

Biodegradable poly(L-lactide)-poly(ethylene glycol) multiblock copolymer: synthesis and evaluation of cell affinity

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Abstract

A series of poly(L-lactide)-poly(ethylene glycol) multiblock copolymers (Multi-PLE) with high molecular weight were synthesized and successfully used to fabricate three-dimensional scaffolds. Using mouse NIH 3T3 fibroblasts as model cells, the cell affinity of various Multi-PLE copolymers was evaluated and compared with that of poly(L-lactide) (PLLA) by means of cell attachment efficiency measurement, scanning electron microscopy observation and MTT assay. On one hand, the results showed that the cell attachment efficiency on Multi-PLE 4/1(4/1 refers to the molar ratio of lactidyl units to ethylene oxide units) films was close to that on PLLA film, however, the other Multi-PLE films exhibited much lower cell attachment efficiency than PLLA film, such as Multi-PLE 2/1 and Multi-PLE 1/1, which had higher PEG content. On the other hand, it was interesting to find that cell proliferation on Multi-PLE4/1 and Multi-PLE2/1 scaffolds was better than that on PLLA scaffold, which was closely related to the improved hydrophilicity of Multi-PLE copolymers due to the incorporation of PEG in comparison with pure PLLA. The Multi-PLE copolymer scaffolds with appropriate hydrophilicity were in favor of mass transportation, and then of cell proliferation and cell affinity. It meant that the cell proliferation would be much improved by increasing the hydrophilicity of the three-dimensional scaffolds, which even outweighed the disadvantages of the cell attachment efficiency reduction with the incorporation of PEG.

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1. Introduction

The biodegradable polymer poly(L-lactic acid) (PLLA) is widely used to construct cell scaffolds for tissue engineering purposes due to its excellent biocompatibility, which can degrade into lactic acid and be eliminated from body by normal metabolic pathway [1]. To realize ideal tissue engineering, it is very important to obtain a sufficient mass of seeded cells and these cells should be uniformly distributed throughout the entire scaffold. However, since the polymer is hydrophobic, the cell suspension is inhibited from penetrating into the pores of the scaffolds [2]. Other transport issues, including nutrient delivery, waste removal, exclusion of materials or cells and protein transport, are also influenced disadvantageously by the hydrophobicity of the scaffolds.

One feasible method of enhancing the hydrophilicity of the PLLA scaffold is to modify the bulk properties of PLLA. Poly(ethylene glycol) (PEG) is often introduced into PLLA chain to improve the hydrophilicity of the PLLA. The diblock and triblock copolymers of PLLA/PEG (PLE) were prepared conveniently by ring opening polymerization of L-lactide in the presence of PEG and selected catalysts [3–8]. To date, there have been many reports focused on the synthesis and characterization of block copolymer of L-lactide and poly(ethylene glycol) [9–12] and on their biomedical applications [13,14]. However, such AB or ABA-type block copolymers have difficulty in getting a PLE copolymer with both high molecular weight and PEG content in case of short PEG blocks (e.g. $M_n < 10\,000$) being used due to the polymerization mechanism that polylactide polymerization is initiated by the hydroxyl end groups of PEG. Thus, it was suggested that multiblock copolymers constructed with alternated short PEG and PLA blocks might overcome this limitation [15–18]. Recently, Huh [16] and Lee et al. [17] had prepared PLLA/PEG

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multiblock copolymers. However, their synthesis methods were very complex and they failed to obtain high molecular weight PLLA/PEG multiblock copolymers with remarkable mechanical strength.

In a previous paper, we had developed a new method to prepare PLE multiblock copolymers with high molecular weight that the PLE triblock copolymers were coupled by succinic anhydride [19]. The resulted multiblock PLE copolymers have good mechanical strength, which enables them to be fabricated into cell scaffolds.

