The effects of bioactive akermanite on physiochemical, drug-delivery and biological properties of poly (lactide-co-glycolide) beads

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Abstract

Poly(lactide-co-glycolide) (PLGA) beads have been widely studied as a potential drug/protein carrier. The main shortcomings of PLGA beads are that they lack bioactivity and controllable drug-delivery ability, and their acidic degradation by-products can lead to pH decrease in the vicinity of the implants. Akermanite (AK) ($Ca_2MgSi_2O_7$) is a novel bioactive ceramic which has shown excellent bioactivity and degradation *in vivo*. This study aimed to incorporate AK to PLGA beads in order to improve the physiochemical, drug-delivery and biological properties of PLGA beads. The microstructure of beads was characterized by SEM. The effect of AK incorporating into PLGA beads on the mechanical strength, apatite-formation ability, the loading and release of BSA, and the proliferation and differentiation of bone marrow stromal cells (BMSCs) was investigated. The results showed that the incorporation of AK into PLGA beads altered the anisotropic microporous struc-

ture into homogenous one and improved their compressive strength and apatite-formation ability in simulated body fluids (SBF). AK neutralized the acidic products from PLGA beads, leading to stable pH value of 7.4 in biological environment. AK led to a sustainable and controllable release of bovine serum albumin (BSA) in PLGA beads. The incorporation of AK into PLGA beads enhanced the proliferation and alkaline phosphatase activity of BMSCs. This study implies that the incorporation of AK into PLGA beads is a promising method to enhance their physiochemal and biological property. AK/PLGA composite beads are a potential bioactive drug-delivery system for bone tissue repair.

Key words: Bioactive akermanite; drug delivery; beads; poly (lactide-co-glycolide); cell differentiation

INTRODUCTION

Controlled release technology is an effective approach to optimize the drug dosage delivered to a specific site for a prolonged duration.[1-3] Biodegradable poly(lactide-co-glycolide) (PLGA) is one of the most widely used synthetic polymers for tissue engineering scaffolds and drug delivery systems as PLGA has high biocompatibility and controllable degradation rates.[4] However, the main shortcomings of PLGA beads are still that they lack bioactivity and controllable drug-delivery ability, and their acidic degradation by-products can lead to pH decrease in the vicinity of the implants, which is detrimental to cell growth.[5-9]

Microsphere-derived scaffolds show very high potentials in both scaffolding and drug delivery.[10] Several techniques have been developed to fabricate polymer beads, including rapid solvent re-

moval by temperature gradient,[11] gas foaming,[12] double emulsification (W/O/W),[13] and solution-induced phase separation.[14] Traditional methods (e.g., emulsion or spraying technique) generate poorly controllable PLGA beads, leading to a wide size distribution and a low loading efficiency.[15] The beads produced using the *thermally induced phase separation* (TIPS) method[16] are structurally different from other known beads, and result in improved characteristics such as a higher mechanical strength, a narrower size distribution, a slower degradation rate and an excellent ability to protect the entrapped drugs/proteins. Johnson et al. described a cryogenic process that involved spraying a polymer/protein suspension into liquid nitrogen or frozen ethanol through an ultrasonic nozzle, and produced uniformly sized beads with high encapsulation efficiency.[17] Low temperature media effectively freeze the protein–polymer droplets, thereby avoiding the protein to contact with water during the microencapsulation process. The TIPS technique for preparing porous polymer beads is a suitable approach for the controllable delivery of drugs.[18]

Previous studies have shown that Akermanite (AK) ($Ca_2MgSi_2O_7$) ceramics are bioactive and biodegradable,[19] and possess improved mechanical strength and bioactivity compared to traditional Ca-P ceramics.[20-23] Due to these advantages of AK ceramics, it is speculated that the incorporation of AK into PLGA beads could significantly improve physiochemical, drug-delivery and biological properties of PLGA beads prepared by the TIPS method. This study is to incorporate different amount of AK powders into the PLGA beads to optimize their physicochemical, drug-delivery and biological properties for potential bone tissue engineering applications.

