International Journal of Pharmaceutics 373 (2009) 85–92

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

A protein/antibiotic releasing poly(lactic-co-glycolic acid)/lecithin scaffold for bone repair applications

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ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form 16 February 2009
Accepted 17 February 2009
Available online 3 March 2009

Keywords:
Poly(lactide-co-glycolide)
Lecithin
Scaffold
Controlled release
Antibacterial

ABSTRACT

Novel poly(lactic-co-glycolic acid) (PLGA)-hybridizing-lecithin scaffolds loaded with drug or protein were prepared with water/oil/water techniques and sintering microspheres technique. In such fabricated composite scaffolds (abbreviated "PLGA/Lec-SMS"), the introduction of lecithin component has been proven capable of largely enhancing Gentamicin (GS) and protein (Bovine Serum Albumin) encapsulation efficiency. The *in vitro* GS and BSA releasing profiles of PLGA/Lec-SMS system were plotted basing over 60 days' and 18 days' data collection, respectively. It indicates a sustained releasing tendency despite a burst at the very beginning. The antibacterial properties of GS-laden scaffolds were determined *in vitro*, and the antibacterial activity of scaffolds was enhanced by incorporating lecithin into PLGA bulks. Additionally, mesenchymal stem cells (MSCs) were seeded onto PLGA-SMS and PLGA/Lec-SMS *in vitro*. The outcome confirmed PLGA/Lec(5%)-SMS functions to improve MSC proliferation and also to enhance general ALP production and calcium secretion which is the vital markers for osteogenesis. In conclusion, this newly designed antibiotic releasing PLGA/Lec-SMS is promising for bone-repairing therapeutics.

1. 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 (Langer and Vacanti, 1993; Griffith and Naughton, 2002; Wang et al., 2007; Ma, 2008). Scaffolds play a paramount role in many tissue engineering strategies especially in bone and cartilage repair and regeneration because they provide a mechanical architecture in which cultured cells are coaxed to grow (Griffith and Naughton, 2002).

Controlled release technology is an effective and favorable method to optimize drug dosage, deliver drug and gene to special site or prolong the delivery duration, which opens up a novel prospect for tissue engineering recently (Megeed et al., 2002; Habraken et al., 2007; Patel et al., 2008). However, the traditional tissue engineering scaffolds with high porosity are short of the ability to control the release kinetics of drugs and bioactive molecules during the process of tissue regeneration.

Poly(lactic-co-glycolic acid) (PLGA) is one of the most widely used synthetic polymers in the fields of tissue engineering and drug delivery (Quaglia, 2008), and it is a biodegradable polymer with good mechanical strength, excellent processibility which can fabricate flexible structure, and as well as tailored degradation rate (Giteau et al., 2008). PLGA microsphere based materials are reported to have broader applications. Traditionally, PLGA microspheres as excellent controlled release carriers hold ideal loading efficiency and allow the drug dosage optimization (Gómez et al., 2007; Sethuraman et al., 2008). Moreover, PLGA microspheres could be used for fabricating sintered microsphere scaffold and as injectable cell carriers (Borden et al., 2002a; Jabbarzadeh et al., 2007; Chung et al., 2008).

Lecithin originates as a substance extracted from mammalian neuronal tissues or brain (Park et al., 2008). It is a typical amphiphilic phospholipid with good biocompatibility and capable of mixing with PLGA, poly(lactic acid) (PLA), or poly-ε-caprolactone (PCL) in their chloroform or dichloromethane solution. Lecithin-containing systems were also found to maintain proteins from denaturation during delivery (Graf et al., 2008; Pestana et al., 2008).

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doi:10.1016/j.ijpharm.2009.02.013

In this study, we take the advantages from both lecithin and PLGA by hybridizing them into composite microsphere scaffolds in which antibiotic and protein were loaded to be delivered to fulfill bone-repairing applications. At the same time, cytocompatibility of the scaffolds and mesenchymal stem cell (MSC) differentiation on the scaffolds were also examined *in vitro*. PLGA sintered microsphere scaffold and PLGA/Lecithin hybridizing sintered microspheres scaffold are abbreviated as "PLGA-SMS" and "PLGA/Lec-SMS", respectively.

2. Materials and methods

2.1. 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 Inc. (Jinan, China). Bovine Serum Albumin (BSA) and poly(vinyl alcohol) (PVA) were obtained from Sigma-Aldrich (Singapore). Lecithin (soy) was supplied by Bio life Science & Technology Inc. (Shanghai, China). Gentamicin (GS) was purchased from Probe Inc. (Beijing, China). All the cell culture-related reagents were purchased from Gibco (Invitrogen, Singapore).

2.2. Microsphere and scaffold preparation

2.2.1. Fabrication of GS/BSA laden PLGA and PLGA/lecithin microsphere

PLGA/lecithin microspheres encapsulating GS/BSA were prepared by the double emulsion-solvent evaporation technique following the method described in the reference (Aubert-Pouëssel et al., 2002). Briefly, GS (60 mg in 3 ml PBS solution) or BSA (1 wt%, 3 ml) was dissolved in phosphate-buffered saline (PBS) as the first water phase (W_1). PLGA (5 g) and lecithin (0, 0.25 g or 0.5 g) were dissolved in 25 ml methylene chloride as oil phase (O). The W_1 solution was then emulsified in oil phase to form the primary W_1/O emulsion via homogenizing at 5000 rpm for 30 s. The resulting emulsion was added dropwise to 1000 ml 1% PVA solution (W_2), and then stirred at 200 rpm for 4 h to allow methylene chloride to evaporate completely. The PLGA microspheres were collected and washed three times with deionized water, and then dried at room temperature for 24 h.