Compared to PLA scaffold, it is believed that the improved hydrophilicity of multiblock PLE should facilitate cell proliferation, however, PEG was reported the most common used compound to reduce the binding of proteins and the cell adhesion on material surface [20]. This contradictory impelled us to investigate the cell affinity of various Multi-PLE copolymers with different PEG content and different hydrophilicity thereof. The objective of this study is to fabricate Multi-PLE films or scaffolds and further evaluate the cell affinity by cell adhesion efficiency determination, SEM observation and MTT assay. The study should result in a better understanding of the relationship between the cell affinity of Multi-PLE copolymers and the content of PEG, and the results could be important in designing their potential applications in tissue engineering.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) (PEG) (CR grade, Tianjing Tiantai fine chemical corp., China) with number average molecular weights of 2000 and 4000 were used after being dried under vacuum for 4 h. L-Lactide (AR grade, PURAC, Netherland) was purified twice by re-crystallization from ethyl acetate. Succinic anhydride (AR grade, Tianjing Juqian fine chemical corp., China) was purified by re-crystallization from acetic anhydride. Chloroform and dichloromethane were dried with CaH_2 and distilled. Diethyl ether was dried over sodium and distilled. Ethyl acetate was dried with P_2O_5 overnight and then distilled. Stannous octoate, *N*-dimethylaminopyridine (DMAP) (AR grade, MERCK, Germany) and dicyclohexylcarbodiimide (DCC) were A.R. grade reagents and were used without further purification.

2.2. Preparation of triblock PLE copolymers

The triblock copolymers were synthesized from L-lactide and PEG ($M_n=2000, 4000$) using stannous octoate as a catalyst [21,22] under 140°C for 24 h. The

obtained raw product was purified by dissolution in chloroform and re-precipitated from diethyl ether, and then dried to a constant weight under vacuum. Measured by ^1H NMR, the molecular weight of this triblock copolymer was calculated.

2.3. Preparation of Multi-PLE copolymers

Triblock PLE copolymers and equimolar succinic anhydride were dissolved in anhydrous dichloromethane. Predetermined amounts of DCC were added to the solution as a coupling agent and DMAP as a catalyst. The mixture was stirred continuously for 48 h at room temperature. A white dicyclohexylurea (DCU) precipitate formed as a reaction byproduct. The precipitated DCU was filtered off. The reaction product in the filtrate was precipitated from diethyl ether, then filtered and dried under vacuum.

2.4. Preparation of film

Polymer films were prepared by casting 10 wt% dichloromethane solutions of polymers into polytetrafluoroethylene (PTFE) mold. After the solvent evaporated at room temperature, the films were removed from the mold and dried under vacuum to a constant weight at room temperature.

2.5. GEL permeation chromatography (GPC) measurement

Gel permeation chromatography (GPC) measurements were carried out on a Waters 510 apparatus equipped with Shodex GPC K-800 series columns. Chloroform was used as the eluent at a flow rate of 1.0 ml/min. Calibration of the molecular weight of the copolymer was based on polystyrene standards.

2.6. Mechanical property measurement

Tensile strength and elongations of the copolymers were measured on a Shinkch Testing Machine at a crosshead speed of 100 mm/min at room temperature. The gauge length and width of dumbbell-like specimens were 20 and 4 mm, respectively. Six specimens were tested for each sample, and then the tensile strength and elongations were averaged from the specimens broken in the middle.

2.7. Hydrophilicity analysis

Contact angles of polymer films were measured on a FACE CA-D Contact Angle Meter (Kyowa Kaimenkagaku Co.) with the determining time being within 10 s. Ten independent determinations at different sites were averaged. Deionized water was used for the measurement.

Water uptake of the copolymer was evaluated after the immersion of polymer films in distilled water for 72 h at room temperature and calculated using the following formula: water uptake (%) = $100(W_w - W_d)/W_d$, where W_d and W_w are the weight of the sample before and after being immersed in water, respectively.

2.8. Scaffold fabrication

Scaffolds were prepared by a solvent-casting and particulate-leaching technique. Polymers were dissolved in dichloromethane (10 wt%), and then the sieved salt (200–280 μm) was added into the polymer solution (porogen weight fraction, 91.7%). The produced mixture was cast into a foursquare PTFE mold ($5 \times 5 \text{ cm}^2$). 48 h were allowed for solvent evaporation, then the formed salt/polymer composite was immersed in 500 ml deionized water for 48 h at room temperature (the water was changed every 6 h) to leach out the salt, followed by air drying for 24 h and vacuum drying for 48 h to obtain the sponge-like scaffold [23]. The thickness of scaffold was 1.48 mm, and the produced scaffolds were stored in a desiccator under vacuum before use.

