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Effect of composition on morphology structure and cell affinity of poly(caprolactone-*co*-glycolide)-*co*-poly(ethylene glycol) microspheres

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ABSTRACT: Poly(caprolactone-*co*-glycolide)-*co*-poly(ethylene gylcol) copolymers (PCEG) with various composition were synthesized by copolymerization of GA, CL, and PEG. PCEG microspheres were fabricated by oil-in-water (o/w) emulsion and solvent-evaporation technique. Effect of chemical composition on hydrophilicity, crystallinity, and degradation of the PCEG was investigated. It was demonstrated that morphology structure of the microspheres was greatly influenced by chemical composition and hydrophilicity of the PCEG polymer. PCEG microspheres could change from a smooth structure to a regular porous structure and an irregular structure. Moreover, the pore size of them increased with increment of PEG content and length. Cell attachment and growth on the PCEG microspheres were evaluated by using mouse NIH 3T3 fibroblasts as model cells *in vitro*. The result showed that the PCEG microspheres with large porous structure were more favorable for cell attachment and growth. Thus the PCEG microspheres with rapid degradation rate and large porous structure possess potential use as injectable scaffolds in tissue engineering. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42861.

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INTRODUCTION

Porous biodegradable polymer scaffolds have been extensively utilized as temporal templates for regeneration of various tissues.[1–4] Highly open porous structured scaffolds with well interconnected pores are required, which not only can achieve sufficient cell density in the scaffold, but also can facilitate in- and out-transport of nutrients and oxygen for subsequent cell proliferation and differentiation. Different morphology of scaffolds could be fabricated by various methods such as the solvent casting and particulate leaching (SCPL) technique,[5–7] emulsion/freeze drying method,[8–10] gas saturation and release method,[11,12] 3D-guided printing method,[13] phase separation techniques,[14–17] electrospinning techniques,[18–20] thermal induced phase separation (TIPS) technique,[17,21] etc. Most of porous scaffolds are fabricated into block or foam shapes for application in tissue engineering, however these kinds of scaffolds are difficult to meet requirements of tissue regeneration with complex shape. Because biodegradable microspheres possess flowability, which can be injected and filled into complicate defect, they are favorite for tissue engineering applications. At the same time, the biodegradable microspheres have other advantages such as benefiting to perform minor-incision operation and carrying some growth factors or drugs. So applications provided by porous biodegradable microspheres can be more versatile than that of the fixed-shape porous scaffolds.[22–26]

Poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA) are widely used in tissue engineering and carriers of drug or protein in delivery system because of their biodegradability, biocompatibility, and the nontoxic degradation products.[27–29] However common disadvantages of them are poor hydrophilicity and some high crystallinity residual, which can induce slow degradation and chronic inflammatory reaction.[30] Many efforts were performed for overcoming the problems. One of the most effective way is to enhance hydrophilicity and lower crystallinity of

Additional Supporting Information may be found in the online version of this article.

the polymer by introducing hydrophilic components into the polymer via copolymerization.[31–34] Poly(ethylene glycol) (PEG) is widely used hydrophilic component and it can be performed as a macromolecular initiator to initiate and copolymerize with caprolactone (CL), lactide (LA), or other lactone monomers by the active hydrogen of the hydroxyl group. As a result various polyester-polyether copolymers can be obtained. Hydrophilicity and degradation rate of them could be adjusted by controlling component ratio of the feeding dose.[35–41]

On the other hand, polymer microspheres can be well fabricated by an oil-in-water (o/w) emulsion and solvent-evaporation technique.[42,43] However the produced microspheres usually possess smooth surface and tight structure, which is disadvantageous for cell adhesion and growth.[44] To obtain porous structured microspheres, many other methods such as solvent evaporation and liquid–solid phase separation method,[45] and porogen adding and gas foaming method[12] were also performed. Although porous structured microspheres could be obtained by these methods, a lot of un-opened "dead pores" were easily formed, and the microspheres could be polluted by the residual porogen.

A kind of amphiphilic polyester-polyether copolymer—poly(caprolactone-*co*-lactide)-*co*-poly(ethylene glycol) tri-component copolymer (PCEL) had ever been reported,[36] and porous PCEL microspheres were fabricated by means of emulsification solvent evaporation method.[46] But, it could be found that pore size of the PCEL microspheres was too small for cells to be seeded and grow inside. So it is necessary to fabricate microspheres with high degradation rate and large sized pores, which will be favorable for being used as injectable scaffolds in tissue engineering.

In the present article, poly(caprolactone-*co*-glycolide)-*co*-poly(ethylene glycol) tri-component copolymer (PCEG) was synthesized. Composition, hydrophilicity, thermal, and crystalline properties of the polymers were characterized. Degradation rate and morphology change of the PCEG films was determined and compared with that of PCEL copolymer. Then, PCEG microspheres were prepared by an oil-in-water (o/w) emulsion and solvent-evaporation technique. Cell affinity of the PCEG microspheres *in vitro* was evaluated by using mouse NIH 3T3 fibroblasts as model cells. Finally, effect of composition on morphology structure, mass loss, and cell affinity of poly(caprolactone-*co*-glycolide)-*co*- poly(ethylene glycol) (PCEG) microspheres were discussed.

EXPERIMENTAL

Materials

Glycolide (GA) (PURAC, the Netherlands) was purified by recrystallization in ethyl acetate (Beijing Beihua Fine Chemicals Company, China), and then was dried and kept over P_2O_5 *in vacuum*. ε-Caprolactone (CL) (Acros Chemica, N.V.) was purified by distillation with CaH_2. Poly(ethylene glycol) (PEG) with M_n of 10,000 ($PEG_{10,000}$) and 4000 ($PEG_{4,000}$) (Yili Chemical Reagent, China) was dried by freeze-drying and then stored over P_2O_5 in vacuum prior to use. Stannous octoate (Sigma, A.R.) was used without further purification. Ethyl acetate was dried over P_2O_5 overnight and distilled before use. All other reagents were of analytical grade and used without further purification.

Synthesis of PCEG and PCEL

PCEG and PCEL were synthesized by ring-opening polymerization of CL with GA or LA by using PEG as initiator and stannous octoate as catalyst under vacumm at 170°C for 24 h, as previously described.[47] After produced raw product was dissolved in chloroform, the solution was precipitated from cold ethyl ether [1:3–1:5 (v/v)] to obtain purified PCEG or PCEL. The PCEG (or PCEL) was named as PCEG (x/y/z)(k) (or PCEL(x/y/z)(k)), where the *x*, *y*, and *z*, respectively represents molar content of caprolactyl, EG, and glycotyl (or lactyl) unit of the copolymer, and *k* represents molecular weight of PEG.