MATERIALS AND METHODS

Preparation of PLGA/akerminate composite beads loaded with BSA

AK powders were synthesized according to the previous publication.[21] Composite beads were prepared by a low temperature-dried method based on thermally induced phase separation principle. Poly (DL-lactide-co-glycolide) (PLGA) (75:25, Sigma, Aldrich, Pty. Ltd, Castle Hill, NSW, Australia) was dissolved in dimethyl carbonate (DMC) (Sigma-Aldrich) to form a 5% (w/v) polymer solution. Defined amounts of AK powders (weight ratios of PLGA to AK: 10:1, 5:1, 2:1, respectively) were added to the PLGA solution and homogenized by vortexing for 10 min, followed by the addition of 100 µl of 20 % (w/v) of bovine serum albumin (BSA) solution in phosphate buffer saline (Fraction V, 99%, Sigma-Aldrich). The suspension was then manually dropped from a syringe with a 23G needle into a vessel containing liquid nitrogen to obtain the PLGA/AK composite beads. These beads were frozen and freeze-dried for 72 hr to remove the solvent to obtain TIPS beads. BSA loaded pure PLGA beads without AK were prepared for the controls following the same procedure described above for the experiment of BSA release. For other experiments of mechanical strength, pH test and cell culture, the pure PLGA and PLGA/AK microspheres without BSA were prepared by the same procedure above without adding BSA.

Preparation of double emulsion PLGA beads

In order to assess the drug delivery properties of TIPS prepared PLGA beads, control PLGA beads were prepared by the double emulsion method according to a previous publication.[24] Briefly, 500 mg of PLGA was dissolved in 8 ml of dichloromethane (CH_2Cl_2, Sigma-Aldrich, Australia), and 1 ml of BSA water solution (0.2 g/ml) added. The mixed solution was emulsified by vortexing for 40 seconds. The primary emulsion (W1/O) was then added drop wise into 100 ml of 1% polyvinyl alcohol (PVA) aqueous solution and mixed for 2 min at 9,500 rpm with a turbine homogeniser (Ultraturrax® T-18, IKE-WERKE, Staufen, Germany), resulting in a secondary emulsion (W1/O/W2). To

this emulsion was further added 100 ml of a 2% isopropanol aqueous solution, which was magnetically stirred for 4 hr at 300 rpm. The beads were sedimented out by centrifugation at 1000 rpm for 1 min, the supernatant decanted and distilled water used to wash off the organic residuals. After three washes, the collected beads were freeze dried for 72 hrs to evaporate any residual solvent.

Characterization and mechanical strength of beads

The morphology and microstructure of the beads were examined using scanning electron microscope (SEM; PHILIPS FEI QUANTA 200, FEI Company, Hillsboro, Oregon, USA). The particle size of the beads was evaluated by an optical microscope (OM; S6D, Leica, Leica Microsystems, Wetzlar, Germany). Mechanical testing of the beads was performed in compression mode on an EnduraTEC ELF3200 (BOSE, Eden Prairie, MN, USA) fitted with a 50N load cell and at a compressive rate of 50 µm/min. The compression tests were conducted at 23°C for all the tests. The elastic response of a microsphere, at the initial stage of the micro-compression, was analyzed in terms of the Hertz theory.

pH change of PBS

In each group a total of 40 mg BEADS were immersed in 2 ml PBS solution for 21 days. Four groups of samples including pure PLGA and composite beads with different PLGA-AK weight ratios were compared. Soaking was carried out at 37°C without stirring and refreshing, and the pH-values of PBS were monitored by an electrolyte-type pH meter (*PINPOINT®* pH Monitor, America). Three times of testing were conducted for each material.