2.9. Cell culture

2.9.1. Preparation of cells

Mouse NIH 3T3 fibroblasts were supplied by the Chinese Academy of Military Medical Sciences. The cells were cultured in 50 ml cell culture flasks containing Dulbecco's Modified Eagles Medium (Gibco) buffered with *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), supplemented with 15% calf serum (Gibco) and 100 U/cm³ each of penicillin and streptomycin. The cell culture was maintained in a gas-jacket incubator equilibrated with 5% CO₂ at 37°C. When the cells had grown to confluence, the cells were digested by 1 ml 0.25% trypsin (Sigma) for 1–2 min, then 3 ml of culture medium was added to stop digestion, and the culture medium was aspirated to cause cell dispersion after counting the cells.

2.9.2. Cell attachment efficiency

PLLA and Multi-PLE films were cut into small disks (15 mm in diameter) with the aid of a cork borer and located into a 24 well cell culture plate (Costar, USA). All the disks were sterilized by Ultraviolet light for 2 h. 200 μl (about $3\text{--}5 \times 10^4$ cells) of cell suspension was evenly placed on the samples. The cell-seeded disks were maintained at 37°C under 5% CO₂ condition for 4 h [24]. The morphology of cell attachment was observed and photographed by invert light microscope (Olympus Optical Co., Ltd.). Subsequently, the culture medium was removed and then the samples were rinsed with PBS three times to remove any of the residual culture medium and unattached cells. After the attached cells

on the disks were digested by trypsin, the cell attachment efficiency was determined by counting the number of cells remaining in the wells. The cell attachment efficiency was expressed as means \pm standard deviations (SD) ($n = 3$ or 4).

2.9.3. Cell culture for SEM observation

PLLA and Multi-PLE porous scaffolds were cut into small pieces ($1 \times 2.5 \text{ cm}^2$), and all pieces were prewetted by 75% ethanol for 2 h, and ethanol was exchanged for an excess amount of phosphate buffered saline (PBS) [25]. The PBS in disks was removed with a pipette. The disks were then sterilized by Ultraviolet light for 2 h, and located in culture dishes 30 mm in diameter. Cell suspension (about 5×10^6 cells/ml) was seeded on the samples until the samples became saturated and then they were cultured for 4 h prior to the addition of 20 ml culture medium into the culture dish.

The cell-seeded scaffolds were replenished with fresh culture medium every second day. After being cultured for two weeks, the scaffolds were taken out from the culture plate and washed with PBS three times, then fixed with 3% glutaraldehyde in PBS for 24 h at 4°C. After through washing with PBS, the samples were dehydrated sequentially in 50%, 70%, 95%, 100% ethanol, each for 2×10 min. Then the fixed samples were freeze-dried, sputter-coated with gold and examined under a scanning electron microscope (Hitachi S-530).

2.9.4. MTT assay

The Multi-PLE and PLLA scaffolds were cut into small disks (7 mm in diameter) with the aid of a cork borer. The disks were placed into a 96 well tissue culture plate and sterilized using the method described above. A cell suspension (40 μl) with a cell density of 5×10^5 cells/disk was seeded evenly into the scaffolds with a micropipette. The cell-seeded scaffolds were maintained at 37°C under 5% CO₂ for 3 h, and then 150 μl /disk of culture medium was added to the wells. The cells were cultured for two days, and then the viability and proliferation of fibroblasts was determined by MTT assay. Original culture medium was removed with a macro-pipette, and 150 μl of fresh culture medium was added to each well. 5 μl of fresh MTT solution (5 mg/ml) was added to each well and the culture plates were incubated at 37 under 5% CO₂ condition for 4 h. The upper medium was carefully removed, and the intracellular formazan was solubilized by adding 200 μl of 0.04 mol/l HCl/iso-propanol into each well. The absorbance of produced formazan was measured at 570 nm with microplate reader (Texcan, Aus). The statistical significance between two sets of data was calculated using Student's *t*-test. Data were taken to be significant, when a *p*-value of 0.05 or less was obtained (showing a 95% confidence limit) [26].