Preparation of Microspheres

PCEG and PCEL microspheres were prepared by an oil-in-water (o/w) emulsion and solvent-evaporation technique.[32] First, 300 mL of 1% (w/v) poly(vinyl alcohol) (PVA) (Beijing Chemical Plant) aqueous solution containing 0.05% (w/v) Tween-60 was stirred at room temperature to form aqueous phase. Then 6 mL of (300 mg) polymer solution in dichloromethane was poured into the aqueous phase to form an emulsion under stirring. After the emulsion was stirred at 1200 rpm for 8 h to remove the solvent at room temperature, the formed microspheres were collected by centrifugation and were washed five times with distilled water. Finally, the product was obtained after lyophilization.

Characterization Methods

Chemical Composition, Molecular Weight, and Inherent Viscosity. Chemical composition of PCEG was determined by ^{1}H NMR measurement using a Bruker DMX400 spectrometer at room temperature, $CDCl_3$ as solvent and tetramethylsilane as internal reference. Molecular weight of PCEG was determined by GPC measurement using a Waters 510 high performance liquid chromatography through a Shodex GPC KF-800 series column at 35°C, while polystyrene samples were used as standard and chloroform was as eluent at a flow rate of 1.0 mL min^{-1}. The viscosity of the copolymer at a concentration of 0.5 g dL^{-1} in chloroform was determined at 30°C ± 0.5°C, and then inherent viscosity of the copolymer was calculated from η_r by the "One-Point Method" and expressed in dL g^{-1}:

$$[\eta]=[2(\eta_{sp}-\ln\eta_r)]^{1/2}/C.$$

Crystallization and Thermal Property. The crystallization of PCEG was analyzed by X-ray diffraction measurement, which was carried out by a Rigaku Dmax-3B X-ray diffractometer equipped with a graphite monochromator-filtered Cu Kα radiation. The X-ray tube worked at 40 kV and 200 mA. The thermal property of PCEG was determined by differential scanning calorimetry (DSC) measurement, which was carried out on a differential scanning calorimeter (Diamond). Thermogram covering a range of −100 to 200°C was recorded at a heating rate of 10°C min^{-1}, to give the melting temperature (T_m), the glass transition temperature (T_g), and ΔH.

Contact Angle and Water Sorption. PCEG films for contact angle measurement was prepared by a solution casting method using 8 wt % solution of PCEG in a chloroform into a poly(tetrafluoroethylene) (PTFE) mould. After solvent evaporation in air at room temperature, the film was removed from the mould and

Table I. Characterizations of PCEG Copolymers

Sample	[C]/[EG]/[G][a] (molar ratio)	M_w/M_n[b] ($\times 10^{-4}$)	$[\eta]$[c] dL g^{-1}	Contact angle (deg)	Water sorption (wt %)
PCEG(30/8/62)(10,000)	29.8/8.4/61.8	4.8/3.6	1.86	69.7 ± 2.2	10.1 ± 0.3
PCEG(28/16/56)(10,000)	27.9/15.8/56.3	5.0/3.9	1.75	59.5 ± 1.1	33.9 ± 1.0
PCEG(25/25/50)(10,000)	25.5/24.9/49.6	4.5/2.7	1.35	45.8 ± 1.2	67.5 ± 1.8
PCEG(23/30/47)(10,000)	22.9/30.5/46.6	5.2/3.7	1.19	42.9 ± 1.8	72.8 ± 4.7
PCEG(28/16/56)(4000)	28.0/15.7/56.3	4.8/3.3	1.07	54.1 ± 3.2	42.4 ± 2.7
PCEG(52/16/32)(10,000)	52.1/15.7/32.2	4.7/3.4	1.32	63.0 ± 1.9	42.3 ± 0.5
PCEL(28/16/56)(10,000)	27.5/16.2/56.3	4.6/3.0	1.59	90.7 ± 2.8	21.4 ± 2.7
PCEG(71/16/13)(10,000)	71.5/15.6/12.9	4.3/7.4	1.41	66.1 ± 1.2	17.3 ± 1.2
PCEG(52/24/24)(10,000)	52.1/23.8/24.1	2.9/4.9	1.05	45.8 ± 2.6	67.5 ± 3.6

[a] Calculated from ^{1}HNMR measurement.
[b] Determined by GPC measurement using polystyrenes as standard.
[c] Calculated by viscosity measurement using chloroform as solvent in concentration of 0.5 g dL^{-1} at 30°C.

dried under vacuum at room temperature for 3 days until constant weight films with thickness of 0.30 mm were obtained. Contact angles to water of the PCEG films were measured on air surface of the films using a FACE CA-D type contact angle meter (Kyowa Kaimenkagaku). The measuring time for every datum was within 10 s, and 10 data were averaged. Some other PCEG films were weighed and then were put in a beaker containing 50 mL of deionized water at 37°C ±1°C. After 3 days the films were taken out and weighed again. Water sorption of the film was calculated as follows:

$$\text{Water sorption } (\%) = [(W_t - W_0)/W_0] \times 100$$

where W_t and W_0 were the weight of water-saturated film and the dried film, respectively. The data were expressed as means ± standard deviations (SD) ($n = 3$ and 10 for water sorption and contact angle, respectively).

Degradation Test *In Vitro*. The PCEG film with thickness of 0.2–0.3 mm was prepared by the similar procedure as aforementioned method, and cut into similar sized small specimens. Then the films were weighed (about 300 mg) and separately immersed in individual test-tube with 15 mL of pH 7.4 phosphate buffer solution (PBS) including 0.05% NaN_3 to prevent bacterial growth. Degradation test of PCEG *in vitro* was performed in a thermo-stated shaking water bath with constant shaking at 37°C ±1°C and buffer solution was renewed every week. At predetermined interval the specimen was taken out and rinsed three times by deionized water. Finally, it was dried to constant weight by freeze-drying. The morphology of specimen was observed by SEM and weight loss of it was calculated as following:

$$\text{Mass loss } (\%) = (W_0 - W_d)/W_0 \times 100$$

where W_0 and W_d represent the initial and dried weight of the degraded specimen, respectively. Here, three samples were measured for each material at each time point.

Scanning Electron Microscopy. PCEG and PCEL microspheres were well dispersed in distilled water and dropped onto a piece of silicon sheet and were freeze-dried. Then the microspheres and immersed films were coated by gold and observed with a Hitachi S-4300 scanning electron microscope (SEM).

Cell Culture and Observation

Mouse NIH 3T3 fibroblasts were supplied by the Chinese Academy of Military Medical Sciences. The cells were incubated at 37°C in DMEM (Gibco) supplemented with 10% FBS (Gibco) and 100 U cm^{-3} each of penicillin and streptomycin in a 5% CO_2 incubator. When the cells had grown to confluence, they were detached by trypsin/EDTA (0.05% w/v/trypsin/0.02% EDTA) (Sigma) and seeded onto PCEG films and microspheres.