2.2.2. Fabrication of GS laden PLGA-SMS and PLGA/Lec-SMS

GS laden PLGA/Lec-SMS (PLGA/Lec-GS-SMS) was fabricated via microsphere sintered technique (Borden et al., 2002a,b). Briefly, the microspheres were stacked in a mold (diameter = 5 mm, height = 10 mm). The mold was transferred to an oven and heated at 55 °C for 4 h, and made the microspheres fuse into a scaffold. The scaffold was then cooled at room temperature and separated from the mold.

2.2.3. Fabrication of BSA laden PLGA-SMS and PLGA/Lec-SMS

PLGA/Lec-SMS with BSA (PLGA/Lec-BSA-SMS) was produced by solvent sintering method (Brown et al., 2008). Briefly, the microspheres were poured into a mold (diameter = 5 mm, height = 10 mm). Fifteen percent acetone/ethanol solution was added into the mold gently, and the microspheres fused together and formed a scaffold. And then the scaffold was separated from the mold, washed with deionic water, and then dried at room temperature for 24 h.

2.2.4. Fabrication of PLGA-SMS and PLGA/Lec-SMS

In order to eliminate the influence of BSA and GS on cell viability and proliferation, PLGA/Lec-SMS and PLGA-SMS without drug/protein were used for cell viability and osteogenesis assay. The fabrication methods for PLGA and PLGA/lecithin microspheres without drug encapsulation were the same as those of drug/protein laden scaffolds described in Section 2.2.1; except that drug/protein contained PBS solution was replaced with pure PBS solution as the first water phase during microsphere preparation process. Null drug/protein encapsulated PLGA-SMS and PLGA/Lec-SMS were fabricated via microsphere sintered technique described in Section 2.2.2.

2.3. Morphological characterization

Morphological characterization was conducted using scanning electron microscopy (SEM, 30XLFEG, Philips, The Netherlands). The microspheres were immobilized on a cupreous stub and coated with gold.

2.4. Degradation studies

PLGA/Lec-SMS and PLGA-SMS were weighted and then immersed in separate plastic bottles with 20 ml of PBS solution (pH value = 7.2). All the samples were incubated at 37 °C for 60 days. The samples were taken out at determined time points and surface water was removed. The wet weight and dry weight of the samples were determined. The pH value of the degradation medium was measured by acidometer (Schott Instruments, Germany). Mass loss and water uptake of scaffolds were calculated according to the following equations:

$$\text{Mass Loss}\,(\%) = \left[1 - \left(\frac{M_d}{M_o}\right)\right] \times 100 \quad (1)$$

$$\text{Water Uptake}\,(\%) = \left[\frac{(M_w - M_d)}{M_d}\right] \times 100 \quad (2)$$

where M_d, M_w and M_o denote dry, wet and original weight of the scaffolds, respectively.

2.5. Determination of encapsulation efficiency

Encapsulation of GS or BSA into microspheres was determined spectrophotometrically after extraction from microspheres. Briefly, accurately weighed amount (20 mg) of microspheres was dissolved in 1 ml methylene chloride, and 5 ml PBS buffer was added subsequently. This solution was stirred at 37 °C for 2 h. After centrifugation, the aqueous supernatant was analyzed using calibration curves on a UV spectrophotometer (Multiskan® Spectrum, Thermo, Finland) according to the method described in the reference (Zhang et al., 1994). Encapsulation efficiency (EE) was expressed by equation as follows:

$$\text{EE} = \left(\frac{\text{actual drug loading}}{\text{theoretical drug loading}}\right) \times 100 \quad (3)$$

2.6. In vitro release test for GS or BSA from sintered microsphere scaffolds

GS or BSA release was determined by suspending 100 mg microsphere sintered scaffolds [the theoretical drug/protein loading amount: 1.2 mg/scaffold (GS) and 0.6 mg/scaffold (BSA)] in 50 ml PBS buffer. Five hundred microlitre of the sample media were collected periodically with equal amount of PBS makeup.

2.7. Antibacterial properties of GS laden PLGA/Lec-SMS

Bacterial viability tests were conducted following the methods described in the reference (Thakur et al., 2008). Briefly, a 100 μl aliquot of *E. coli* K12 JM109 (New England BioLabs Inc., USA) reconstituted in nutrient broth and previously sub-cultured was spread onto an agar plate. PLGA/Lec-SMS and PLGA-SMS (9 mm diameter)