3. Results and discussion

3.1. Synthesis and characterization of Multi-PLE copolymers

Triblock and multiblock PLE copolymers of various compositions could be synthesized according to Scheme 1. The ring-opening polymerization of lactide could be performed by using hydroxyl-containing compounds as initiators and Sn(Oct)₂ as catalyst, thus, PEG with hydroxyl end groups could be used as macroinitiator for lactide polymerization to form ABA-type triblock copolymer. Triblock PLE copolymers with different PEG or PLA block lengths could be obtained by using different molecular weight PEG as initiators and different LA/EG molar ratios. Thereafter, multiblock PLE copolymers were synthesized by coupling the triblock PLE copolymers with succinic anhydride and DCC. The yield of the triblock was about 95% and that of multiblock was about 85%. For the sake of simplicity, the obtained triblock PLE copolymers were expressed as Tri-PLE a/b(c), for example, Tri-PLE2/1(2000), where 2/1 referred to LA/EG molar ratio and the molecular weight of PEG segment was 2000. The average molecular weights of the polymers were determined by GPC and listed in Table 1. All the multiblock PLE copolymers exhibited higher molecular weight than the corresponding triblock PLE copolymers. For example, the average molecular weight of the Tri-PLE1/1(2000) and Multi-PLE1/1(2000) was 8500 and 53 800, respectively. It meant that it was a simple and efficient way that Tri-PLE copolymers were coupled by succinic anhydride to obtain high molecular weight PLE copolymer with high PEG content.

3.2. Properties of Multi-PLE copolymers

The mechanical properties of the PLE copolymers were evaluated by measuring tensile strength and elongation at break. As shown in Table 2, most triblock copolymers could not be cast into film for measurement.

Although the mechanical properties of triblock copolymers were slightly improved if the chain length of PEG or the content of PLA was increased, these Tri-PLE copolymers still had not enough mechanical strength to be made into scaffolds. Therefore, it was hard to obtain a triblock PLE copolymer of both high mechanical strength and good hydrophilicity. However, the films of all the Multi-PLE copolymers showed good mechanical properties even when the copolymer contained a rather high content of short PEG segments. For example, the film of Multi-PLE1/1(2000) presented a remarkable tensile strength of about 28.0 MPa and a considerable elongation of near 500%. This made Multi-PLE copolymers suitable for using as biomedical materials.

The surface and bulk hydrophilicity of various multiblock PLE copolymers was determined by contact angle and water uptake, and the data are presented in Table 3. It could be seen that the contact angles of the Multi-PLE copolymers were smaller than that of homo-PLLA. This meant that the introduction of PEG segments enhanced the surface hydrophilicity of the copolymers. However, the improvement of surface

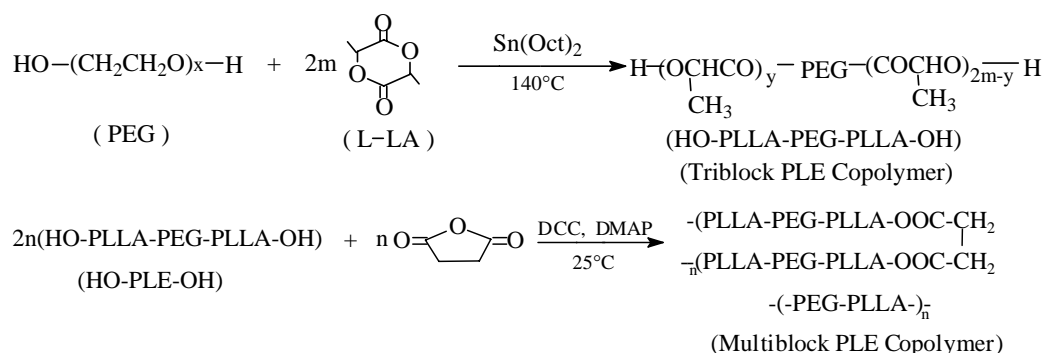
Table 1
Characterization of Tri- and Multi-PLE copolymer

Polymers	[η] ^a (dl/g)	LA/EG (molar ratio) ^b	Mw ^c (10 ⁻³)	Mw/Mn ^c
Tri-PLE1/1(2000)	0.20	0.96/1	8.5	1.15
Tri-PLE2/1(2000)	0.30	1.91/1	16.5	1.53
Tri-PLE4/1(2000)	0.43	4.05/1	33.4	1.58
Tri-PLE1/1(4000)	0.36	0.93/1	14.3	1.42
Tri-PLE2/1(4000)	0.46	1.90/1	28.0	1.56
Tri-PLE4/1(4000)	0.66	3.97/1	49.8	1.57
Multi-PLE1/1(2000)	1.20	0.96/1	53.8	1.42
Multi-PLE2/1(2000)	1.17	2.02/1	46.7	1.06
Multi-PLE4/1(2000)	0.90	3.86/1	54.7	—
Multi-PLE1/1(4000)	0.71	0.96/1	40.3	—
Multi-PLE2/1(4000)	0.82	1.80/1	55.3	1.55
Multi-PLE4/1(4000)	0.95	3.95/1	66.8	—

^a Measured in chloroform solution at 30°C.