Apatite-formation ability of beads in simulated body fluids

The simulated body fluids (SBF) were prepared according to the procedure described by Kokubo [25] and Table 1 shows the ion concentrations of the SBF solution and human blood plasma. Pure PLGA and AK/PLGA composite beads were soaked in SBF at 37°C for 21 days with refreshing SBF every week, and the ratio of the sample weight to the SBF volume (mg/ml) was 3:5. After soaking, samples were removed from the SBF, gently washed with deionized water, dried at room temperature, and characterized by SEM and energy-dispersive spectrometer (EDS) (Oxford INCA Energy 400 EDS, Oxford Instruments, Oxford, UK).

Determination of encapsulation efficiency

Encapsulation efficiency of BSA into beads was determined spectrophotometrically after extraction from beads. Briefly, accurately weighed amount (20 mg) of beads was dissolved in 1 ml methylene chloride, and 1 ml PBS buffer was added. The solution was stirred at 37°C for 24 hr. After centrifugation, the protein concentration in the aqueous phase was then determined by a bicinchonic acid (BCA) protein assay kit (Thermo Fisher Scientific Inc., Waltham, MA). Encapsulation efficiency (EE) was calculated by equation as follows: EE = actual BSA loading/theoretical BSA loading×100%

In-vitro release of BSA from beads

In vitro release of BSA from double emulsion, pure and composite PLGA TIPS beads was carried out by immersing 20 mg of beads in 1 ml of PBS at 37°C for an extended period of time on a rotat-

ing shaker. The supernatants were periodically removed after centrifugation to assay for BSA and replaced with an equal amount of PBS. The BSA in the supernatant solution was quantified using the BCA assay as mentioned above.

Cell culture

Isolation and culture of BMSCs was conducted following previously published protocols.[26] Bone marrow aspirates were obtained from patients (mean age, 65 years) undergoing elective knee and hip replacement surgery. Informed consent was given by all patients involved and the research protocol had been approved by the Human Ethics Committees of Queensland University of Technology and The Prince Charles Hospital. Bone marrow sample (10 ml) was mixed with 5,000 units of heparin to prevent coagulation, and diluted with 10 ml of phosphate buffered saline (PBS). Cells were fractionated by centrifuging at 800 x *g* for 10 min over a density gradient solution (Lymphoprep; Axis-Shield PoC AS, Rodelokka, Norway). The resulting mononuclear cells appearing as a buffy interface was kept and cultured in low-glucose Dulbecco's Modified Eagle Medium (LG-DMEM; GIBCO, Invitrogen, Mt Waverley, Vic, Australia) supplemented with 10% v/v foetal bovine serum (FBS; Thermo Scientific, Melbourne, Vic, Australia) and 1% v/v penicillin and streptomyocin (P/S; GIBCO) at 37^{o}C in humidified atmosphere with 5% CO_2. Initial medium change was performed on the fifth day to remove non-adherent cells, after which media refreshed twice a week. Passage 3 hBMSCs were used in this study.

Cell proliferation

hBMSCs were seeded onto 6-well TCPS (tissue culture polystyrene) plates at a density of 1×10^4 cells/well and incubated in the culture medium in a humidified incubator at 37^oC and 5% CO_2. Cells were allowed to adhere and grow for 2 hours. Subsequently, 20mg different weight ratios of PLGA/AK beads sterilized by 75% ethanol were added to each well. After cultured for 4 and 7 days without refreshing medium, cells were harvested and accounted. In the control groups, cells were cultured alone or with pure PLGA beads only.

Alkaline phosphatase activity

Cells were allowed to adhere and grow for 3 days in the osteogenic medium (DMEM supplemented with 10% FBS, 100 nM dexamethasone, 50 mg/ml ascorbic acid, and 10 mM b-glycerophosphate). Then, 20 mg pure TIPS BEADS or AK/PLGA BEADS were added into the cell cultures respectively. Cells were cultured in osteogenic differentiation media in for 21 days. Alkaline phosphatase (ALP) activity was determined using pNPP assay (p-nitrophenyl phosphate liquid substrate, Sigma-Aldrich). Briefly, BMSCs were washed with PBS, then were lysed in 0.5 ml PBS containing 0.1 M glycine, 1 mM $MgCl_2$ and 0.05% Triton X-100 for 10min at 4 ºC. The lysate was incubated with p-nitrophenyl phosphate (pNPP) solution at 37^oC for 30 min, and then subjected to a spectrophotometer on which the absorbance at 405 nm was measured and recorded to indicate ALP activity.[27]