Various PCEG films were cut into disks with a diameter of 15 mm and placed in a 24-well culture plate. After the films were irradiated by ultraviolet light for 1 h, cells were seeded at a density of 4–5 $\times$ 10^4 cells/well and cultured for 6 h. Then the culture medium was removed and the films were rinsed with PBS for three times in order to remove the unattached cells. The attached cells on the films were digested by trypsin/EDTA and the number of cells in the wells was counted. Finally, cell attachment efficiency was calculated according to the following formula: Cell attachment efficiency (%) = $100(N_1/N_0)$, where N_1 and N_0 are the number of attached cells and seeded cells, respectively.

About 10 mg of dry PCEG microspheres were soaked into PBS (pH 7.4) for 1 h to make the microspheres disperse well. After removing PBS, the PCEG microspheres were irradiated by ultraviolet light for 1 h. Then the microspheres were mixed with 100,000 of NIH 3T3 fibroblasts containing 500-μL culture media and transplanted into a glass flask, which was previously siliconized. The media were changed once every 2 days.

After NIH 3T3 fibroblasts had been cultured for 6 h on various microspheres respectively, the cell attachment efficiency of various microspheres was evaluated by the similar method as mentioned above.

After NIH 3T3 fibroblasts had been cultured for 7 days on microspheres, the microspheres were washed with PBS and fixed with 2.5% glutaraldehyde at 4°C for 24 h. And then the microspheres were dried and sputter-coated with gold. Finally, the

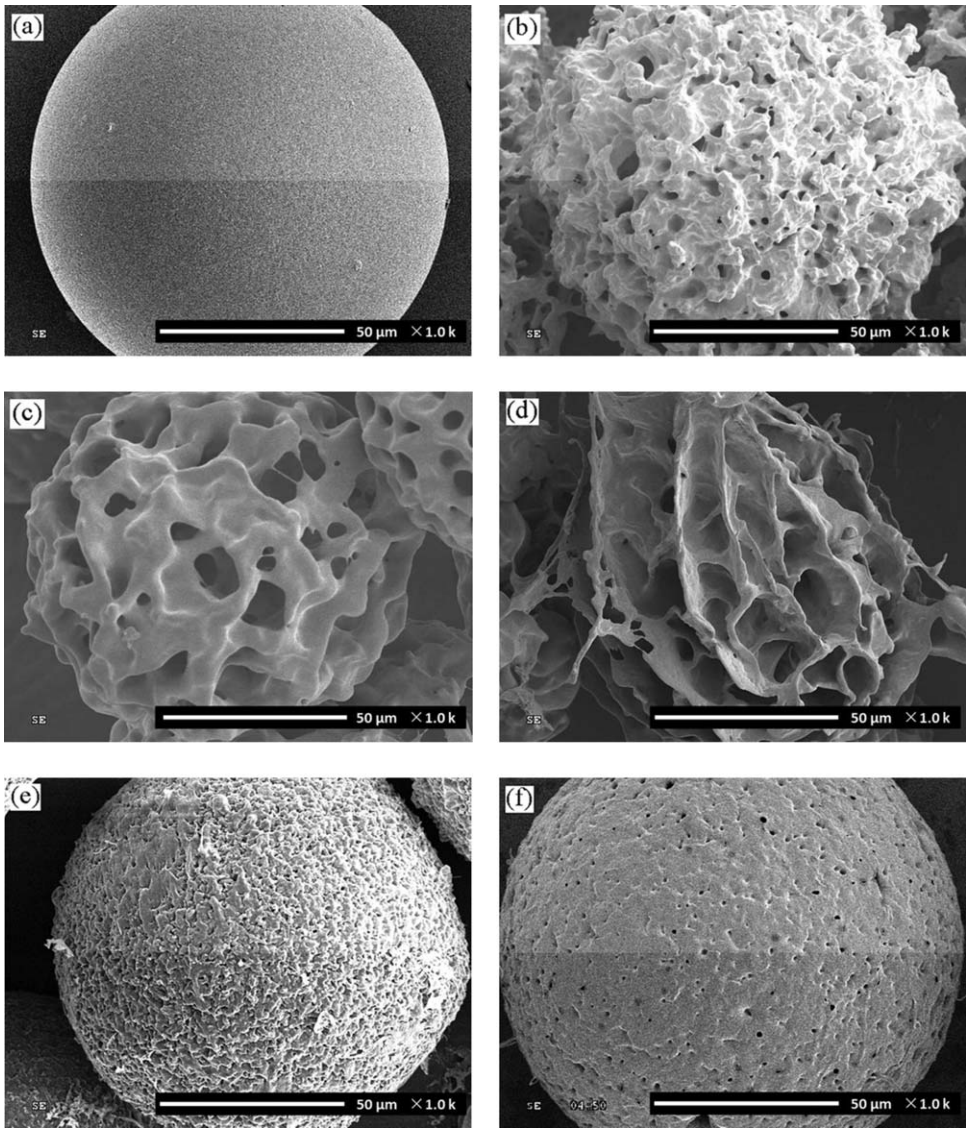

Figure 1. Effect of content and length of PEO segment on morphology of PCEG microspheres. (a) PCEG(30/8/62)(10,000); (b) PCEG(28/16/56)(10,000); (c) PCEG(25/25/50)(10,000); (d) PCEG(23/30/47)(10,000); (e) PCEG(70/15/15)(10,000); (f) PCEG(28/16/56)(4000).

morphology of the cells on microspheres was observed by SEM (Hitachi S-4300, Japan).

Viability and proliferation of 3T3 fibroblasts were determined by MTT assay after cultured on different microspheres for 1, 4, 7, and 10 days, respectively. At each predetermined interval, after the original culture medium was removed, 400 μL of fresh culture medium was added to each glass flask, and followed by incubation at 37°C and 5% CO_2 for 4 h. The upper medium was removed carefully and the intracellular formazan was dissolved by adding 400 μL of 0.04 mol L^{-1} HCl/iso-propanol to each glass flask. The absorbance of produced formazan was measured at 570 nm with micro-plate reader (ZS-2, Beijing).

RESULTS AND DISCUSSION

Two series of PCEG copolymers were synthesized. One was the polymers composed of similar molecular weight of PEG (4000 or 10,000) but molar ratio of glycotyl (G) to caprolactyl (C) to ethylene glycol (EG) was changed, the other had similar molar ratio of [G] to [C] to [EG] but different molecular weight of PEG (4000 and 10,000). The results obtained from Table I indicated that composition of all produced PCEG copolymers was basically consistent with components ratio of the feeding dose. It meant that composition ratio of the PCEG could be easily controlled by adjusting the feeding dose of copolymerization.