^b Calculated from ¹H NMR measurement.

^c Measured (GPC), using polystyrenes as standards.



Scheme 1. Synthesis of the triblock and multiblock PLE copolymers.

Table 2
Comparison of mechanical properties of homo-PLLA and PLE copolymers

Polymers	Tensile strength (MPa)	Elongation (%)	Polymers	Tensile strength (MPa)	Elongation (%)
Tri-PLE1/1(2000)	— ^a	— ^a	Multi-PLE1/1(2000)	28.0	485
Tri-PLE2/1(2000)	— ^a	— ^a	Multi-PLE2/1(2000)	35.6	666
Tri-PLE4/1(2000)	— ^a	— ^a	Multi-PLE4/1(2000)	34.7	282
Tri-PLE1/1(4000)	— ^a	— ^a	Multi-PLE1/1(4000)	12.5	734
Tri-PLE2/1(4000)	4.0	6.0	Multi-PLE2/1(4000)	22.1	469
Tri-PLE4/1(4000)	11.7	6.8	Multi-PLE4/1(4000)	25.2	59.3
PLLA	19.2	2.2			

^aThe polymer could not be cast into film for measurement.

Table 3
Hydrophilicity of Multi-PLE copolymers and homo-PLLA

Polymers	Contact angle (deg)	Water uptake (%)
Multi-PLE1/1(2000)	20.8	44.3
Multi-PLE2/1(2000)	60.8	21.4
Multi-PLE4/1(2000)	71.8	15.1
Multi-PLE1/1(4000)	15.0	42.9
Multi-PLE2/1(4000)	59.4	28.9
Multi-PLE4/1(4000)	71.7	23.1
PLLA	78.0	0.5

hydrophilicity was not so significant as expected, especially when the content of PEG segments was low.

The bulk hydrophilicity of the copolymer was detected by water uptake and the results are shown in Table 3. It could be seen that the water uptake of homo-PLLA was near zero, while those of the multiblock copolymers were above 20%. The water uptake of the Multi-PLE copolymers could be enhanced with the content of PEG segments in the copolymer increasing. When scaffolds made of homo-PLLA and Multi-PLE were immersed in water, the homo-PLLA scaffold floated on the water for its hydrophobic nature, and the Multi-PLE scaffold adsorbed water rapidly and sank. This phenomenon elucidated that Multi-PLE copolymer should be a more powerful candidate for scaffold materials in comparison with pure PLLA.

3.3. Attaching efficiency determination and morphological observation of 3T3 fibroblasts on materials

In this research mouse 3T3 fibroblasts were used as model cells to test the cell affinity of the Multi-PLE copolymer. The cell attachment efficiency on Multi-PLE materials and PLLA was compared in Table 4. It could note that the cell attachment efficiency for Multi-PLE copolymer films decreased with the PEG content in the copolymers increasing, and no cells were found attaching to PLE films when the LA/EG ratio was increased to 1/1. Moreover, the highest cell attachment efficiency on Multi-PLE was only 57%, which was just close to and not better than that on PLLA film. It could be explained that PEG was not good for cell adhesion and cells were

liable to attach on relatively hydrophobic surface [27]. This result was further identified by the morphology observation of 3T3 fibroblasts. As shown in Fig. 1, fewer cells attached to Multi-PLE films if the copolymer contained higher PEG fraction. And it could be observed that the cells were liable to attach on the spherulite structure (Fig. 1C), which was assigned to the PLLA crystal. One reason for this was that PLLA crystalline domain provided a hydrophobic surface, and another reason was that the crystalline would result in a rough surface, which facilitated the cell attachment [28,29].