Statistic analysis

Six completely independent experiments for mechanical testing, BSA release and cell culture, were performed for every assay and the results were expressed as means ± standard deviations. Statis-

tical significance was calculated using one way analysis of variance (one-way ANOVA). Comparison between the two means was performed using Turkey test and the significance was determined by $p<0.05$.

RESULTS

Microstructural characteristics of the pure PLGA beads

SEM pictures showed that TIPS beads showed a regular spherical shape with a narrow diameter distribution (Fig. 1 A), and contained a highly order interconnected porosity with radial channel-like pores opening both on the surface and in the interior of the beads (Fig. 1 C and Fig. 1 D). The size of beads was in the range of 1.2 mm to 2.3mm and were controlled by varying the size of needles used (Table 2).

Microstructural characteristics of the PLGA/AK composite TIPS beads

SEM images showed that AK ceramic particles were incorporated successfully into the PLGA TIPS beads (Fig. 2). The ceramic particles dispersed throughout the foam structure of the beads (Fig. 2). The pore structure is less ordered with fewer channels or ladder-like pores compared with pure PLGA BEADS, when the ratio of PLGA to AK reached 5:1 (Fig. 2 C). When the PLGA/AK weight ratio increased to 2:1, the random and round pores became more evident as the sharp pore edges dis-appeared (Fig. 2 D).

Effect of AK addition on the compressive modulus of the PLGA/AK beads

AK modification significantly enhanced the mechanical strength of PLGA beads when the ratio of PLGA/AK is 2:1 ($p<0.05$) (Fig. 3). The compressive strength of BEADS with the maximum AK increased about 30% in comparison to the pure PLGA BEADS or those with low amount of AK (Fig.3), although these BEADS had a higher porosity.

pH variation of PBS solutions against time

For pure PLGA beads, the pH was did not noticeably change and maintained around 7.0 in the first 11 days of soaking, and then showed a sharp decrease from 7.0 to 5.46 when the soaking continued from 11 to 21 days. For the composite beads, the pH values of the PBS solutions showed a quickly increase in the first 2 days of soaking, and then relatively slowly decreased, and finally maintained within a narrow range from 7.44 to 7.76 until the end of the soaking period. (Fig. 4).

Apatite-formation ability of the Beads in SBF

SEM images of the beads after soaking in SBF solution for 21 days are shown in Fig. 5. For the pure PLGA beads, the surface showed a smooth microstructure. In contrast, on the surface of the PLGA/AK composite beads, a rough deposit layer was formed. At a high magnification, it was revealed that the layer was composed of micro/submicro-sized particles, which assembled into large aggregates with a typical morphology of apatite (Fig.7). In addition, some pores with the nano-level diameter were observed, which indicated that the layer was microporous. To determine the chemical composition of the microporous deposits, the surfaces of the beads were further characterized by EDS. The EDS spectra of the pure PLGA BEADS and the PLGA/AK composite BEADS after incubation in SBF solution for 21 days are shown in Fig. 6. The pure PLGA microsphere surface only

showed high peaks of C and O elements from PLGA (Fig. 6A), while Ca and P peaks were detected on the surface of the composite microsphere and the atom ratio of Ca to P was about 1.65, which was close to that of carbonated apatite (Fig. 6B). However, there is no very obvious apatite formation for pure PLGA and PLGA/AK microspheres at the earlier stage of soaking (7 day).