Morphology of PCEG Microspheres

According to the method described previously,[23,38] a series of PCEG microspheres were made from PCEGs with different content and length of PEG segment. To study effect of PEG on morphology of PCEG microspheres, molar ratio of [C]/[G] remained the same. It could be seen that morphology structure of the microspheres closely depended on composition of the polymer. In the case of PCEG composed of lower PEG content (<10%), the produced microspheres had a tight surface, as shown in Figure 1(a). With PEG content increasing to 16%, surface morphology

Table II. Cell Attachment Efficiency on Films and Microshperes of PCEG Copolymers

Polymer	Film (%)	Microspheres (%)
PCEG(70/15/15)(10,000)	45.2 ± 2.0	56.6 ± 2.1
PCEG(28/16/56)(10,000)	44.5 ± 1.6	60.3 ± 1.9
PCEG(25/25/50)(10,000)	38.0 ± 2.3	58.4 ± 1.5
PCEG(28/16/56)(4,000)	44.6 ± 2.6	52.2 ± 1.2

of the produced microspheres changed to rough and porous [Figure 1(b)]. When PEG content increased to 25%, highly porous microspheres with well interpenetrating pores, whose diameter reached to about 10–20 μm, could be obtained [Figure 1(c)]. However irregular structured microspheres would be formed when PEG content reached to 30%, as shown in Figure 1(d).

On the other hand, it could be also found that the length of PEG segment had also an effect on morphology structure of the PCEG microspheres. In the case of PCEG composed of similar composition but different length of PEG segment (4000 and 10,000), pore size and surface roughness of the microspheres increased with the length of PEG segment increasing, as shown in Figure 1(b,f).

Because hydrophilicity of a polymer has great influence on morphology structure of its microspheres and degradation behavior, surface and bulk hydrophilicities of the PCEG were identified respectively by measurements of contact angle to water and water sorption, as summed in Table I. The contact angle reduced from 69.7° to 42.9° and water sorption increased from 10.1% to 72.8% with $PEG_{10,000}$ content increasing from 8 to 30 mol %. It could be seen from the results that when the PCEG polymer was composed of similar segment length of PEG, its surface and bulk hydrophilicity had increased with increasing PEG content. On the other hand, the hydrophilicity of PCEG with similar composition had reduced with increasing segment length of PEG. For example, the contact angle of PCEG(28/16/56) increased from 54.1 to 59.5° and water sorption reduced from 42.4 to 33.9% with segment length of PEG increasing from 4000 to 10,000, respectively.

Above results suggested that different morphologies of the microspheres had been produced by different chemical composition and hydrophilicity of the polymer. With hydrophilicity of the polymer increasing, morphology of the produced microspheres changed from smooth to porous to irregular. This result was also proven by comparing of PCEG and PCEL microspheres, as shown in Supporting Information (Figure S1). It was considered that during formation of the microspheres, with solidification of PCEG in the aqueous phase, the hydrophilic PEG segment had a tendency to orientate to aqueous phase and swelled in the aqueous phase. As a result, pore-structured microspheres were formed after the water saturated microspheres were dried by lyophilization under vacuum. The mechanism is similar with the formation of porous structure of PCEL microspheres.[32] So the more PEG content of PCEG was, the bigger pore-size of porous microspheres was. However, if the hydrophilicity of the PCEG was too high, swelling of the microspheres in the aqueous phase would be too big to form regular pores and finally an irregular structure had been formed. The exact example was the PCEG(23/30/47)(10,000) microspheres, as shown in Figure 1(d).

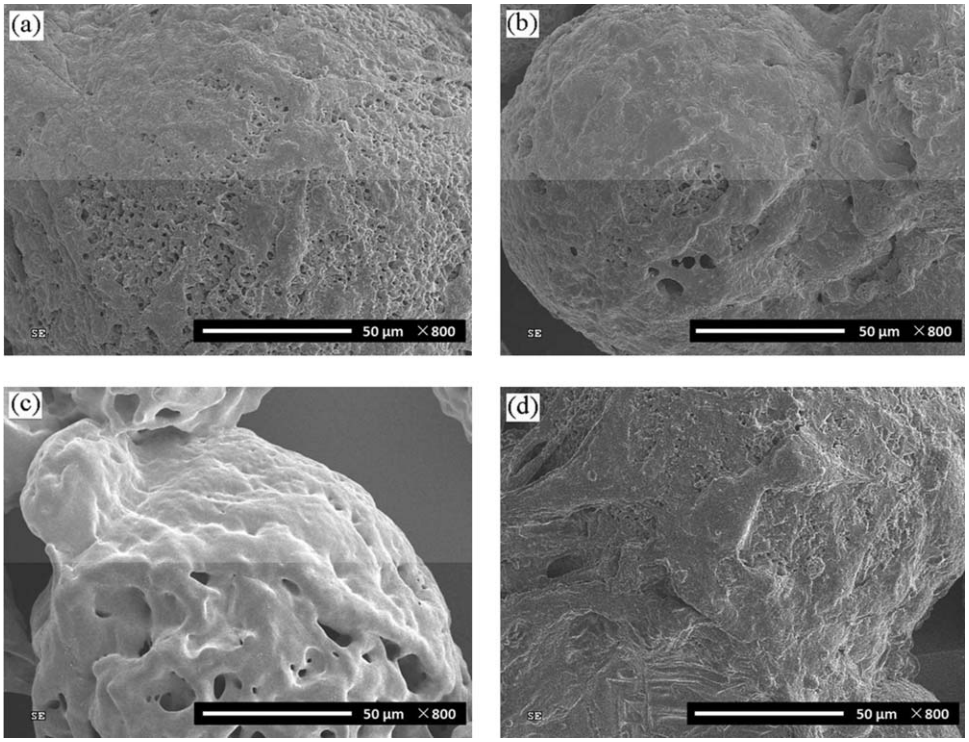

Figure 2. Morphology of NIH 3T3 fibroblasts on different PCEG microspheres cultured for 7 days (×800). (a) PCEG(70/15/15) (10,000); (b) PCEG(28/16/56)(10,000); (c) PCEG(25/25/50)(10,000); (d) PCEG(28/16/56)(4000).

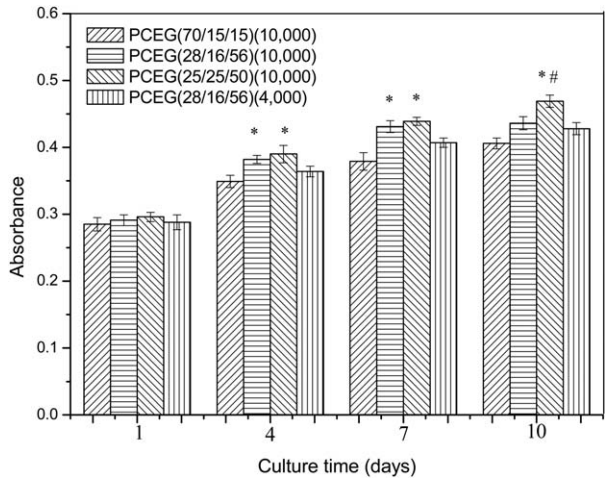

Figure 3. MTT-tetrazolium assay of NIH 3T3 fibroblasts cultured on various PCEG microspheres within different period. The values represent the mean ± standard deviation ($n = 4$). $^{*}P < 0.05$; significant against the proliferation and viability of NIH 3T3 fibroblasts on PCEG(70/15/15)(10,000) and PCEG(28/16/56)(4000) microspheres at the corresponding day. $^{\#}P < 0.05$; significant against the proliferation and viability of NIH 3T3 fibroblasts on PCEG(28/16/56)(10,000) microspheres at the corresponding day.