We had inspected the ability of cells to attach on PLLA with different molecular weights. These two PLLA had different crystallizability that PLLA with molecular weight of 23,000 ($\Delta H_m = -58.6 \text{ J/g}$) showed much higher crystallizability than PLLA with molecular weight of 116,000 ($\Delta H_m = -7.2 \text{ J/g}$). From Table 4, it could be clearly seen that higher PLLA crystallizability resulted in higher cell attachment efficiency, which was attributed to its rougher surface caused by the contraction of PLLA crystals (Figs. 2A and B). Thereafter, it was easy to understand that the cell attachment efficiency on Multi-PLE4/1(4000) (57.0%) was larger than that on Multi-PLE4/1(2000) (43.9%), even they had the same LA/EG ratio, because the crystallizability of PLLA blocks in PLE4/1(4000) was stronger for its longer chain length and more PLLA spherular crystals were formed on the film surface as proven by Figs. 2C and D.

3.4. Affinity of 3T3 fibroblasts in the porous scaffold

3.4.1. SEM observation of PLLA and PLE scaffolds seeded by 3T3 fibroblasts

3T3 fibroblasts were cultured in the PLLA scaffold and Multi-PLE2/1(2000) scaffold. After 2 weeks, SEM observation of the scaffolds was performed. The SEM pictures showed that the cells on the surface and cross-section (Figs. 3A and C) of PLE2/1(2000) scaffold spread well and secreted a great deal of extracellular matrix because of its hydrophilicity facilitating the migration of cells into the scaffold. However, the cells tended to aggregate together on the surface of PLLA

Table 4
Cell attachment efficiency on Multi-PLE copolymers and homo-PLLA

Polymers	PLE 1/1(4000)	PLE 2/1(4000)	PLE 4/1(4000)	PLE 4/1(2000)	PLLA (23,000)	PLLA (116,000)
Cell attachment efficiency ^a	0	— ^b	57.0 ± 1.6%	43.9 ± 2.4%	64.8 ± 2.9%	43.2 ± 3.8%

^a Cell concentration: 2×10^5 cells/ml.

^b Cannot be detected using the given cell counting method.

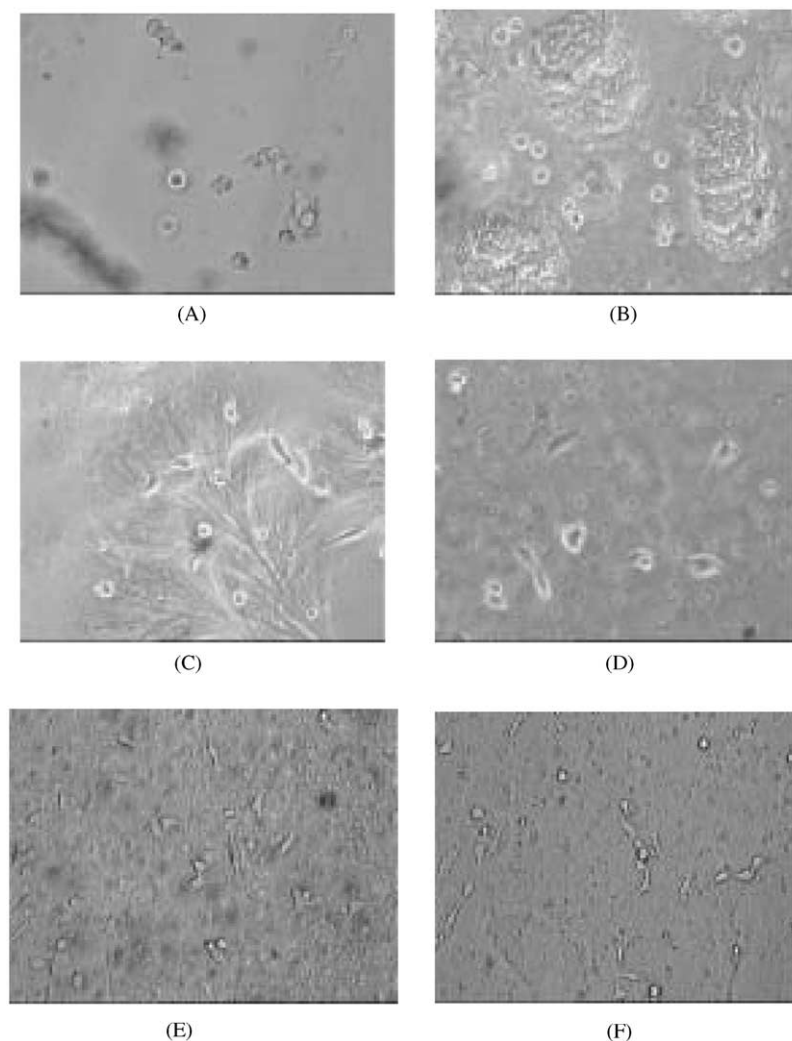


Fig. 1. Comparison of morphology ($\times 250$) of mouse 3T3 fibroblasts on the surface of Multi-PLE copolymer and homo-PLA samples. (A) Multi-PLE1/1(4000); (B) Multi-PLE2/1(4000); (C) Multi-PLE4/1(4000); (D) Multi-PLE4/1(2000); (E) PLLA ($M_n = 23,000$); (F) PLLA ($M_n = 116,000$).