Encapsulation efficiency and release profile of BSA loaded in the beads

In this study, the BSA was used to evaluate the release kinetics of the BEADS. The encapsulation efficiency values of the double emulsion BEADS, the pure PLGA TIPS BEADS and The TIPS PLGA/AK BEADS were listed in Table 3. Traditional double emulsion method was less efficient in entrapping the BSA, whereas the beads by TIPS showed much higher levels of encapsulation. The comparative BSA release profiles of the BEADS over 38 days are shown in Fig. 8. The release kinetics from the double emulsion PLGA beads exhibited a significant burst release: 47.3% of the total BSA loaded amount was released from the PLGA BEADS during the first 2 days. In contrast, the release profiles of the pure TIPS PLGA and the composite TIPS BEADS showed a low initial burst release (Table 3).

Cell proliferation

The proliferation of hBMSCs was affected by the different compositions of the composite BEADS used (Fig. 9). The pure PLGA BEADS inhabited cell proliferation significantly compared with the PLGA/AK composite BEADS. At day 4, composite BEADS (PLGA/AK 5:1 and 2:1) enhanced the proliferation of hBMSCs significantly compared to the pure PLGA BEADS ($p<0.05$, Fig. 9), but no difference was found between the low level AK composite BEADS (PLGA/AK 10:1) and the pure PLGA BEADS ($p>0.05$, Fig. 9). However, the cell proliferation in this low AK composite BEADS (PLGA/AK

10:1) showed a relatively higher level at the day 7 than that of the pure PLGA BEADS group. However, the highest ratio of PLGA to AK (2:1) showed slight inhibition effect on the cell growth compared with the ratio of 5:1 BEADS ($p<0.05$, Fig. 9).

ALP activity

hBMSCs were cultured in osteogenic media under different composite BEADS environments for 21 days. The secretion of ALP, a maker of osteogenic differentiation, was determined by ALP activity assay (Fig. 10). A high ALP activity was detected in positive control and PLGA/AK composite BEADS groups at Day 7 in comparison to pure PLGA BEADS in both day 7 and day 14. (Fig. 10). Interestingly, hBMSCs on the PLGA/AK BEADS(PLGA/AK 2:1) displayed significant higher level of ALP secretion compared to the pure PLGA BEADS and PLGA/AK BEADS (10:1) in day 7, but reversely deceased the activity of ALP in day 14 ($p<0.05$, Fig. 10).

DISCUSSION

The TIPS method has been widely used to make various beads as it can produce highly porous interior structures. Since the DMC solvent used in this study has a freezing temperature of -1ºC, once the polymer solution droplets are sprayed into the liquid nitrogen, the polymer solution will be separated into a polymer-rich phase and a polymer-poor phase due to the thermally induced phase separation. The polymer phase is expelled from the solvent phase during the crystallization to form the matrix of the porous beads. The TIPS beads have a relatively smooth but still porous skin surface due to the initial fast evaporation in air or fast freezing in liquid nitrogen. Sublimation of the solvent during the freeze drying caused interior pores, which are a three-dimensional fingerprint of the geometry of the solvent-derived crystals. This porous structure of the beads is characteristic of scaffolds made by solid-liquid thermally induced phase separation. On the other

hand, the addition of hard particulate phases into polymer matrices and the structural changes in pore morphology has been reported to influence the mechanical properties of TIPS derived foams, namely, composite and monolithic foams with the inclusion of bio-glass particles,[28] and hydroxyapatite in poly(a-hydroyesters). In this study, with the addition of increasing AK content to the PLGA beads, the interference between the crystallizing solvent by the solid AK particles resulted in more irregular and anisotropic crystal growth of the solvent, leading to less ordered pore structure with fewer channels or ladder-like pores. The mechanical properties were also changed by the addition of AK. The PLGA/AK composite beads showed much higher Young's modulus than the pure PLGA BEADS. Although the Young's modulus of the obtained microspheres (about 250MPa) is lower than that of cortical bone of human being (7-30GPa)[29], AK-modified PLGA BEADS could be used to filler materials in non-load bearing parts of human being.