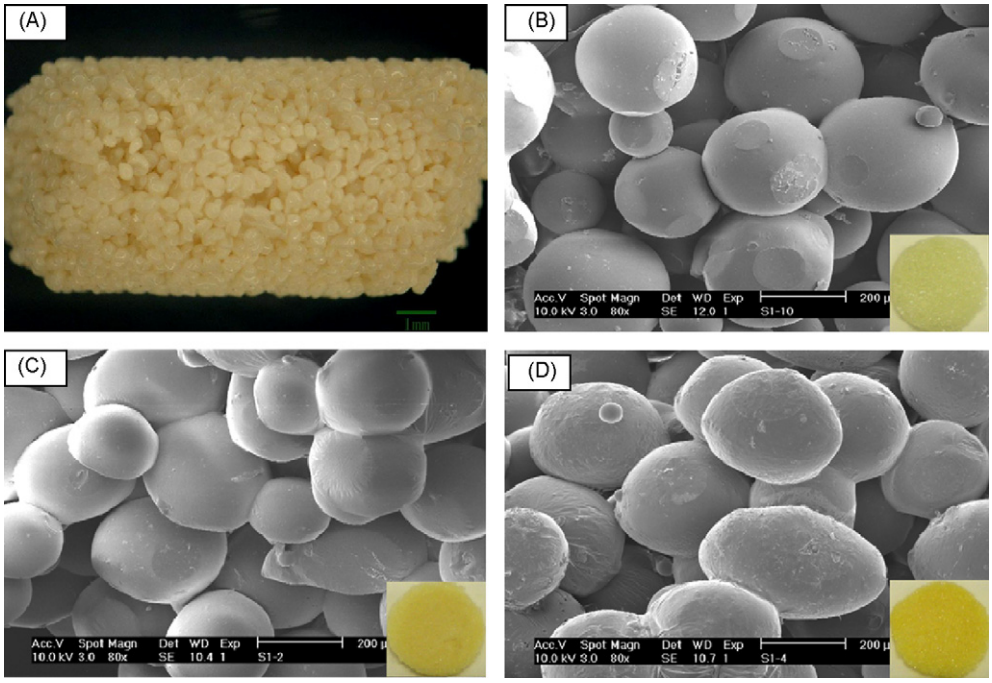

Fig. 1. Phase contrast microscope image and SEM images of PLGA-SMS (B), PLGA/Lec(5%)-SMS (A and C) and PLGA/Lec(10%)-SMS (D).

which were taken out from simulated body fluid (SBF) solution after 0, 7 and 14 days' incubation were placed on agar plates, allowing sufficient time for the drug to diffuse into the surroundings. The plates were incubated for 6 h at 37 °C, and then the zone diameters were measured.

2.8. Cell culture and cell seeding

Rabbit mesenchymal stem cells were harvested using the method descried in the reference (Yokoyama et al., 2005). Briefly, harvested synovium from rabbit was digested in a 3 mg/ml collagenase D solution in DMEM at 37 °C. After 3 h, digested cells were filtered through a 70 μm filter. The digested cells were propagated in Dulbecco's modified eagle's medium (DMEM) with supplements of 1.5 mg/ml sodium bicarbonate, 4.5 mg/ml glucose, 10% (v/v) fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. After 3 days, the medium was changed to remove non-adherent cells. Passage 3 MSCs were used in this study.

Three different microspheres scaffolds (PLGA/SMS, PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS) were placed in 24-well plates. The fabricated scaffolds were sterilized by 70% ethanol for 2 h and washed with PBS solution for three times. All the scaffolds were pre-wetted in culture medium for 12 h. Fifty microlitre of cell suspension (1×10^6 cells/scaffold) were seeded onto the pre-wetted scaffolds. The scaffolds were left in the humidified incubator for 2 h to allow cells to attach to the scaffolds, and 750 μl of culture medium were added to each well.

2.9. Cell viability and proliferation

Cell viability was evaluated with WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulfonate assay, Roche Diagnostics, Germany) assay following the manufacturer's instruction. Cell proliferation on the scaffolds was detected on Day 3, 7 and 14 using a "Live/Dead" assay (Molecular Probes, Invitrogen Singapore) following the manufacturer's instruction.

2.10. Osteogenesis

Osteogenic differentiation was performed by incubating MSC laden scaffolds into osteogenic media (DMEM supplemented with 10% FCS, 100 nM dexamethasone, 50 mg/ml ascorbic acid, and 10 mM β-glycerophosphate) for 14 days. Alkaline phosphatase (ALP) activity assay was conducted following the method described in the reference (Wang et al., 2008). Briefly, MSCs that had been pre-washed with PBS were lysed in 0.5 ml PBS containing 0.1 M glycine, 1 mM $MgCl_2$ and 0.05% Triton X-100. The lysate solution was incubated with p-nitrophenyl phosphate (pNPP) solution at 37 °C for 30 min and then subjected to a spectrophotometer on which the absorbance at 405 nm was measured and recorded to indicate ALP concentration. A quantificational Alizarin red-based assay of mineralization by osteoblasts was performed according to the reference (Gregory et al., 2004).

2.11. Statistical analysis

Experiments were repeated three times and results were expressed as means ± standard deviations. Statistical significance 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 $p < 0.05$.

3. Results and discussions

3.1. Preparation and morphology

In order to cater for the need of drug or protein release, the double emulsion technique which is widely applied in fabricating drug delivery system was introduced in PLGA/Lecithin scaffolds fabrication, and at the same time, microsphere sintered technique developed by Borden and Laurencin et al. was also utilized (Borden et al., 2002a; Kofron et al., 2007). Fig. 1 shows the morphologies of PLGA-SMS and PLGA/Lec-SMS. Through controlling sintering time and sintering temperature, microspheres maintain spherical shape.