The above results revealed that the porous structure of the PCEG microspheres depended on both elements of content and length of the PEG segment. The more the PEG content of PCEG and the longer PEG segment, the bigger the pore-size of porous microspheres. Therefore, various porous structured PCEG microspheres could be fabricated by controlling composition of the PCEG polymer.

Cell Affinity of PCEG Microspheres

Influence of composition on cell affinity of PCEG microspheres was studied by mouse NIH 3T3 fibroblasts culture. The attachment, morphology, and proliferation of NIH 3T3 fibroblasts cultured on various microspheres were investigated and compared.

Table II showed cell attachment efficiency of various PCEG films and microspheres. It could be seen that cell attachment efficiency on various PCEG microspheres was higher than that on the relative PCEG films. On the other hand, the cell attachment efficiency on PCEG film decreased with PEG content increasing, but there was no statistic difference on the PCEG microspheres. Moreover, for PCEG microspheres it increased with molecular weight of PEG segment increasing when composition of the PCEG was same.

Figure 2 showed morphology of NIH 3T3 fibroblasts cultured for 7 days on various PCEG microspheres. It could be seen that the cells exhibited different shape on the microspheres. Height of the cells on the PCEG(28/16/56)(10,000) and PCEG(25/25/50)(10,000) microspheres [Figure 2(b,c)] was lower than that on the PCEG(70/15/15)(10,000) and PCEG(28/16/56)(4,000) microspheres [Figure 2(a,d)], which meant the cells much tightly anchored to the PCEG(28/16/56)(10,000) and PCEG(25/25/50)(10,000) substrate. After culturing for 7 days NIH 3T3 fibroblasts proliferated promptly on the PCEG(28/16/56)(10,000) and almost covered whole surface of the microshperes, even some cells on the PCEG(25/25/50)(10,000) microspheres spread into big pores [Figure 2(c)].

Proliferation and viability of NIH 3T3 fibroblasts on various microspheres was determined by MTT assay after culturing for 1, 4, 7, and 10 days, as shown in Figure 3. It could be seen that after culturing for 1 day, the proliferation and viability of NIH 3T3 fibroblasts on various PCEG microspheres were not statistically different. However, after culturing for 4 and 7 days the proliferation and viability of the cells on PCEG(28/16/56)(10,000) and PCEG(25/25/50)(10,000) was higher than that on PCEG(70/15/15)(10,000) and PCEG(28/16/56)(4,000) microspheres. Moreover, after culturing for 10 days the cells showed the highest viability and the rapidest proliferation on PCEG(25/25/50)(10,000).

These results revealed that the composition and structure of PCEG microspheres had an important effect on cell attachment and growth. Because PEG was not good for cell adhesion,[48] cell attachment of PCEG microspheres should be worse than that of films. However, because rough surface or porous structure would be favorable for cells attachment and growth,[49–52] the cell attachment on rough and porous microspheres was better than that on the dense films. As a result, although PEG content on surface of the PCEG microspheres was higher, the disadvantage could be overcome by increasing roughness and porous structure of the microspheres. In addition, if the high level of PEG weakened cell adhesion, which could be improved by loading bioactive substances such as growth factors into the microspheres.[53–55]

Therefore, cell attachment and growth of PCEG microspheres were better than that of PCEG films. Moreover, cell growth on porous PCEG microspheres was faster than on the non-porous PCEG microspheres. This could be attributed to hydrophilicity and large interconnected pores of PCEG microspheres, which allowed cells and culture medium to penetrate more easily into the microshperes and provided more space for cell attachment and growth. Therefore, the porous PCEG microspheres

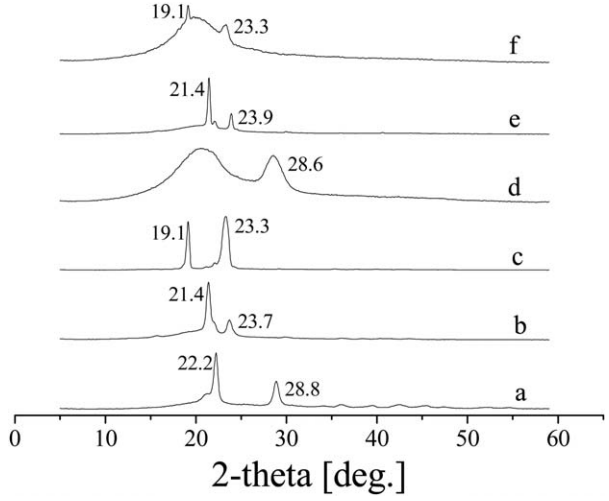

Figure 4. X-ray diffraction spectra of PCEG copolymers and their components. (a) PGA; (b) PCL; (c) PEG_{10000}; (d) PCEG(28/16/56)(10,000); (e) PCEG(71/16/13)(10,000); (f) PCEG(52/16/32)(10,000).

Table III. Thermal Property of PCEG Copolymers

Samples	T_g (°C)	ΔH_m (J g^{-1})
PCEG(28/16/56)(10,000)	−21.79	16.00
PCEG(52/16/32)(10,000)	−40.41	3.24
PCEG(71/16/13)(10,000)	−59.08	47.17
PCEG(52/24/24)(10,000)	−43.74	20.74

would be a kind of potential injectable scaffold used in tissue engineering.

Crystallinity of PCEG

Because degradability of a polymer depended on hydrophilicity and crystallinity of it, the crystallinity of polymers was studied by X-ray diffraction measurements, as shown in Figure 4. Some thermal data of the PCEG polymers determined by DSC measurement were summarized in Table III and Figure 5. The results indicated glass transition temperature of polymers significantly decreased with C component of them increasing. All of the PCEG copolymers and homopolymers of PGA, PCL, and PEG showed crystalline patterns, which meant that the PCEG copolymer has a certain degree of crystallinity. However, further comparing the X-ray diffraction spectra it could easily be found that spectrum of the PCEG polymer strongly was affected by the main component of the polymer. For example PCEG (28/16/56)(10,000) (curve d) and PCEG(71/16/13)(10,000) (curve e) displayed major peaks at $2\theta = 28.6°$ that was similar with PGA crystalline patterns (curve a) and major peaks at $2\theta = 21.4°$ and 23.9° that was like PCL crystalline patterns (curve b), respectively. However, for PCEG(52/16/32)(10,000) (curve f) although it was still composed of a large amount of C content (50%), lower crystallinity of the polymer indicated that crystalline patterns was greatly affected by the PEG segment. The difference of the crystalline pattern may be the reason why water sorption of PCEG(52/16/32)(10,000) (42.4%) was higher than that of PCEG(28/16/56) (10,000) (33.9%).

