sponsor: Ministry of Education, Singapore; contract grant number: ARC 10/06

solution. It has been applied to enhance the hydrophilicity of plain PLGA materials. Wang et al. employed lecithin as an organic template to control the nucleation and growth of inorganic minerals.[13] Additionally, lecithin-containing systems were also found to maintain proteins from denaturation during delivery.[14,15]

Besides the traditional forms of sponge-like porous scaffolds made of PLGA, which are either fabricated via solvent casting/particulate leaching (SCPL) or thermally induced phase separation (TIPS), Borden et al. have developed an innovative technology to sinter thermoplastic microspheres together into a single macro-construct that, as a novel tissue-engineering scaffold, achieves superior mechanical strength with unlimited size of fabrication.[16–18] Herein, we developed a novel sintered PLGA/lecithin hybrid microspherical (PLGA/Lec-SMS) scaffold for bone tissue engineering. Furthermore, the improved performance on osteoconductivity is assessed via an *in vitro* transplantation of human fetal osteoblasts in a series of these PLGA/Lec-SMS scaffolds.

MATERIALS AND METHODS

Materials

PLGA (lactic/glycolic 1:1; Mw 31,000 Da; inherent viscosity 0.30 dL/g in chloroform at 30°C) was purchased from Daigang Biomaterials. (Jinan, China). Poly(vinyl alcohol) (PVA) was obtained from Sigma-Aldrich (Singapore). Lecithin (soy) was supplied by Boao (Shanghai, China). All the cell culture–related reagents were purchased from Gibco (Invitrogen, Singapore).

PLGA/lecithin membrane preparation

Lecithin was mixed with 2 g PLGA solution in 10 mL methylene chloride according to a 1:100, 1:20, or 1:10 lecithin to PLGA weight ratio. The mixture was vortexed until uniform solution formed, and then was poured into a glass dish. The PLGA/lecithin composite membrane was removed when the solvent completely evaporated in air for 8 h.

PLGA/lecithin composite microspheres preparation

As shown in Figure 1, lecithin (0.05 g, 0.25 g, or 0.5 g) was mixed with 5 g PLGA solution in 25 mL methylene chloride by stirring till uniform solution was formed. The mixture was added dropwise to a stirred 1% PVA solution. The resultant emulsion was vortexed with a stirring speed of 200 rpm for 12 h to allow the solvent to be completely evaporated. PLGA/lecithin blend microspheres were isolated by vacuum filtration and were washed three times in deionized water. The round-shaped microspheres were chosen with sieves and stored in a dessicator for future use.

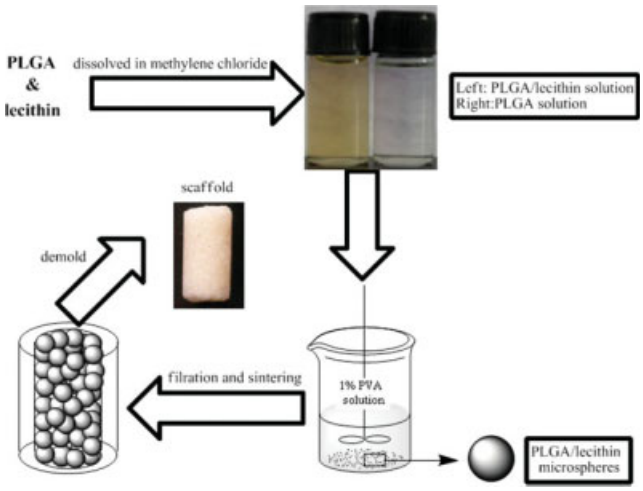

Figure 1. Scheme for preparation process of microspheres sintered scaffold. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Three-dimensional PLGA/Lec-SMS fabrication

PLGA/Lec-SMS were fabricated by pouring microspheres with different diameter into a cylindrical silica gel mold (diameter = 10 mm, height = 20 mm) and heated at 60°C, 65°C, or 70°C for 6, 12, or 24 h, respectively. After the molds were cooled down to the room temperature, the samples were removed from the molds.

Morphological characterization

Morphological characterization was conducted using scanning electron microscopy (SEM, 30XLFEG, Philips, The Netherlands). The microspheres were adhered on a cupreous stub by double-faced adhesive tape and coated with gold and further analyzed.

Static contact angles

Static contact angles of PLGA and PLGA/lecithin membranes (1×1 cm^2) were measured with a contact angle analyzer (First 10 A° angstroms, Virginia) using the sessile drop technique. The measurements were carried out at room temperature in air with deionized water as the probe liquid. Twenty-five microlitre liquid droplets were deposited onto the sample surface through a gauge-dispensing needle at a rate of 5 mL/s. Each contact angle reported here was an average of at least five measurements and the contact angles were determined with direct optical images by a camera.

ATR-FTIR spectroscopy and X-ray photoelectron spectroscopy

ATR-FTIR spectroscopy was conducted with a Nicolet Magna-IR 550 spectroscopy. PLGA or PLGA/lecithin microspheres (2 mg) were measured with 200 mg of KBr as a pellet pressed with 10 ton press. Each spectrum was corrected

for the background to obtain the sample vibrational spectrum. XPS spectra were recorded on a Kratos spectrometer (Japan) operated using Al Kα (1486.6 eV) monochromatic X-ray source.

Porosity determination of scaffolds

Porosity of PLGA/Lec-SMS was determined following the method described in the reference.[19] Briefly, ethanol was used as the liquid phase and kept at 25°C. A bottle filled with ethanol was weighed (W_1). Then a scaffold sample weighing W_S was immersed into the bottle and weighed (W_2). ρ is the density of ethanol at 25°C The size of the cylindrical scaffold including radius (R) and height (H) was measured. The porosity (P) was calculated using the equation as follows:

$$P = 1 - (W_1 - W_2 + Ws)/\rho)/((\pi \times R^2) \times H) \quad (1)$$

Mechanical behavior test

Compressive strength and compressive modulus of the cylindrical scaffolds (diameter = 10 mm, height = 20 mm) were measured using a universal material testing machine (Instron 5567, Instron) at a crosshead speed of 5 mm/min for compressive strength tests and 50 mm/min for DTS tests.