(Fig. 3B). And few cells could be found on the cross-section of PLLA scaffolds due to its hydrophobicity impeding the cells to migrate into the internal pores of the scaffold (Fig. 3D).

3.4.2. Effects of MTT assay

The proliferation of 3T3 fibroblasts in Multi-PLE and PLLA scaffolds after being cultured for 2 days, was compared by MTT assay. The data are shown in Fig. 4. It could be seen that no viable cells were detected in the Multi-PLE1/1(4000) scaffold. One possible reason for

this phenomenon was the difficulty of cell attachment. The other reason was thought due to the high water uptake of Multi-PLE1/1(4000). The water uptake of Multi-PLE1/1(4000) could reach 42.9%, which caused the swelling of the scaffolds and the contraction of the pores, and blocked the cells penetrating into scaffolds. However, as shown in Fig. 4, all the other multiblock PLE scaffolds showed higher cell viability and faster proliferation than PLLA scaffold. The absorbance values on Multi-PLE4/1(4000), Multi-PLE2/1(2000) and Multi-PLE4/1(2000) were statistically different

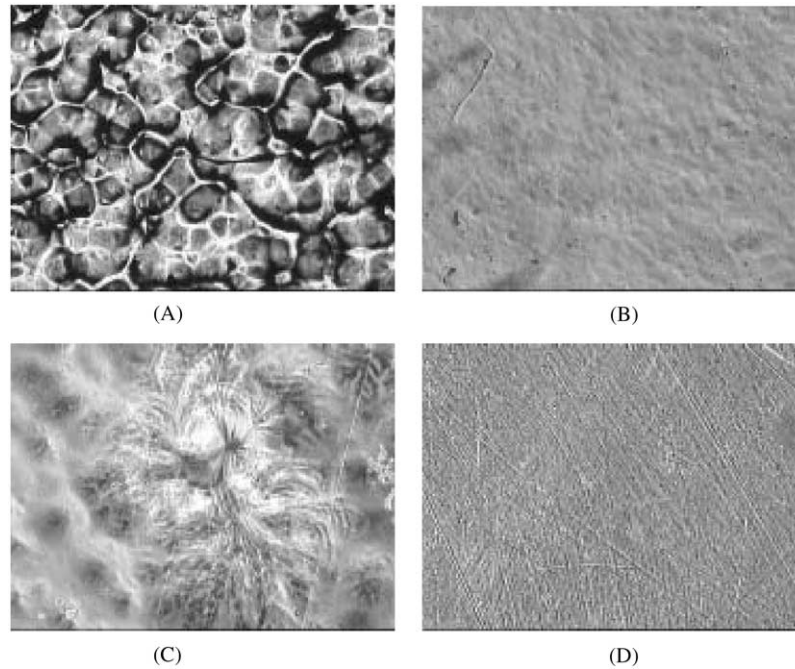


Fig. 2. The morphology of the surface of homo-PLLA and Multi-PLE copolymer samples ($\times 250$): (A) PLLA ($M_n=23,000$); (B) PLLA ($M_n=116,000$); (C) PLE4/1(4000); (D) PLE4/1(2000).