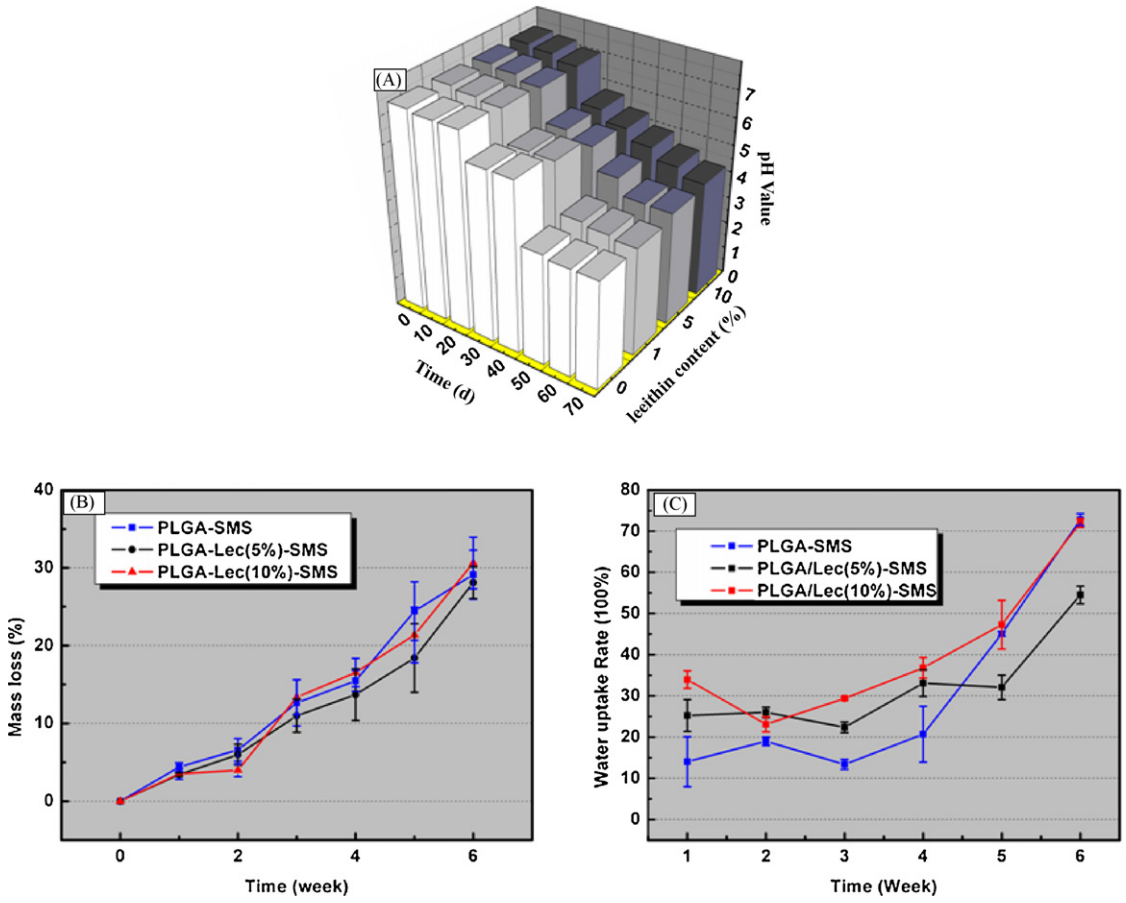

Fig. 2. Degradation properties of PLGA-SMS and PLGA/Lec-SMS: (A) pH value varieties; (B) mass loss; (C) water uptake rate.

Meanwhile, due to the surface fusion function induced by heating or sintering solution (acetone–ethanol mixture solution), microspheres were connected into an integrated scaffold and the bonding among adjacent microspheres could be easily observed. Compared with the pure PLGA microspheres, PLGA/lecithin microspheres displayed slightly wrinkling morphologies. All the scaffolds have the pore sizes between 30–400 μm, and microsphere sintered scaffolds with similar structure have been confirmed to be able to facilitate cell ingrowth and proliferation (Borden et al., 2002b).

One of the crucial challenges in designing systems for protein delivery is maintaining protein stability. However, heating is one of the principal physical factors for inducing protein denaturation. In this study, a substituted method–solvent fusion method was adopted for protein laden scaffold fabrication. The microsphere sintering via solvent fusion only lasted a short time, and mainly occurred on microspherical surface. So solvent fusion has weak influence on protein encapsulated in the interior of PLGA microspheres.

3.2. Degradation and water absorption

Fig. 2(A) shows the pH varieties during the degradation experiment. The pH values of every group exhibited a slight drop for the first 30 days. However, a significant decrease appeared for the last 30 days. The pH values of all the groups fell to around 4.5, which indicated that lecithin cannot hinder the decrease of pH values. Therefore, some further modified methods need to be performed in order to compensate the pH drop of PLGA. Fig. 2(B) shows the weight loss of PLGA-SMS and PLGA-Lec-SMS. Weight loss of all groups increased with the progress of degradation experiments. No significant difference was found among these three groups.

The temporal variations of water absorption are exhibited in Fig. 2(C). During the first 4 weeks, the degradation of PLGA was not remarkable, and lecithin may assume an active role in water uptake. The differences in water absorption between PLGA/Lec-SMS and PLGA-SMS were significant during the first 4 weeks. The remarkably higher rate of water absorption of PLGA/Lec-SMS in the first 4 weeks was mainly ascribed to the balance between the dissolution of oligomers in the solution and the water uptake of the residual materials (Fu et al., 2000). However, after 4 weeks for all samples, with the degradation of PLGA, water uptake rate of PLGA-SMS increased significantly.