Cells culture and seeding

Human fetal osteoblasts (hFOBs) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained in DMEM/Ham's F12 (1:1) culture medium with supplements of 2.5 mM L-glutamine, 0.3 mg/mL G418, and 10% (v/v) FBS. The fabricated scaffolds were sterilized with 70% ethanol for 2 h and washed with PBS solution three times. Scaffolds were pre-wetted in the culture medium for 12 h. Fifty microlitre of the cell suspension (1×10^6 cells/scaffold) was seeded into the pre-wetted scaffolds. The scaffolds were left in the humidified incubator for 2 h to allow cells to attach on the scaffolds, and then 750 μL of culture medium were added to each scaffold. The scaffolds with cells were cultured at 33.5°C in a humidified incubator of 5% CO_2 for 14 days.

Cell viability

The viability of hFOBs on PLGA-SMS, PLGA/Lec(5%)-SMS, and PLGA/Lec(10%)-SMS was tested using the WST-1 assay (4-[3-(4-lodophenyl)-2-(4-nitrophenyl)- 2H-5-tetrazolio]-1,3-benzene disulfonate assay, Roche Diagnostics, Germany). Briefly, Osteoblasts were incubated for 1, 3, 7, and 14 days before analysis. The WST-1 reagent was added to the cell-cultured scaffolds, and incubated at 37°C, 5% CO_2 for 4 h. Analyses were performed in triplicate. The level of dye formed was then measured using microplate reader at a wavelength of 450 nm with a reference wavelength of 625 nm. The loaded cell viability on the scaffolds was detected on Day 14 using a "Live/Dead" assay (Molecular Probes, Invitrogen Singapore) following the manufacturer's instruction.

Osteogenic analysis

The culture conditions were altered to investigate differentiation by elevating the cultivation temperature to 39.5°C. hFOBs were harvested at various time points and subjected to osteogenic tests, including alkaline phosphatase (ALP) assay, calcium deposition, and phenotype checking. Both the ALP and total DNA tests were based on analyses of cell lyses, while the corresponding measurements were conducted using pNPP assay (*p*-nitrophenyl phosphate liquid substrate, Sigma Diagnostics) and Hoechst assay (Hoechst 33258, Molecular Probes, Invitrogen), respectively, following the manufacturers' instructions. Via the measured DNA quantities, the exact numbers of the committed cells could be counted using a conversion rate of 6.6 pg DNA per cell, and the ALP outcomes were further normalized against the cell numbers using this value. A quantificational Alizarin red–based assay of mineralization by osteoblasts was performed according to the reference.[20]

Gene expression

The gene expression of ALP, type I collagen, and osteocalcin (OC) in the cells on the composite PLGA and PLGA/lecithin composite scaffolds were evaluated using a real time RT-PCR. Total RNA was extracted following TRIzol protocol and subjected to RT with SuperScript™ First-Strand Synthesis System (Promega). The yielded complementary DNA (cDNA) was then subjected to PCR examining gene expressions of ALP, type I collagen, OC, and β-actin. The adopted primer sequences and PCR conditions are listed in Table I. The quantitative polymerase chain reaction (qPCR) was conducted with SYBR green assay (iQ supremix, Bio-rad). The gene expressions were quantified with a calculation of $2^{-\Delta C}{}_T$, where C_T represents the cycle number when an arbitrarily placed threshold was reached, and $\Delta C_T = (C_{T,\text{target gene}} - C_{T,\beta\text{-actin}})$.

Statistical analysis

Experiments were repeated three times and results were expressed as means ± standard deviations. Statistical sig-

TABLE I
Validated Primer Sequences for Real-Time PCR

Gene	Direction	Sequence (5′-3′)
ALP	Forward	GGGAACGAGGTCACCTCCAT
	Reverse	TGGTCACAATGCCCACAGAT
OC	Forward	AGCAAAGGTGCAGCCTTTGT
	Reverse	GCGCCTGGGTCTCTTCACT
Collagen I	Forward	CCTGCGTGTACCCCACTCA
	Reverse	ACCAGACATGCCTCTTGTCCTT
β-actin	Forward	CCTGGCACCCAGCACAAT
	Reverse	GGGCCGGACTCGTCATACT

nificance was calculated using one-way analysis of variance (one-way ANOVA). Comparison between the two means was determined using the Tukey test and statistical significance was defined as $p < 0.05$.

RESULTS

Morphology

The PLGA/Lec-SMS and PLGA-SMS were fabricated as demonstrated in Figure 1 and the morphologies of them are exhibited in Figure 2. After introducing lecithin into PLGA bulks, composite microspheres still maintain in spherical shape. Slightly wrinkling morphologies are observed on the surfaces of PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS.

Wettability

The surface hydrophilicity of PLGA and PLGA with different lecithin as characterized by static water contact angle is presented in Figure 3. All the contact angles of different composite surfaces declined significantly (from 83° to nearly 0°), which indicates that the hydrophilicity enhances with the increasing content of lecithin in the hybrid films. When the lecithin content reaches 40 wt % in PLGA bulks, the contact angle is almost 0°.