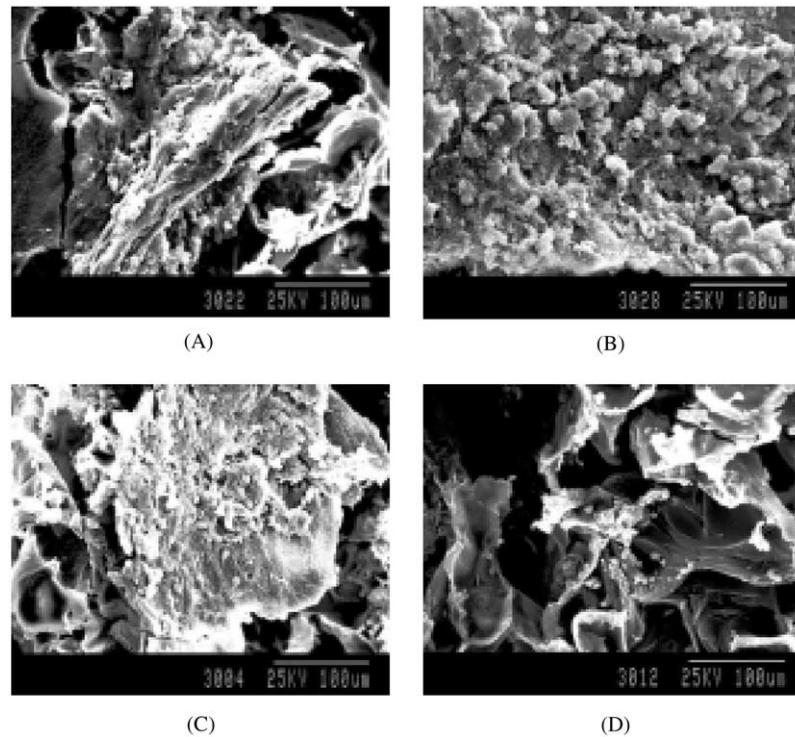


Fig. 3. SEM image ($\times 300$) of the Multi-PLE and homo-PLLA scaffolds after 3T3 fibroblasts being cultured for two weeks (A) Surface of Multi-PLE2/1(2000), (B) Surface of homo-PLLA, (C) Cross section of Multi-PLE2/1(2000), (D) Cross section of homo-PLLA.

from that on PLLA ($P < 0.05$). This could be attributed to the better hydrophilicity of Multi-PLE compared to PLLA, which allowed the cells and culture medium to penetrate more easily into the scaffolds. This was helpful

for cell growth. These results showed that the cell proliferation would be much improved by increasing the hydrophilicity of the three-dimensional scaffolds, and which even outweighed the disadvantages of the cell

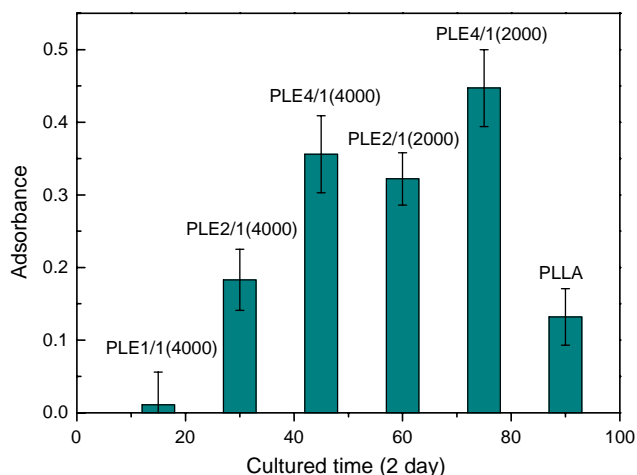


Fig. 4. MTT-tetrazolium assay after the mouse 3T3 fibroblasts cultured on Multi-PLE and homo-PLLA scaffolds.

attachment efficiency reduction with the incorporation of PEG.

4. Conclusions

In the present work biodegradable Multi-PLE copolymers that had a high content of short PEG segments, high hydrophilicity and good mechanical properties were synthesized and used as cell scaffolds for tissue engineering. The cell affinity of Multi-PLE films and scaffolds was studied and compared with the affinity of controlled PLLA. The results showed that the cell attachment efficiency on the Multi-PLE 4/1 films was close to that on homo-PLLA film. With the increase of the content of PEG segments in the copolymer, the cell attachment efficiency decreased because PEG segments could reduce cell adhesion. The results of MTT assay indicated that the cell affinity and proliferation of Multi-PLE copolymer scaffolds were much better than that of PLLA scaffold, except for that of Multi-PLE1/1(4000) copolymer. The water uptake of the Multi-PLE1/1(4000) was so high that pores of the scaffold were blocked. The SEM observation showed that cells grew only on the surface of PLLA scaffold because of its hydrophobicity, but cells grew on the entire Multi-PLE scaffolds. From the results of these cell-cultivation experiments, it could be concluded that the cell affinity of the Multi-PLE copolymers scaffolds was improved when appropriate amounts of PEG blocks were introduced into PLA polymer chain.

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