3.3. Drug/protein encapsulation efficiency

Table 1 displays GS and BSA encapsulation efficiency of PLGA-SMS and PLGA/Lec-SMS. With the increase of lecithin content from 0 to 10%, GS encapsulation efficiency increased from (27.1 ± 9.2)% to (43.2 ± 7.1)%. And almost 70% of GS molecules were lost for PLGA-SMS. However, the scaffolds with lecithin exhibited higher

Table 1
Theoretical loading and encapsulation efficiency of scaffolds.

Batch	Drug/Protein Theoretical Loading (%)	Encapsulation Efficiency (%)
PLGA-SMS	1% GS	(27.1 ± 9.2)%
PLGA-Lec (5%)-SMS	1% GS	(45.5 ± 9.6)%
PLGA-Lec (10%)-SMS	1% GS	(43.2 ± 7.1)%
PLGA-SMS	1%BSA	(38.1 ± 4.5)%
PLGA-Lec (5%)-SMS	1%BSA	(63.9 ± 1.3)%
PLGA-Lec(10%)-SMS	1%BSA	(64.6 ± 1.8)%

encapsulation efficiency and almost 50% of the drug was encapsulated into the microspheres. GS is a hydrophilic drug, and it is easy to escape from PLGA bulks during microspherical fabrication. Lecithin is a composite of lipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), inositol phospatides, phosphatidylserine (PS). Except for PC and PE (net charge = 0), the net charge of other lipids is negative, which will favor arresting of positively charged hydrolysis products of GS (Lodish et al., 2004). BSA encapsulation efficiency of PLGA-SMS and PLGA/Lec-SMS scaffold is tremendously different as exhibited in Table 1. Compared with that of PLGA-SMS scaffold, BSA encapsulation efficiency of PLGA/Lec-SMS is almost doubled.

PLGA is aliphatic polyester that has no functional groups in its backbone for drug conjugation. Many methods have been developed to improve the drug encapsulation efficiency. Ho et al. (2008) introduced hydroxyapatite (HA) into PLGA microspheres and BSA encapsulation efficiency of PLGA/HA microspheres were significantly increased. Similarly in this study, after introducing lecithin into PLGA bulks, both BSA and GS encapsulation efficiency were remarkably elevated.

3.4. In vitro drug delivery

As shown in Fig. 3(A), GS release from PLGA microsphere sintered scaffolds consisted of an initial burst phase and a following gradual drug release. It can be observed that release rate of PLGA/Lec(10%)-SMS was remarkably high during the initial burst phase in the initial 6 days, but the scaffold displayed lower release rate with the decrease of lecithin content. Around 56.6% of total amount of GS was released from PLGA/Lec(10%)-SMS at the end of Day 6. However, only 31.0% and 45.8% of total drug loading were released from PLGA/Lec(5%)-SMS and PLGA-SMS. The significant initial burst release of PLGA/Lec-SMS scaffolds may be due to the rapid diffusion of more GS molecules on composite microspherical surface. After the initial burst phase, gradual drug release occurred through dissolution and diffusion of the remaining drug within the microspheres, which was controlled by the degradation and erosion of polymer matrix.

GS release behavior of PLGA/Lec-SMS with different microsphere sizes was also investigated. The scaffolds built by the microspheres with the diameter range between 400 μm and

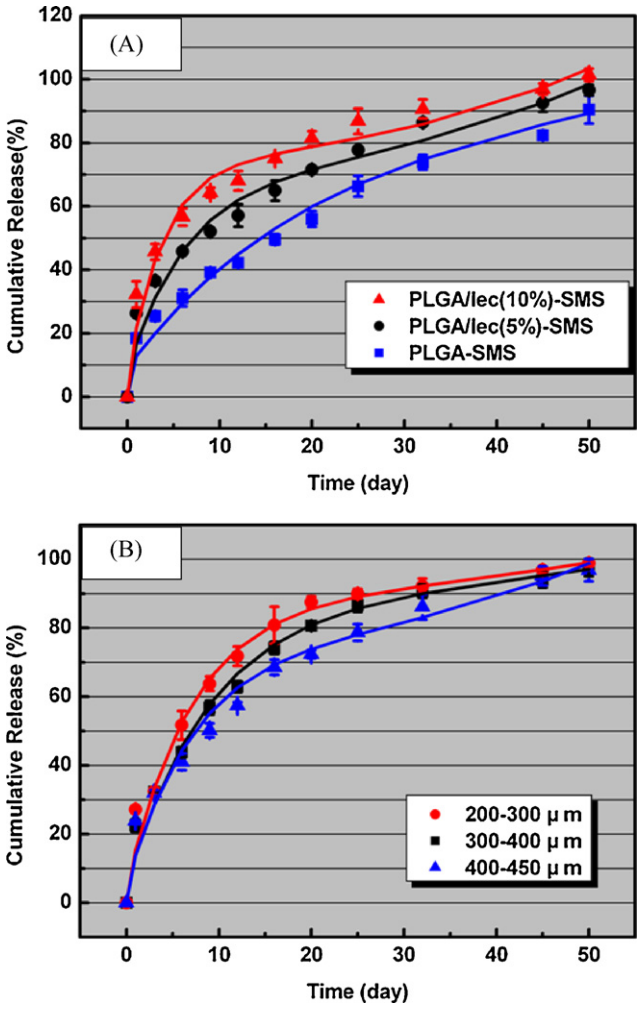

Fig. 3. Cumulative release of GS from PLGA-SMS and PLGA/Lec-SMS: (A) scaffolds with different lecithin content; (B) scaffolds with different microspheres sizes.

Table 2
Antibacterial zone diameter of GS laden PLGA-SMS and PLGA/Lec-SMS.