ATR-FTIR and XPS

Figure 4 shows typical ATR-FTIR spectra of the PLGA/lecithin and pure PLGA. The band at 2859 cm^{-1}

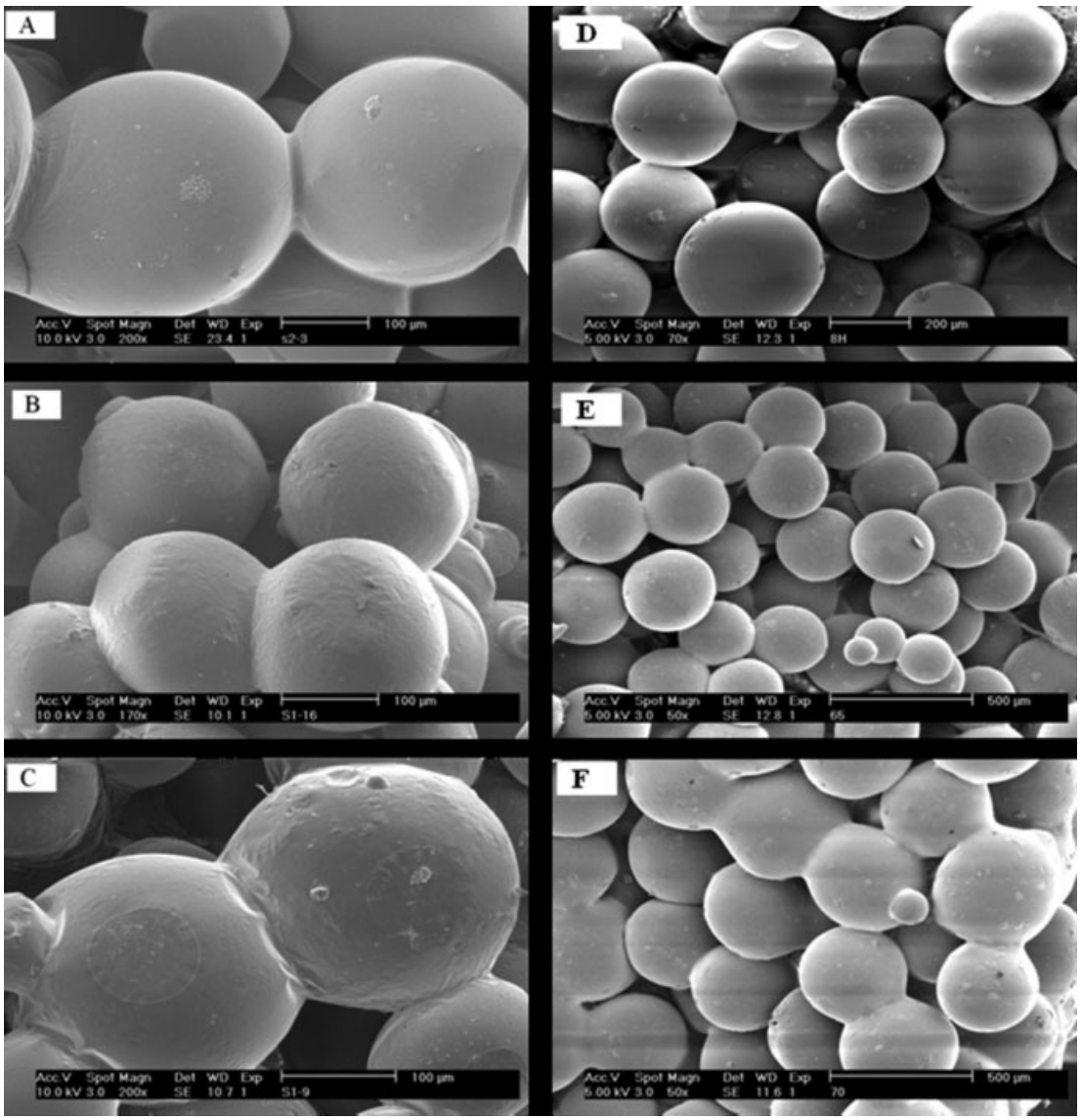

Figure 2. SEM images of (A) PLGA-SMS, (B) PLGA/Lec(5%)-SMS, (C) PLGA/Lec(10%)-SMS , and PLGA/Lec(1%)-SMS at different temperature 60°C (D), 70°C (E), and 75°C (F).

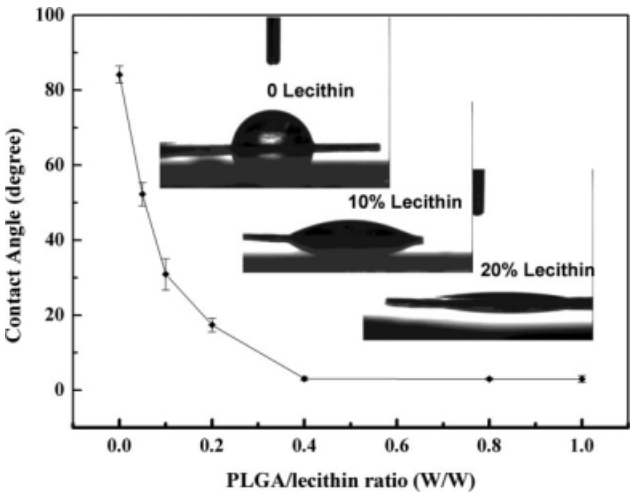

Figure 3. Wettability of PLGA microspheres sintered scaffolds with different lecithin content.

representing symmetric CH_2 emerges on the spectrum of PLGA/lecithin (10%).[21] In addition, the peak at 1095 cm^{-1} broadens, due to the coalescent of peaks locating at 1095 and 1092 cm^{-1} ascribing to C-O stretching of PLGA and symmetric PO_2^- of lecithin.[22] The vibration peak at about 1600 cm^{-1} which ascribes to the amide of lecithin appears on PLGA/lecithin composite. As shown in Figure 5 and Table II, XPS spectra of PLGA/lecithin (5%) and PLGA/lecithin (10%) exhibit three separated peaks, which correspond to P2p (133eV), P2s (188eV), N1s (400eV). With the increasing of the lecithin content, the composition of P increases due to the presence of more lecithin on the surfaces of PLGA bulks.