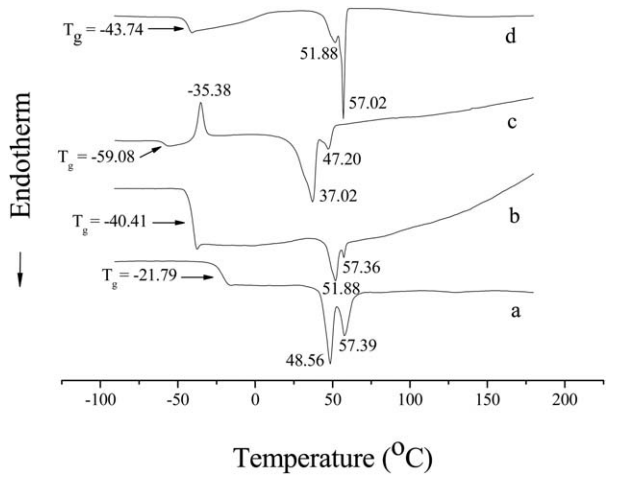

Figure 5. DSC spectra of PCEG copolymers. (a) PCEG(28/16/56)(10,000); (b) PCEG(52/16/32)(10,000); (c) PCEG(71/16/13)(10,000); (d) PCEG(52/24/24)(10,000).

The results indicated that the physical properties of the polymers including glass transition temperature and crystallinity were more similar to that of monomers with high content. Polymer with different properties could be prepared by adjusting its components.

Mass Loss and Morphology Change of PCEG

Degradability is an important factor for tissue engineering scaffolds. Biomaterials with different degradation rate are needed for repair of tissue defects of different types and positions. So mass loss and morphology change of the PCEG films were determined by immersion in PBS *in vitro* at 37°C ± 1°C. It was observed that physical appearance of all the PCEG films changed from initially translucent to whitish during the immersion. For PCEG(28/16/56)(10,000), it had broken into a few pieces after 10 weeks, and then more smaller bits with time increasing. Within the period molecular weight of the PCEG(28/16/56)(10,000) had changed from 50,000 to 33,000. However, molecular weight of PCEG(52/16/32)(10,000) decreased from 47,000 to 34,000.

The change of mass of the polymer during the immersion was also determined. We could see from Figure 6 that the mass loss of PCEG(52/16/32)(10,000) within the first 2 weeks was 4.1% and at the end of 24 weeks was 45.4%, but that of PCEG(28/16/56)(10,000) had greatly increased from 17.8 to 90.2%, respectively. It was clear that mass loss of PCEG(28/16/56)(10,000) was much faster than that of PCEG(52/16/32)(10,000).

From Table III it is easy to see that crystallinity of PCEG(28/16/56)(10,000) was much higher than that of PCEG(52/16/32)(10,000). According to the usual rule that degradation rate decreased with the increasing crystallinity of the copolymers with similar structure.[56] However, the result was just an opposite. We think the reason is hydrophilicity of PCEG(52/16/32)(10,000) was worse than PCEG(28/16/56)(10,000) because of effect of more hydrophobic PCL. The water difficultly permeated into the polymer and degradation reaction difficultly performed. It revealed that hydrophilicity had greater effect on degradation of the PCEG copolymer than crystallinity.

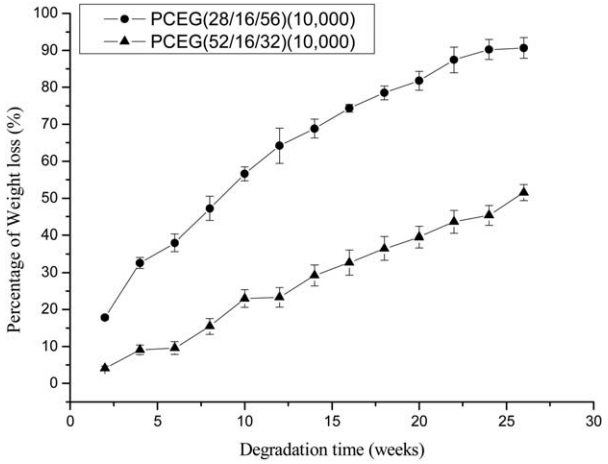

Figure 6. Weight loss of PCEG copolymer films after immersion in the PBS. The values represent the mean ± standard deviation ($n = 3$).