Samples	Antibacterial Zone Diameter (mm)		
	Day 0	Day 7	Day 14
PLGA-SMS	(16.5 ± 0.5)	(15.0 ± 0.5)	(9.8 ± 0.7)
PLGA/Lec(5%)-SMS	(15.8 ± 0.8)	(14.1 ± 0.2)	(10.2 ± 0.7)
PLGA/Lec(10%)-SMS	(14.0 ± 0.5)	(12.8 ± 0.8)	(9.5 ± 0.5)

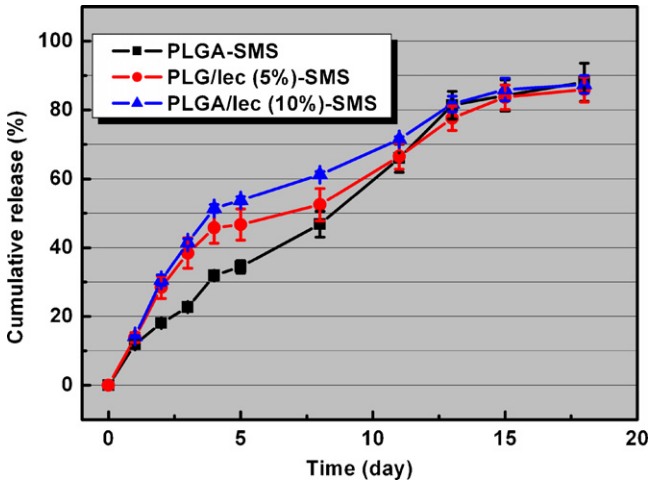

Fig. 4. Cumulative release of BSA from PLGA-SMS and PLGA/Lec-SMS.

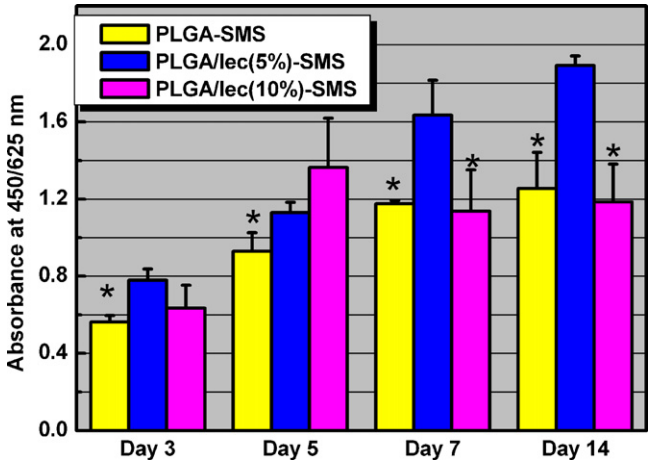

Fig. 5. Cell viability after 1, 3, 7 and 14 days of culture on samples of PLGA-SMS, PLGA/Lec-SMS. (*) indicate statistical significance when compared with the PLGA/Lec(5%)-SMS ($p < 0.05$).