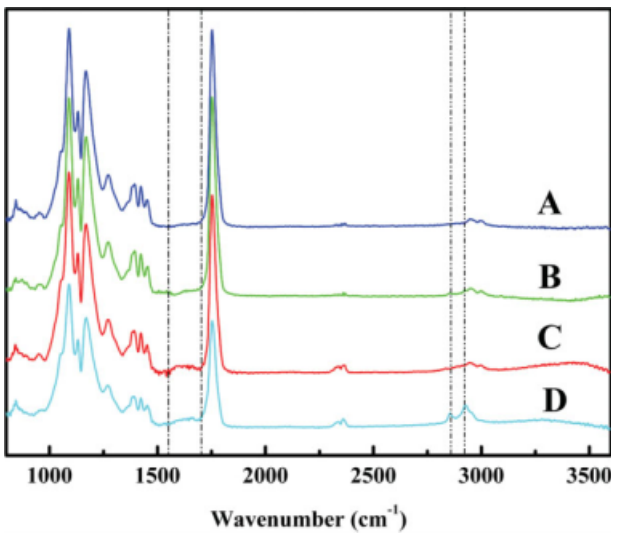

Figure 4. ATR-FTIR spectra of (A) PLGA, (B) PLGA/Lec(1%), (C)PLGA/Lec(5%), and (D) PLGA/Lec(10%). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

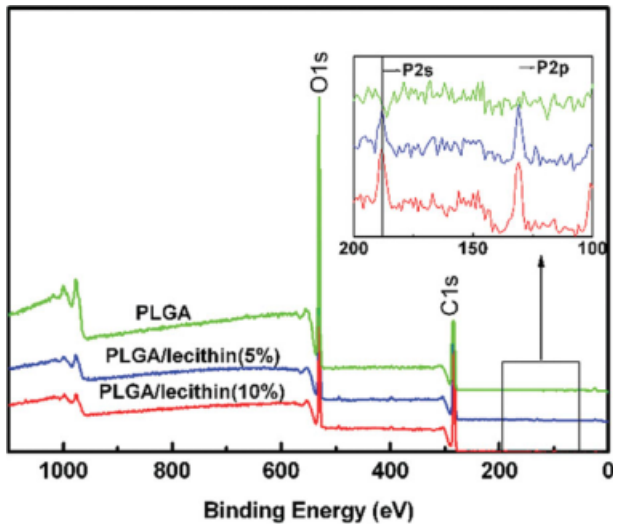

Figure 5. XPS survey scan spectra of surfaces of various PLGA and PLGA/Lec microspheres. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Porosity

Table III reveals the porosity of PLGA/Lec-SMS with different fabrication conditions. The range of porosity of all the scaffolds is from 30 to 50%. With the increase of duration and temperature, the porosities of scaffolds decrease remarkably, which may be due to the higher degree of bonding between adjacent microspheres.

Mechanical properties

Table III exhibits compressive strength and compressive modulus of the scaffolds built by microspheres with different sizes. With the diameters of microspheres increasing from 200–300 μm to 400–450 μm, the compressive strength of scaffold decreases from 5.16 ± 0.43 to 4.15 ± 0.30 MPa, and the compressive modulus also decreases from 150.68 ± 23.46 to 114.78 ± 20.18 MPa.

The sintering time is another factor which influences the mechanical properties of the scaffolds. At the same sintering temperature, the mechanical behav-

TABLE II
Elemental Composition of PLGA/Lecithin Microspheres Scaffolds

Variables	Composition (%)			
	C	O	P	N
PLGA	62.69	37.31	0	0
PLGA/lecithin (5%)	74.57	23.91	0.85	0.67
PLGA/lecithin(10%)	78.55	19.52	1.17	0.76

TABLE III
Effects of lecithin content, microspheres size, sintering temperature and sintering time on compressive strength and compressive modulus, and porosity of PLGA-SM and PLGA/lec-SMS

Variables	Compressive strength (MPa)	Compressive modulus (MPa)	Porosity (%)
Lecithin content			
0%	4.15 ± 0.27	111.18 ± 19.89[a]	43.51 ± 5.86
1%	4.09 ± 0.17[a]	114.74 ± 14.28[a]	40.15 ± 7.72
5%	4.37 ± 0.46	122.85 ± 21.56	43.20 ± 2.05
10%	4.75 ± 0.37	141.52 ± 10.34	38.15 ± 6.67
Microsphere size			
200–300 μm	5.16 ± 0.43	150.68 ± 23.46	37.33 ± 4.97
300–350 μm	4.80 ± 0.45	137.99 ± 19.97	38.57 ± 5.19
350–400 μm	4.37 ± 0.46	122.85 ± 21.56	43.20 ± 2.05
400–450 μm	4.15 ± 0.30[b]	114.78 ± 20.18	39.37 ± 0.68
Sintering temperature			
60°C	4.37 ± 0.46[c,d]	122.85 ± 21.56[c,d]	43.20 ± 2.05
65°C	5.95 ± 0.78[c]	165.77 ± 32.20	37.89 ± 1.50
70°C	7.45 ± 0.38	250.35 ± 24.15	31.43 ± 1.70
Sintering time			
6 h	4.37 ± 0.46[e,f]	122.85 ± 21.56[e]	43.20 ± 2.05
12 h	5.13 ± 0.20[e]	146.19 ± 34.95	38.56 ± 1.28
24 h	6.41 ± 0.53	184.48 ± 16.42	37.50 ± 1.11

[a]Indicated statistical significance when compared with the scaffolds with 10% lecithin.
[b]Indicated statistical significance when compared with the scaffolds with microsphere size between 200 and 300 μm.
[c,d]Indicated statistical significance when compared with the scaffolds sintered at 70 and 65°C, respectively.
[e,f]Indicated statistical significance when compared with the scaffolds sintered for 24 and 12 h, respectively.

iors of PLGA/Lec(5%)-SMS with similar microspheres size are significantly greater as the sintering time extending from 6 to 24 h.

The morphology of the sintering microspheres scaffolds (Figure 2) with different sintering temperature directly reflects the condition of microspheres bond. The microspheres exhibit no distinct bonding area at 60°C. After elevating the sintering temperature to 65°C, moderate bonding area is observed. And the microspheres sintered at 70°C present compact bonding. Strong bonding among adjacent microspheres is responsible for high mechanical properties of scaffolds. As sintering temperature increased from 60°C to 70°C, compressive strength and compressive modulus significantly increased from 4.37 ± 0.46 MPa and 122.85 ± 21.56 MPa to 7.45 ± 0.38 MPa and 250.35 ± 24.15 MPa, respectively.