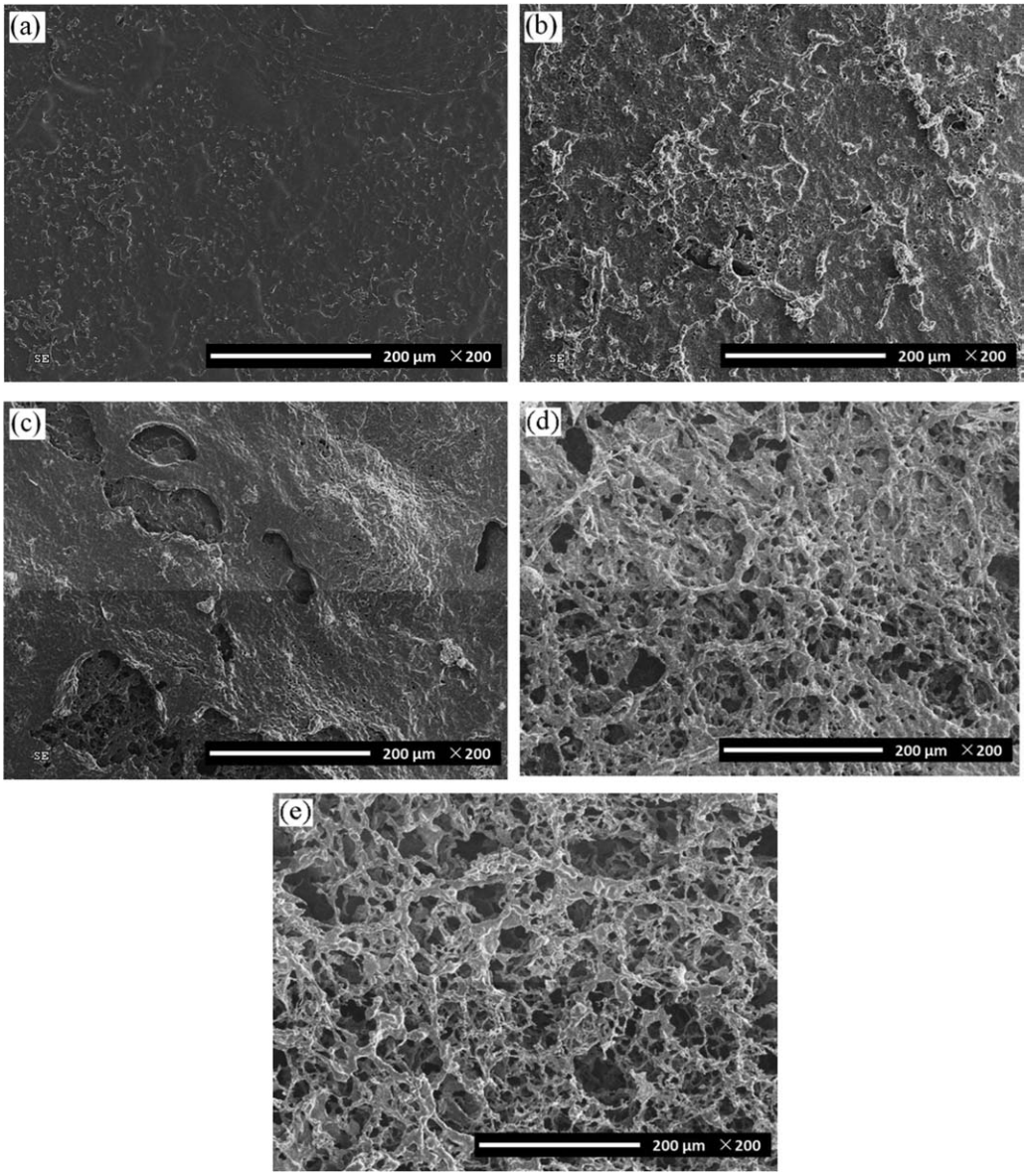

Figure 7. The SEM micrographs of PCEG(28/16/56)(10,000) film after different weeks of immersion in the PBS. (a) 2 w; (b) 6 w; (c) 12 w; (d) 18 w; (e) 24 w.

Further comparing PCEG(28/16/56)(10,000) and PCEL (28/16/56)(10,000), although both polymers had similar molar ratio of [C]/[EG]/[G] and [C]/[EG]/[L], the mass loss rate of PCEG(28/16/56)(10,000) was much faster than that of PCEL(28/16/56)(10,000), as shown in Figure 6 and Supporting Information Figure S2. Comparing hydrophilicity of them, it also could be seen from Table I that the contact angle and water sorption of PCEG(28/16/56)(10,000) were 59.5° and 33.9% respectively, but for that of PCEL(28/16/56)(10,000) were 90.7° and 21.4% respectively. So the main reason might be that PGA has better hydrophilicity and faster degradation rate than that of PLA.

The change of surface morphology of PCEG(28/16/56)(10,000) and PCEL(28/16/56)(10,000) films with immersing time was also different, as shown in Figure 7 and Supporting Information Figure S3, respectively. It could be seen that with increasing immersing time the surface morphology of PCEG(28/16/56)(10,000) film (Figure 7) has become rougher and more porous. Some pores could be found on the surface after 6 weeks, and amount and size of the pores greatly increased with immersing time. But for PCEL(28/16/56)(10,000) film no pore could be found on the surface even after 24 weeks of immersion (Supporting Information Figure S3). The result demonstrated the degradation rate of PCEG(28/16/56)(10,000) was much faster than that of PCEL(28/16/56)(10,000).

The above results revealed that mass loss rate of different polymers were greatly influenced by hydrophobicity. Besides, replacing lactide with glycolide can effectively improve degradation rate of the produced copolymer, which provided tissue engineering scaffold materials with faster degradation.

CONCLUSION

By copolymerization of GA, CL, and PEG, a series of PCEG copolymers with different composition were synthesized and PCEG microspheres were fabricated by oil-in-water (o/w) emulsion and solvent-evaporation technique. Morphology structure of the microspheres was greatly affected by chemical composition and hydrophilicity of the PCEG polymer. With PEG content increasing from 8 to 30%, the morphology structure of PCEG microspheres changed from smooth to a porous structure and until an irregular structure. The hole size of the microspheres increased with segment length and content of the PEG increasing. The pore-size of PCEG microspheres was bigger than that of PCEL when both of them were composed of similar EG and C content. On the other hand, degradation rate of PCEG *in vitro* was much faster than that of PCEL. The cell affinity of porous PCEG microspheres was better than that of nonporous PCEG microspheres. The PCEG microspheres with rapid degradation and large porous structure could be fabricated by adjusting the composition of copolymer, which would be a potential injectable scaffold for tissue engineering.