Degradable polymer scaffolds with potential therapeutic applications including microsphere-based controlled release systems for proteins and nuclear acids have been studied intensively as well as polymer scaffolds with seeded cells for tissue regeneration and repair. However, acidic by-products due to polymer degradation however can lead to inflammatory responses and additively cytotoxicity to the seeded or surrounding cells. A build-up of acidic by-products of the implanted polymer scaffolds is also known to be deleterious to some proteins and nucleic acids. Thus it is very important to control the pH value of the environment during the polymer degradation. Fu et al.[30] studied the pH value change of the PLGA beads and showed a gradient acidic environment from the center of the beads to the edges, with a minimum pH value as low as 1.5 within the particles. In our study, there was a visible decrease of pH value in the PBS solution containing the pure PLGA beads, indicating that some acidic by-products were produced during the soaking. In addition, we demonstrated that the pH value of the PBS solutions containing the PLGA/AK composite beads was stable and showed little change during a 21-day soaking period, which suggested

that the incorporation of AK into the PLGA neutralized the acidic byproducts and stabilized the pH value of the PBS solutions. Previous studies clarified that the AK-derived scaffolds could gradually release Ca, Mg and Si ions, and these ions increased with the soaking time.[21] The released ions are able to form basic hydrates, which can possibly explain the pH value stabilization ability of AK. Therefore, it can be concluded that the AK is effective in neutralizing the acidic by-products of PLGA, and the pH-stabilization ability of the PLGA/AK composite beads could be controlled by adjusting the amount of the AK in the composite beads.

The addition of AK also enhanced the apatite precipitation on the BEADS as shown in this study. Different weight ratios of the composite beads reflected different precipitation patterns: with the increase of the AK content, more apatite particles were formed and an apatite layer was observed on the PLGA/AK 2:1 BEADS. Ionic exchange happened between AK and the SBF when the PLGA/AK composite beads were immersed in the solution, which would drive the formation of Si-OH groups on the material surface and induce the apatite nucleation. Such apatite nuclei would grow subsequently at the expense of P ions in the SBF solution and form an apatite layer.[23] In this study, the observation of the beads indicated that a microporous apatite layer or isolated apatite deposits were indeed formed on the composite beads while nothing was formed on the pure PLGA beads. Previous study has revealed that akemanite can induce osteogenic differentiation and bone formation.[22] The in vivo studies suggest that the apatite-formation ability is a critical factor in facilitating the chemical fixation of biomaterials to bone tissue, and ultimately leading to the in vivo success of the bone grafting materials. In this study, the results revealed that the PLGA/AK composite beads could induce the formation of apatite layer on their surface and it can be expected that an apatite layer may be formed on such composite beads when they are used in vivo.

Regarding the BSA loading efficiency in different BEADS, TIPS BEADS, as a small amount of water was used to dissolve the BSA and there was no need to wash the beads, showed reduced leaching

out or loss of the encapsulated BSA. The only little loss of the BSA was due to the residual left in the viscous polymer solution smeared on the internal surface of the dispensing syringe after the TIPS process. As to the releasing profile, the low initial burst release of TIPS BEADS may be associated with the reduced release surface area due to the critical size of the BEADS rather than due to the porosity formed on the surface of the beads.[12] Furthermore, the porous pure TIPS PLGA BEADS displayed a closely linear release trend as the high pore interconnectivity maintained the constant pH value environment throughout the beads in contrast to the double emulsion BEADS. As incorporating AK into the polymer further modified the BSA release profiles of BEADS by neutralizing the acidic degradation of PLGA, the typical three-phase release was not distinguished. With the increase of the AK content, the release rate of the BSA increased gradually, and the PLGA/AK BEADS with weight ratio 2:1 begun to accelerate at the later-period of release. These findings indicate that the BSA release could be controlled by changing the amount of the AK in PLGA beads.