The variation of lecithin content in PLGA/Lec-SMS also influences the scaffold mechanical properties. With the increase of lecithin content from 0 to 10%, the compressive strength increases from 4.15 ± 0.27 to 4.75 ± 0.37 MPa, and the compressive strength also increases from 111.18 ± 19.89 to 141.52 ± 10.34 MPa.

Cell viability

hFOBs were used to evaluate the osteogenesis of the PLGA-SMS and PLGA/Lec-SMS. Cell viability was analyzed using WST-1 assay. As shown in Figure 6, a relative increase in absorbance from Day 3 to Day 14 was recorded, indicating a trend of cell viability on all scaffolds. On the third day, the viability of hFOBs within the PLGA/Lec(5%)-SMS were observed to be significantly higher than those within the PLGA-SMS and PLGA/Lec(10%)-SMS. By contrast, there were no significant difference on cell viability between PLGA-SMS and PLGA/Lec(10%)-SMS. After 14 days of culture, PLGA/Lec(5%)-SMS exhibited remarkable cell viability. The cell viability of PLGA/Lec(5%)-SMS was nearly 30% higher than that of other groups. Moreover, the cell viability of osteoblasts cultured on the PLGA-SMS and PLGA/Lec(10%)-SMS was com-

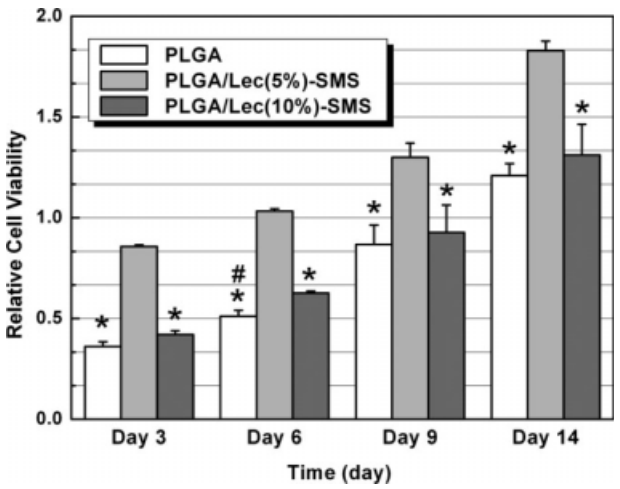

Figure 6. Relative HFOBs viability in PLGA/lecithin microspheres scaffolds. (*) and (#) indicated statistical significance when compared with PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS, respectively.

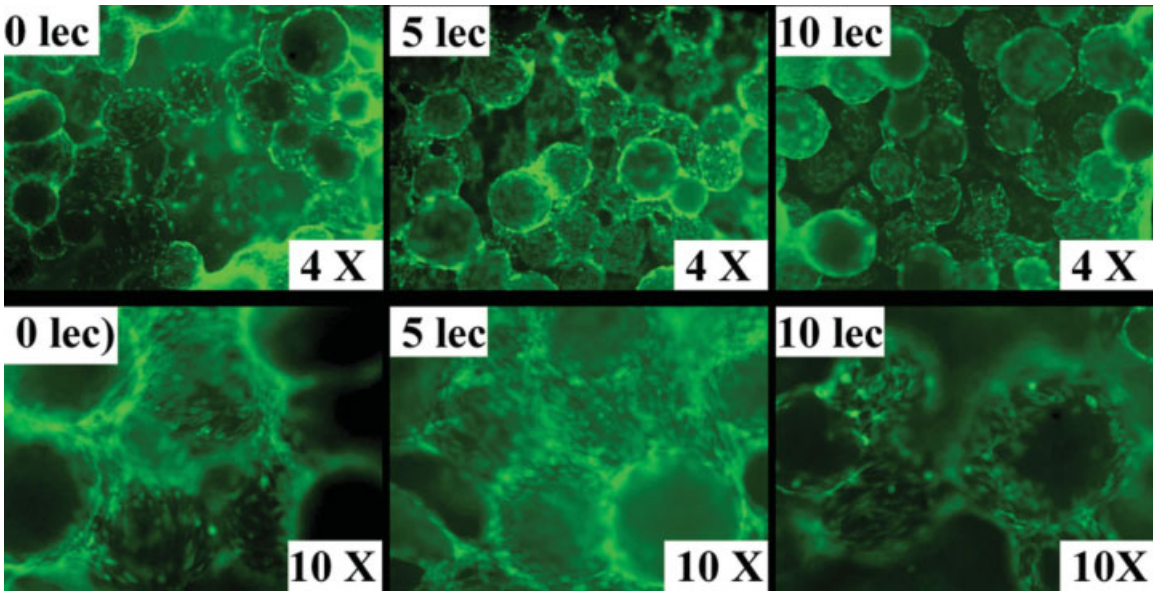

Figure 7. Cell morphology and distribution of PLGA, PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS after 14 days culture indication with the fluorescent "Live/Dead" assay. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

parable. Cell morphology and distribution on the scaffolds (Figure 7) were recorded by fluorescence microscope. As seen from the micrograph, cells grew well and proliferated around the microspheres and their bonding area at Day 14.

Osteogenesis

As a standard maker of osteoblasts differentiation, ALP was determined by pNPP assay (Figure 8) Positive ALP production was first detected at Day 3, followed by significant upregulation until Day 14. On the third day, PLGA/Lec(10%)-SMS exhibited higher ALP activity than other group. However, the osteoblasts on the PLGA/Lec(5%)-SMS showed significant higher level of ALP activity compared to other groups after 14 days of culture.