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REFERENCES

1. Mooney, D. J.; Park, S.; Kaufmann, P. M.; Sano, K.; Mcnamara, K.; Vacanti, J. P.; Langer, R. *J. Biomed. Mater. Res.* **1995**, *29*, 959.
2. Tabata, Y.; Miyao, M.; Yamamoto, M.; Ikada, Y. *J. Biomater. Sci. Polym. E* **1999**, *10*, 957.
3. Jonnalagadda, J. B.; Rivero, I. V. *J. Mech. Behav. Biomed.* **2014**, *40*, 33.
4. Mikos, A. G.; Sarakinos, G.; Lyman, M. D.; Ingber, D. E.; Vacanti, J. P.; Langer, R. *Biotechnol. Bioeng.* **1993**, *42*, 716.
5. Huang, R.; Zhu, X. M.; Tu, H. Y.; Wan, A. *Mater. Lett.* **2014**, *136*, 126.
6. Thadavirul, N.; Pavasant, P.; Supaphol, P. *J. Biomed. Mater. Res. A* **2014**, *102*, 3379.
7. Mikos, A. G.; Thorsen, A. J.; Czerwonka, L. A.; Bao, Y.; Langer, R.; Winslow, D. N.; Vacanti, J. P. *Polymer* **1994**, *35*, 1068.
8. Jung, S. H.; Jang, J. W.; Kim, S. H.; Hong, H. H.; Oh, A. Y.; Rhee, J. M.; Kang, Y. S.; Khang, G. *Tissue Eng. Regen. Med.* **2008**, *5*, 643.
9. Grant, N.; Zhang, H. F. *J. Colloid. Interfaces Sci.* **2011**, *356*, 573.
10. Ho, M. H.; Kuo, P. Y.; Hsieh, H. J.; Hsien, T. Y.; Hou, L. T.; Lai, J. Y.; Wang, D. M. *Biomaterials* **2004**, *25*, 129.
11. Harris, L. D.; Kim, B. S.; Mooney, D. J. *J. Biomed. Mater. Res.* **1998**, *42*, 396.
12. Kim, T. K.; Yoon, J. J.; Lee, D. S.; Park, T. G. *Biomaterials* **2006**, *27*, 152.
13. Park, A.; Wu, B.; Griffith, L. G. *J. Biomater. Sci. Polym. E* **1998**, *9*, 89.
14. Akbarzadeh, R.; Yousefi, A. M. *J. Biomed. Mater. Res. B* **2014**, *102*, 1304.
15. Collins, M. N.; Birkinshaw, C. *Carbohydr. Polym.* **2013**, *92*, 1262.
16. Liu, X. H.; Ma, P. X. *Biomaterials* **2009**, *30*, 4094.
17. Hu, X. X.; Shen, H.; Yang, F.; Bei, J. Z.; Wang, S. G. *Biomaterials* **2008**, *29*, 3128.
18. Garrigues, N. W.; Little, D.; Sanchez-Adams, J.; Ruch, D. S.; Guilak, F. *J. Biomed. Mater. Res. A* **2014**, *102*, 3998.
19. Xin, X. J.; Hussain, M.; Mao, J. J. *Biomaterials* **2007**, *28*, 316.
20. Bashur, C. A.; Dahlgren, L. A.; Goldstein, A. S. *Biomaterials* **2006**, *27*, 5681.
21. Yang, F.; Qu, X.; Cui, W. J.; Bei, J. Z.; Yu, F. Y.; Lu, S. B.; Wang, S. G. *Biomaterials* **2006**, *27*, 4923.
22. Chen, C.; Yu, C. H.; Cheng, Y. C.; Yu, P. H. F.; Cheung, M. K. *Biomaterials* **2006**, *27*, 4804.
23. Hong, Y.; Gao, C. Y.; Xie, Y.; Gong, Y. H.; Shen, J. C. *Biomaterials* **2005**, *26*, 6305.
24. Lerouxel, E.; Weiss, P.; Giumelli, B.; Moreau, A.; Pilet, P.; Guicheux, J.; Corre, P.; Bouler, J. M.; Daculsi, G.; Malard, O. *Biomaterials* **2006**, *27*, 4566.
25. Liu, Y. F.; Huang, K. L.; Peng, D. M.; Liu, S. Q.; Wu, H. *J. Polym. Sci. Polym. Chem.* **2007**, *45*, 2152.
26. Qutachi, O.; Vetsch, J. R.; Gill, D.; Cox, H.; Scurr, D. J.; Hofmann, S.; Muller, R.; Quirk, R. A.; ShakeSheff, K. M.; Rahman, C. V. *Acta Biomater.* **2014**, *10*, 5090.
27. Virto, M. R.; Elorza, B.; Torrado, S.; Elorza, M. D. A.; Frutos, G. *Biomaterials* **2007**, *28*, 877.
28. Jaklenec, A.; Wan, E.; Murray, M. E.; Mathiowitz, E. *Biomaterials* **2008**, *29*, 185.
29. Ashton, R. S.; Banerjee, A.; Punyani, S.; Schaffer, D. V.; Kane, R. S. *Biomaterials* **2007**, *28*, 5518.
30. Wang, Z. X.; Wang, S. G.; Marois, Y.; Guidoin, R.; Zhang, Z. *Biomaterials* **2005**, *26*, 7387.
31. Pistel, K. F.; Bittner, B.; Koll, H.; Winter, G.; Kissel, T. *J. Control Release* **1999**, *59*, 309.
32. Li, G. M.; Cai, Q.; Bei, J. Z.; Wang, S. G. *Polym. Adv. Technol.* **2002**, *13*, 636.
33. Stanek, L. G.; Heilmann, S. M.; Gleason, W. B. *Carbohydr. Polym.* **2006**, *65*, 552.
34. Wang, S. G.; Cui, W. J.; Bei, J. Z. *Anal. Bioanal. Chem.* **2005**, *381*, 547.
35. Li, X.; Liu, K. L.; Li, J.; Tan, E. P. S.; Chan, L. M.; Lim, C. T.; Goh, S. H. *Biomacromolecules* **2006**, *7*, 3112.
36. Lang, M. D.; Bei, J. Z.; Wang, S. G. *J. Biomater. Sci. Polym. E* **1999**, *10*, 501.
37. Hu, Y.; Jiang, X. Q.; Ding, Y.; Zhang, L. Y.; Yang, C. Z.; Zhang, J. F.; Chen, J. N.; Yang, Y. H. *Biomaterials* **2003**, *24*, 2395.
38. Bae, Y. H.; Huh, K. M.; Kim, Y.; Park, K. H. *J. Control Release* **2000**, *64*, 3.

39. Ben-Shabat, S.; Kumar, N.; Domb, A. J. *Macromol. Biosci.* **2006**, *6*, 1019.
40. Ohya, Y.; Nakai, T.; Nagahama, K.; Ouchi, T.; Tanaka, S.; Kato, K. *J. Bioact. Compat. Polym.* **2006**, *21*, 557.
41. Xie, L.; Xu, H.; Wang, Z. P.; Li, X. J.; Chen, J. B.; Zhang, Z. J.; Yin, H. M.; Zhong, G. J.; Lei, J.; Li, Z. M. *J. Polym. Res.* **2014**, *21*, 357.
42. Gilchrist, S. E.; Rickard, D. L.; Letchford, K.; Needham, D.; Burt, H. M. *Mol. Pharmaceut.* **2012**, *9*, 1489.
43. Hu, X. X.; Shen, H.; Yang, F.; Liang, X. J.; Wang, S. G.; Wu, D. C. *Appl. Surf. Sci.* **2014**, *292*, 764.
44. Singhvi, R.; Stephanopoulos, G.; Wang, D. I. C. *Biotechnol. Bioeng.* **1994**, *43*, 764.
45. DeGroot, J. H.; Kuijper, H. W.; Pennings, A. J. *J. Mater. Sci. Mater. M* **1997**, *8*, 707.
46. Chen, D. R.; Chen, H. L.; Bei, J. Z.; Wang, S. G. *Polym. Int.* **2000**, *49*, 269.
47. Cai, Q.; Bei, J. Z.; Wang, S. G. *Polymer* **2002**, *43*, 3585.
48. Wan, Y. Q.; Chen, W. N.; Yang, J.; Bei, J. Z.; Wang, S. G. *Biomaterials* **2003**, *24*, 2195.
49. Goshima, J.; Goldberg, V. M.; Caplan, A. I. *Clin. Orthop. Relat. R* **1991**, *262*, 298.
50. Nakahara, H.; Goldberg, V. M.; Caplan, A. I. *Clin. Orthop. Relat. R* **1992**, *276*, 291.
51. Fewster, S. D.; Coombs, R. R. H.; Kitson, J.; Zhou, S. *Nanobiology* **1994**, *3*, 201.
52. Wan, Y. Q.; Wang, Y.; Liu, Z. M.; Qu, X.; Han, B. X.; Bei, J. Z.; Wang, S. G. *Biomaterials* **2005**, *26*, 4453.
53. Shen, H.; Hu, X. X.; Yang, F.; Bei, J. Z.; Wang, S. G. *Acta Biomater.* **2010**, *6*, 455.
54. Ho, M. H.; Hou, L. T.; Tu, C. Y.; Hsieh, H. J.; Lai, J. Y.; Chen, W. J. *Macromol. Biosci.* **2006**, *6*, 90.
55. Shen, H.; Hu, X. X.; Bei, J. Z.; Wang, S. G. *Biomaterials* **2008**, *29*, 2388.
56. Wang, S. G.; Chen, H. L.; Cai, Q.; Bei, J. Z. *Polym. Adv. Technol.* **2001**, *12*, 253.