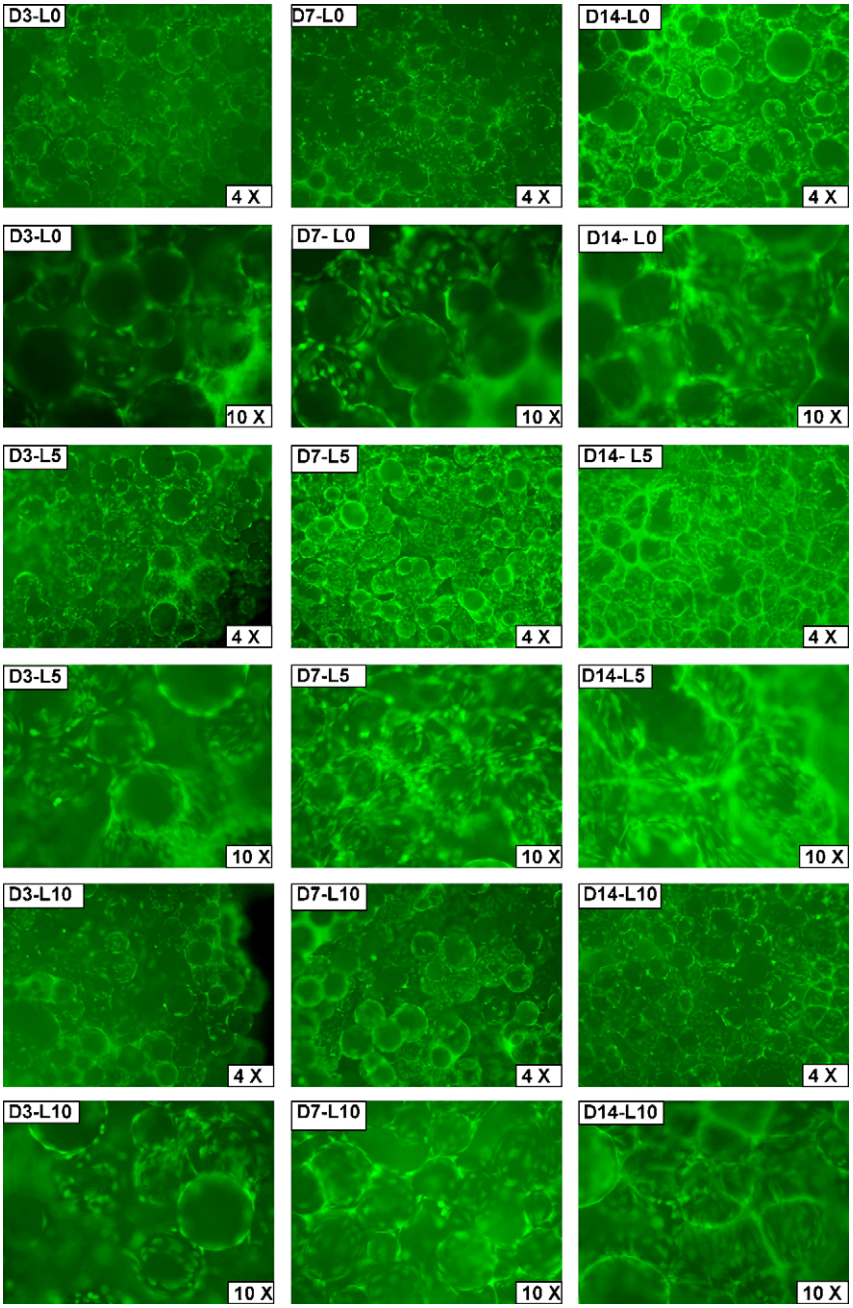

Fig. 6. Fluorescence photographs of cell proliferation on PLGA-SMS and PLGA/Lec-SMS after 3, 7 and 14 days of culture. D_3, D_7 and D_{14} are MSCs culture time (day) on the scaffolds; L_0, L_5 and L_{10} are the abbreviation of PLGA-SMS, PLGA/Lec(5%)-SMS, and PLGA/Lec(10%)-SMS.

450 μm indicated a low drug delivery rate [Fig. 3(B)]. By contrast, the scaffolds with microsphere sizes between 200 μm and 300 μm showed a high drug delivery rate. During the initial release period, the surface area/volume ratio dominates the release kinetics, and the microspheres with larger size possess low surface area/volume ratio, which leads to the relatively lower release rate. Berkland et al. (2003) also reported similar results that microspheres size could affect the drug distribution and polymer degradation, which had direct relation with drug delivery kinetics. After 30 days release, the release rate of the scaffolds with larger microsphere size slightly elevated. This was expected due to the degradation behavior of PLGA. Generally, the rate of PLGA degradation was faster for lager microspheres, because of an increased buildup of the acidic production which accelerated the rate of autocatalytic PLGA degradation.

3.5. Antibacterial assay

As shown in Table 2, up to Day 14, the antibacterial rings approximated to the diameter of all scaffolds, which indicated that most of GS molecules were released into SBF solution. PLGA/Lec-SMS displayed larger antibacterial rings than PLGA-SMS after incubating in SBF solution for 0, 7 and 14 days, and the antibacterial rings were maintained for more than 5 days. The data of antibacterial rings accorded with the results of GS release (Fig. 3) and GS encapsulation efficiency (Table 1). Compared with PLGA-SMS, PLGA/Lec-SMS showed more remarkable antibacterial properties mainly due to significantly higher encapsulation efficiency.

Infection is one of the most serious problems in conventional treatment of acute and chronic bacterial osteomyelitis includes sur-

gical removal of necrotic bone tissue (Heidenau et al., 2005). Drug delivery systems that sustain high local concentrations of antibiotics for several weeks are necessary for treatment of this disease. Many different materials have been utilized for local antibiotic delivery such as calcium phosphate (Laurent et al., 2008), bioactive glass (Xia and Chang, 2008), mesoporous silica (Balas et al., 2006; Shi et al., 2009a) and so on. However, these ceramic materials integrate with drug by physical adsorption, and present a very sharp initial burst. Some polymers, for example, Polymethylmethascrylate (PMMA) are also widely used for antibiotic delivery systems, whereas PMMA is not biodegradable, and in addition, PMMA cements are known to cause tissue necrosis due to high exothermic polymerization reaction temperatures (Nicholas et al., 2007). In this study, the biodegradable PLGA/Lec-GS-SMS systems have been shown to have high GS encapsulation efficiency and excellent antibacterial properties, indicating that they could be promising to facilitate the healing of bone removal induced by bacterial osteomyelitis.

3.6. *In vitro* protein delivery

BSA was used as a protein model for the evaluation of potential protein (such as growth factors and cytokines) release function of the scaffolds. The profiles of BSA (cumulative *in vitro*) release over 18 days from both PLGA-SMS and PLGA/Lec-SMS are shown in Fig. 4, where all the curves indicate comparable shape with an analogous tendency despite a minimal bursting initiation. Reflected by this tendency, the release proceeded in a remarkably high rate during first five days, especially for lecithin-containing scaffolds, and accordingly 30–60% of total release had been accomplished by then; while after the curve inflexion at around Day 4–5, the subsequent release rate lowered down. Up to Day 18, the total BSA release reached around 90% from all samples.

3.7. *Cell viability and proliferation*

Cell viability and cell growth were analyzed using WST-1 assay after 3, 5, 7 and 14 days of culture (Fig. 5), and Live/Dead assay after 3, 7 and 14 days of culture (Fig. 6), respectively. On the third day, the cell proliferation within all the scaffolds did not show significant difference. After 7 days of culture, MSCs on PLGA/Lec(5%)-SMS exhibited remarkable cell viability and proliferation, which was significantly higher than that of PLGA-SMS and PLGA/Lec(10%)-SMS. This phenomenon indicated that the content of lecithin in PLGA influenced cell proliferation. It has been confirmed that high lecithin content will lead to high hydrophilicity which would effect on cells attachment and proliferation (Zhu et al., 2007). A more detailed observation (Fig. 6) of the cells on scaffolds suggested that, for the sintering microspheres scaffolds, cells preferred growing around the gaps among the microspheres.