A critical important function of osteoblasts is participating in biological mineralization. The calcium deposition by osteoblasts was accessed by a quantificational Alizarin red−based assay (Figure 9). The results demonstrated that the calcium content of all the scaffolds increased continuously over the culture period. After 7 days and 14 days of culture, the calcium deposition on PLGA/Lec(5%)-SMS scaffold was higher than that on PLGA-SMS and PLGA/Lec(10%)-SMS. Regarding PLGA/Lec(10%)-SMS and

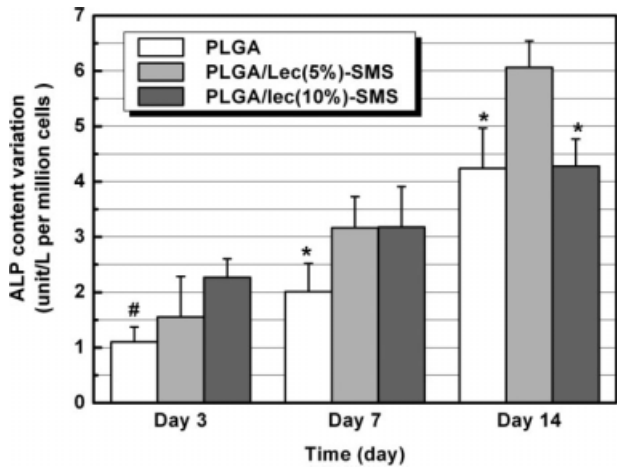

Figure 8. Alkaline phosphatase activity of osteoblasts cultured on PLGA-SMS and PLGA/Lec-SMS for 14 days. (*) indicated statistical significance when compared with PLGA/Lec(5%)-SMS.

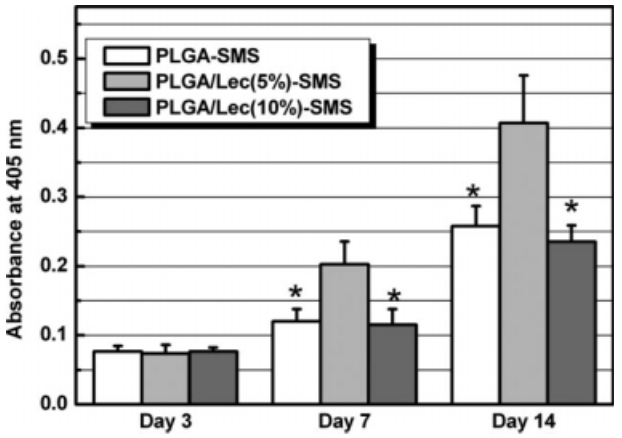

Figure 9. Calcium deposition of osteoblasts on PLGA-SMS and PLGA/Lec-SMS for 14 days. (*) indicated statistical significance when compared with PLGA/Lec(5%)-SMS.

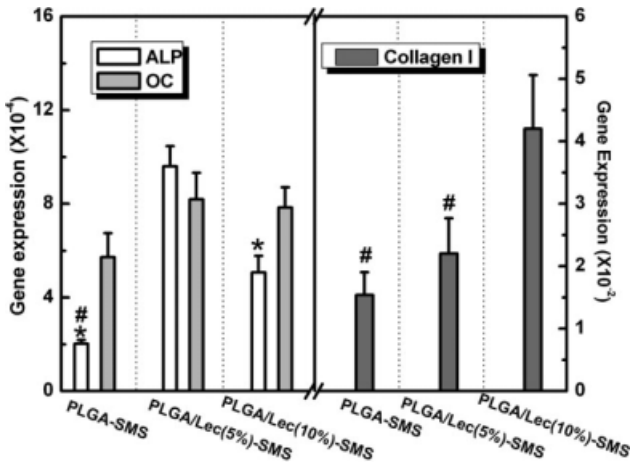

Figure 10. Osteoblastic markers gene expression of cells cultured on PLGA-SMS and PLGA/Lec-SMS. (*) and (#) indicated statistical significance when compared with PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS, respectively.

PLGA-SMS, no significant difference in calcium deposition between them was observed.

To further analyze cell differentiation on scaffolds, Real-time PCR was used to assess mRNA expression of typical osteoblastic markers (Figure 10). The osteoblasts within PLGA/Lec(5%)-SMS displayed significantly higher expression of ALP than that of other scaffolds, which is consistent with the results of ALP activity assay by pNPP. No difference was observed in transcripts for OC in cells with all the groups. In addition, a lecithin-dependent increase in the expression level of type I collagen was observed.

DISCUSSION

It is well known that the mechanical properties of scaffold are of importance for bone regeneration. The scaffold should have suitable mechanical strength and act as temporary substitution of bone during bone recovery process. Many approaches have been utilized to enhance mechanical properties of PLGA scaffold, and the most popular one is hybridizing the second phase such as inorganic nano-powders and fibers with PLGA matrix.[23–25] Herein, sintering microsphere technique was used to manufacture scaffolds. The sintered microsphere scaffolds are built up by bonding microspheres via surface fusing. And the bondings among adjacent microspheres, mainly determined by sintering conditions (time and temperature), is paramount for scaffold mechanical properties. The bondings among microspheres can decrease microsphere sizes, but provide the bridges for adjacent microspheres and finally construct an integrated scaffold. A small bonding area results in weak coalescents among microspheres, consequently the whole scaffold owns poor capacity to resist outside forces; while a large bonding area induces decreased microsphere diameter and scaffold porosity accordingly. Therefore, the key factor in scaffolds fabrication is to control the degree of bonding among the microspheres by regulating sintering conditions. With moderate porosity and high mechanical behaviors, PLGA-SMS and PLGA/Lec-SMS, with microspheres size above 300 μm, which were sintered at 60°C for 6 h were used for biocompatibility evaluation in this study.