Cell proliferation assay on different beads reflected indirectly the effect of acidity variation in medium on the cell growth as well. The low pH value environment introduced by the pure PLGA beads inhibited the cell growth, while composite beads (PLGA/AK 5:1 and 2:1) enhanced the proliferation of hBMSCs significantly compared to the pure PLGA beads and low content of AK (PLGA/AK 10:1). The 10:1 PLGA/AK could not entirely neutralize degraded acidic monomers, which might cause the fall of cell number in day 4. By day 7, in the low AK composite group cell proliferation was recovered, which may be related to the release of more Ca, Mg, and Si ions from akermanite ceramics and neutralize the acidic products from PLGA.

In addition to the pH value effect, the ions released from the AK also affected favourably on the cell growth and differentiation. Previous study showed that Ca, Mg, and Si ionic concentrations in SBF increased with increasing soaking time due to the dissolution of the Ca, Mg, and Si ions from the akermanite ceramic. Ca ions could induce osteoblast proliferation and chemotaxis through

binding to a G-protein coupled extracellular calcium sensing receptor.[31] Mg supplementation of a bioceramic stimulated adhesion of osteoblastic cells to commonly used orthopedic implants.[32] The Si, Mg and Ca ions from dissolution at a certain concentration range also promoted hBMSC growth [23]. Studies also showed that the Si ions from bioactive glass dissolution at a certain concentration range increased cell proliferation and differentiation.[33,34] A low concentration of Si could facilitate cell proliferation, but a high concentration gave the opposite results.[33,34] Our results suggested that the Si, Mg and Ca ions released from the PLGA/AK BEADS may play an important role in regulating hBMSC proliferation and differentiation. The content of AK in the PLGA/AK BEADS determines the positive or negative regulation of hBMSCs in proliferation and differentiation in term of ALP activity.

CONCLUSIONS

Our results indicate that PLGA/AK composite beads can improve the structural, physic-chemical, mechanical and biological properties in comparison to traditional pure PLGA beads. This composite microsphere can efficiently control the environmental pH value, loading and releasing BSA proteins, and regulate the human BMSC cell growth and osteogenic differentiation.

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Figure captions

Fig. 1. Representative photograph and SEM images showing the internal and the external microstructures of PLGA beads prepared by TIPS: (A) photograph of the PLGA TIPS beads; (B) SEM image of the beads at a low magnification; (C) SEM image at a high magnification showing the surface of a microsphere; (D) SEM image of the internal porous structure of a microsphere.

Fig. 2. SEM images of the external surfaces of the beads with different PLGA/AK weight ratios: (A) pure PLGA microsphere; (B) microsphere with 10:1 ratio of PLGA to AK ; (C) microsphere with 5:1 ratio of PLGA to AK; and (D) microsphere with 2:1 ratio of PLGA to AK.

Fig. 3. Young's modulus of the PLGA/AK beads.

Fig. 4. The time-dependent pH value changes of the PBS solutions with soaked beads.

Fig. 5. SEM images of the beads after soaking in SBF for 21 days: (A) and (B) pure PLGA beads; (C) and (D) PLGA/AK (10:1) composite beads.

Fig. 6. EDS spectra of the surfaces of beads: (A) from a pure PLGA microsphere; (B) from a PLGA/AK (10:1) composite microsphere.

Fig. 7. SEM images show the apatite deposits on the surfaces of the beads with different PLGA/AK weight ratios: A (1) 10:1; B (1) 5:1; C (1) 2:1 respectively in SBF for 21 days

Fig. 8. BSA release profiles from pure PLGA TIPS beads and TIPS BEADS with different PLGA / AK weight ratios, compared with double emulsion-derived beads.

Fig. 9. Proliferation of hBMSCs cultured on pure PLGA TIPS beads, and composite TIPS BEADS with different PLGA/AK ratios for 4 and 7 days.

Fig. 10. ALP activity of hBMSC in day 7 and day 14 day in the culture with PLGA/AK composite BEADS. In culture with PLGA/AK BEADS, the ALP activity in BMSCs increased to higher level compared to the culture with the pure PLGA BEADS.