As a widely used drug and protein delivery system, the low cell toxicity of PLGA microspheres was already confirmed (Shi et al., 2009b,c). At the same time, the bioactivity of lecithin is undoubted. Baca et al. (2006) reported a nano silica-cell composite material which was assembled by phospholipids. Phospholipids take an important role for enhancing the cell viability and create a biocompatible environment for cells enwrapped by silica. Wuthier et al. (1992) also found a phospholipids complex composed by acidic phospholipids, calcium, and inorganic phosphate, which were identified as the nucleational core of these tissues. Our study has shown that appropriate lecithin content in PLGA bulks can elevate cell viability and proliferation.

3.8. *Osteogenesis*

In this study, MSCs on the scaffolds went through osteogenic differentiation. As a standard maker of osteogenic differentiation, ALP

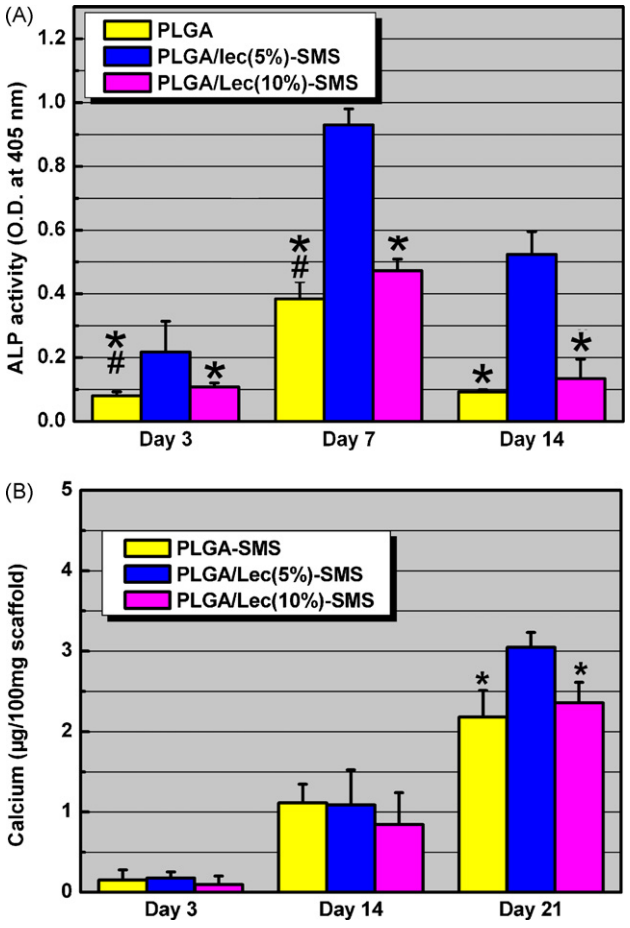

Fig. 7. Osteogenesis assay: (A) Alkaline phosphatase activity of MSCs cultured on PLGA-SMS and PLGA/Lec-SMS for 14 days. (B) Calcium deposition of osteoblasts on PLGA-SMS and PLGA/Lec-SMS for 14 days. (*) and (#) indicated statistical significance when compared with PLGA/Lec(5%)-SMS and PLGA/Lec(10%)-SMS ($p < 0.05$).

was determined by pNPP assay [Fig. 7(A)] Positive ALP production was first detected at Day 3, followed by significant upregulation until Day 7 and downregulation on Day 14. MSCs on the PLGA/Lec(5%)-SMS displayed significant higher level of ALP secretion compared to other groups.

A critical important function of osteogenic cells is participating in biological mineralization. The calcium deposition by osteogenic cells was accessed by a quantificational Alizarin red-based assay [Fig. 7(B)]. 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 PLGA-SMS, no significant difference in calcium deposition between them was observed.

4. Conclusion

In this study, PLGA/Lecithin hybridized sintered microsphere scaffolds with the functions of protein/antibiotic release were developed. The PLGA/Lec-SMS scaffold showed similar morphologies and degradation profiles to the PLGA-SMS scaffold and some further modifications may still need to investigate to compensate the pH change during degradation of PLGA. PLGA/Lec-SMS showed good GS and BSA encapsulation efficiency, which was much higher than PLGA-SMS. The antibacterial properties of GS-laden scaffolds were determined *in vitro*, and the antibacterial activ-

ity of PLGA/Lec-SMS was remarkable which mainly attributed to high GS encapsulation efficiency. Additionally, MSC were seeded onto PLGA-SMS and PLGA/Lec-SMS *in vitro*. The outcome confirmed PLGA/Lec(5%)-SMS improved MSC proliferation, and also enhanced general ALP production and calcium secretion which are the vital markers for osteogenesis. This indicated that the newly designed antibiotic releasing PLGA/Lec-SMS is promising for bone-repairing therapeutics.

Acknowledgements

Xuetao Shi was a China Scholarship Council (CSC) scholarship recipient (2007U33046). This research was financially supported by the National Basic Research Program of China (Grant 2005CB623902), Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period (2006BA116B04), the State Key Program of National Natural Science of China (50732003), and also supported by AcRF Tier 1 RG 64/08, Ministry of Education, Singapore.

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