PLGA/Lec-SMS with comparable mechanical properties as cancellous bone (Table IV) could be manufactured simply and controllably, which caters for the need on mechanical behavior for cancellous bone regeneration.[26] On the other hand, cell migration and vascularization requires more porosity.[26] The pore volume of sintered microsphere scaffolds is between 30 and 50%. Previous studies revealed that the scaffolds fabricated by sintered microspheres methods can provide moderate structure for osteoblasts proliferation.[27] Additionally, these kinds of scaffolds can also stimulate endothelial cell growth and maintain its characteristic phenotype, indicating that it has a potential for promoting new blood vessel formation.[28]

Lecithin is a composite of lipids such as phosphatidylcholine, phosphatidylethanolamine, inositol-phospatides, phosphatidylserine, and some other biomolecules. In this study, lecithin was incorporated into PLGA bulks to improve biocompatibility of scaffolds. Firstly, lecithin-containing scaffolds were found to effectively enhance cell proliferation. Via controlling the content of lecithin blending with PLGA, the surface wettability can be regulated, and ultimately the optimal surface characteristics for cell proliferation was achieved (Figure 7). Cell attachment to biomaterial surface is influenced by their surface properties such as wettability, chemical functionalities, roughness, and surface charger. A surface hydrophilic–hydrophobic balance is a paramount factor that influences the cytocompability of biomaterials. Surface wettability affects protein adsorption and activity, which in turn accelerates or restrains the cell adhesion

TABLE IV
Compressive Strength and Compressive Modulus of Cortical Bone, Cancellous and PLGA Based Microspheres Scaffolds

Variables	Compressive strength (GPa)	Compressive modulus (MPa)
Cortical bone	7–30	100–230
Cancellous bone	0.05–0.5	2–12
PLGA/lecithin microspheres sintered scaffolds	0.1–0.3	4–8

and proliferation. More recently, Lim et al.[29] demonstrated moderate hydrophilic surface significantly increased cell adhesion, and at the same time, osteoblasts on moderate hydrophilic surfaces exhibited increased mineralized area. Ma et al.[30] also revealed the similar results by studying the cytocompability of cells on hydrophilic groups−grafted PLLA surfaces. Other studies reported that the emergence of groups such as amino ($-NH_2$), carboxylic acid (−COOH), or hydroxyl (−OH) and moderate wettability with water contact angles of 40°−70° presented by polymer surface benefit cell attachment.[31]

Secondly, osteoblasts on PLGA/Lec-SMS exhibited significantly higher osteogenic marker expression (Figure 8 and Figure 10). ALP is expressed mainly on cell surfaces or in matrix vesicles. It specifically degrades the organic phosphoesters in bone and cartilage, which inhibits cartilage mineralization and promotes the calcium deposition in bone.[32,33] The expression of ALP increases before mineralization and subsequently decreases after the initiation of mineralization. OC is an important-late bone marker that regulates the formation and growth of bone minerals and therefore indicates the finalization of osteogenesis.[33] Type I collagen holds overwhelming majority (over 90%) among the bony structural proteins and takes major responsibility for the tensile strength of the tissue and also provides templates for mineralization.[33] In summary, the gene expression data (Figure 10) suggest a more rapid differentiation of osteoblasts on PLGA/Lec(5%)-SMS.

In addition, Lecithin also promotes mineralization. Lipids are present in the extracellular matrix at sites of mineralization and are associated with the mineral phase such as regulating the growth of preformed mineral crystal.[26] Wuthier's group[34] identified a phospholipids complex composed of acidic phospholipids, calcium, and inorganic phosphate. The phospholipids were found in mineralizing tissues and were identified as the nucleational core of these tissues. And furthermore, acidic phospholipids which present in mineralization sites were considered as important factors responsible for the rapid accumulation Ca^{2+}.[35] Therefore, these theories that are based on the experiments *in vitro* or *in vivo* support the data from our studies. By introducing lecithin into PLGA matrix, a high-level calcium deposition of osteoblasts on the composite scaffolds was observed (Figure 9). And at the same time, some mineralization-related protein such as Type I collagen and OC also display higher level expression (Figure 10). However, the expression of osteoblastic markers of cells on PLGA/Lec(10%)-SMS decreased compared to PLGA/Lec(5%)-SMS. This outcome could be attributed to the wettability of scaffold. PLGA/Lec(10%)-SMS exhibits significantly high hydrophilicity, and the contact angle is below 40°. The extra high surface hydrophilicity would influence cells attachment, which leads to low cells viability as shown in Figure 7. Therefore, some mineralization-related data for PLGA/Lec(10%)-SMS are incomparable to PLGA/Lec(5%)-SMS.

CONCLUSIONS

Toward an ultimate goal of bone regeneration, we have introduced lecithin as a modifying additive in PLGA bulk, based on which a series of novel PLGA/Lec-SMS scaffolds are developed and tested. The desired hydrophilic modification has been achieved without sacrificing material bulk property; while as demanded for bone substitution, the construction mechanics and porosity are also optimized by altering lecithin fractions associating with various sintering conditions. Human fetal osteoblasts are employed to evaluate the biofunctional performance of the scaffolds *in vitro*. The result confirms that 5% lecithin in PLGA/Lec-SMS scaffolds yields the best cyto-compatibility as well as the greatest osteoconductivity, with indications of cell viability, ALP activity, calcium deposition, and phenotype checking with osteogenic markers.

The authors thank Dr. Jinghua Hao at NTU for helpful revision.

References

1. Langer R, Vacanti JP. Tissue engineering. Science 1993;260: 920–926.
2. Wang D-A, Varghese S, Sharma B, Strehin I, Fermanian S, Gorham J, Fairbrother DH, Elisseeff JH. Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. Nat Mater 2007;6:385–392.
3. Griffith LG, Naughton G. Tissue engineering-current challenges and expanding opportunities. Science 2002;295:1009–1014.
4. Habraken WJEM, Wolke JGC, Jansen JA. Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007;59:234–248.
5. Tessmar JK, Göpferich AM. Matrices and scaffolds for protein delivery in tissue engineering. Adv Drug Deliv Rev 2007; 59:274–291.
6. Guo D, Xu K, Han Y. The influence of Sr doses on the in vitro biocompatibility and in vivo degradability of single-phase Sr-incorporated HAP cement. J Biomed Mater Res A 2008;86:947–958.
7. Shi X, Wang Y, Ren L, Gong Y, Wang D-A. Enhancing alendronate release from a novel PLGA/hydroxyapatite microspheric system for bone repairing applications. Pharm Res 2009;26:442–430.
8. Hwang CM, Khademhosseini A, Park Y, Sun K, Lee SH. Microfluidic chip-based fabrication of PLGA microfiber scaffolds for tissue engineering. Langmuir 2008;24:6845–6851.
9. Shen H, Hu X, Yang F, Bei J, Wang S. Combining oxygen plasma treatment with anchorage of cationized gelatin for enhancing cell affinity of poly(lactide-co-glycolide). Biomaterials 2007;28:4219–4230.

10. Croll TI, O'Connor AJ, Stevens GW, Cooper-White JJ. Controllable surface modification of poly(lactic-co-glycolic acid) (PLGA) by hydrolysis or aminolysis I: Physical, chemical, and theoretical aspects. Biomacromolecules 2004;5:463–473.
11. Jiang T, Abdel-Fattah WI, Laurencin CT. In vitro evaluation of chitosan/poly(lactic acid-glycolic acid) sintered microsphere scaffolds for bone tissue engineering. Biomaterials 2006;27: 4894–4903.
12. Park SI, Kim JH, Lim JH, Kim CO. Surface-modified magnetic nanoparticles with lecithin for applications in biomedicine. Curr Appl Phys 2008;8:706–709.
13. Wan P, Zhao Y, Tong H, Zhu Z, Shen X, Hu J. The inducing effect of lecithin liposome organic template on the nucleation and crystal growth of calcium carbonate. Mater Sci Eng C 2009;29:222–227.
14. Pestana KC, Formariz TP, Franzini CM, Sarmento VHV, Chiavacci LA, Scarpa MV, Egito EST, Oliveira AG. Oil-in-water lecithin-based microemulsions as a potential delivery system for amphotericin B. Colloid Surf B 2008;66:253–259.
15. Graf A, Ablinger E, Peters S, Zimmer A, Hook S, Rades T. Microemulsions containing lecithin and sugar-based surfactants: Nanoparticle templates for delivery of proteins and peptides. Int J Pharm 2008;350:351–360.
16. Borden M, Attawia M, Khan Y, Laurencin CT. Tissue engineered microsphere-based matrices for bone repair: design and evaluation. Biomaterials 2002;23:551–559.
17. Cushnie EK, Khan YM, Laurencin CT. Amorphous hydroxyapatite-sintered polymeric scaffolds for bone tissue regeneration: Physical characterization studies. J Biomed Mater Res A 2008;84:54–62.
18. Kofron MD, Cooper Jr. JA, Kumbar SG, Laurencin CT. Novel tubular composite matrix for bone repair. J Biomed Mater Res A 2007;82:415–425.
19. Goh YQ, Ooi CP. Fabrication and characterization of porous poly(L-lactide) scaffolds using solid-liquid phase separation. J Mater Sci: Mater Med 2008;19:2445–2452.
20. Gregory CA, Gunn WG, Peister A, Prockop DJ. An alizarin red-based assay of mineralization by adherent cells in culture: Comparision with cetylpyridinium chloride extraction. Anal Biochem 2004;329:77–84.
21. Tantipolphan R, Rades T, Mcquillan AJ, Medlicott NJ. Adsorption of bovine serum albumin (BSA) onto lecithin studied by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. Int J Pharm 2007;337:40–47.
22. Zhu N, Cui FZ, Hu K, Zhu L. Biomedical modification of poly(L-lactide) by blending with lecithin. J Biomed Mater Res A 2007;82:455–461.
23. Petricca SE, Marra KG, Kumta PN. Chemical synthesis of poly(lactic-co-glycolic acid)/hydroxyapatite composites for orthopaedic applications. Acta Biomater 2006;2:277–286.
24. Torres FG, Nazhat SN, Sheikh Md-Fadzulla SH, Maquet V, Boccaccini AR. Mechanical properties and bioactivity of porous PLGA/TiO2 nanoparticle-filled composites for tissue engineering scaffolds. Composites Sci Tech 2007;67: 1139–1147.
25. Zhang X, Hua H, Shen X, Yang Q. In vitro degradation and biocompatibility of poly(L-lactic acid)/chitosan fiber composites. Polymer 2007;48:1005–1011.
26. Hollinger JO, Thomas E, Bruce D, Charles S. Bone Tissue Engineering. Boca Raton, Florida: CRC Press; 2005.
27. Borden M, Attawia M, Laurencin CT. The sintered microsphere matrix for bone tissue engineering: In vitro osteoconductivity studies. J Biomed Mater Res 2002;61:421–429.
28. Jabbarzadeh E, Jiang T, Deng M, Nair LS, Khan YM, Laurencin CT. Biotech Bioeng 2007;98:1094–1102.
29. Lim JY, Shaughnessy MC, Zhou Z, Noh H, Vogler EA, Donahue HJ. Biomaterials 2008;20:1776–1784.
30. Ma Z, Gao C, Gong Y, Shen J. Chondrocyte behaviors on poly-L-lactic acid (PLLA) membranes containing hydroxyl, amide or carboxyl groups. Biomaterials 2003;24:3725–3730.
31. Arima Y, Iwata H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. Biomaterials 2007; 28:3074–3082.
32. Xue W, Krishna BV, Bandyopadhyay A, Bose S. Processing and biocompatibility evaluation of laser processed porous titanium. Acta Biomater 2007;3:1007–1018.
33. Wang D-A, Williams CG, Yang F, Cher N, Lee H, Elisseeff JH. Bioresponsive phosphoester hydrogels for bone tissue engineering. Tissue Eng 2005;11:201–213.
34. Wuthier RE, Wu LNY, Sauer GR, Genge BR, Yoshimori T, Ishikawa Y. Bone Miner 1992;17:290–295.
35. Wuthier RE, Eanes ED. Effect of phospholipids on the transformation of amorphous calcium phosphate to hydroxyapatite in vitro. Calcified Tissue Int 1975;19:197–210.