

A novel porous cells scaffold made of polylactide–dextran blend by combining phase-separation and particle-leaching techniques

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Received 5 December 2001; accepted 6 May 2002

Abstract

In this study, a kind of biodegradable material was developed by blending polylactide (PLA) with natural biodegradable dextran, and a novel sponge-like scaffold made of it was fabricated thereof using solvent-casting and particle-leaching technique. To obtain a uniform blend of PLA and dextran by simple solvent-casting method, hydroxyls of dextran should be protected via trimethylsilyl (TMS) groups to make dextran soluble in organic solvents. Benzene was found among the few solvents that could dissolve this TMS-protected dextran (TMSD) well, however, it was not a good solvent for PLA. Therefore, a homogeneous mixed solution of PLA and TMSD could be obtained when a mixture of dichloroform (DCM) and benzene ($v/v = 6/4$) was used. By this technique, PLA–dextran blend films and even PLA films were observed a microporous structure (pore size around 5–10 μm) formation throughout the films under scanning electron microscope (SEM). Scaffolds that were prepared by dissolving PLA and TMSD in mixed solvent of DCM and benzene and using salt as porogen, were observed the formation of micropores (pore size around 5–10 μm) in the cellular walls of macropores (pore size around 100–200 μm). This microporous structure was closely related to the phase separation occurring during films or foams formation, which was mainly due to the different solubility of PLA and TMSD in benzene, as well as the different evaporation rates of DCM and benzene. In comparison with PLA, the surface and bulk hydrophilicity of PLA–dextran blend films or foams were significantly improved after the TMS groups were removed in methanol, and the results of cell culture on these polymeric substrates exhibited an enhancement on cell attachment and proliferation. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Polylactide; Dextran; Cells scaffold; Phase separation; Particle leaching

1. Introduction

Many strategies in tissue engineering have focused on using synthetic biodegradable polymers such as temporary scaffolds to stimulate isolated cells to regenerate tissues with defined size and shape. The temporary scaffold should be tissue compatible, biodegradable and with mechanical properties closely matched to the target tissues [1–6]. Aliphatic polyesters such as polylactide (PLA), polyglycolide (PGA) and their copolymer of poly(lactide-co-glycolide) (PLGA) are among the few synthetic polymers that meet these requirements and have been used in the tissue engineering of cartilage [7,8], bone [9,10], tendon [11], skin [12,13], liver [14] and heart valve [15]. Cell scaffolds should have a three-

dimensional porous structure and its porosity should be at least 90% in order to provide a high surface area for maximizing cell seeding and attachment, sufficient space for extracellular matrix (ECM) regeneration and minimal diffusion constraints during the *in vitro* culture [16]. Transport issues are very important in designing a scaffold, an open porous structure are desirable to admit the nutrient delivery, waste removal, vascularization and tissue ingrowth [17].

Several methods have been developed to fabricate highly porous biodegradable cell scaffolds, including fiber-bonding [18], solvent-casting and particle-leaching [19,20], phase-separation [21], emulsion freeze drying [22], gas-foaming [23] and 3D-printing technique [24]. Among these methods, solvent-casting and particle-leaching method is a convenient way to fabricate sponge-like scaffolds and the process is reproducible. Water-soluble particles are used as porogens in this method, such as salts [25], glucose [26] and gelatin

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microspheres [27], as well as ice particles [28], which provide easy control on pore structure, pore size and porosity.

However, scaffolds that are fabricated by particle-leaching method usually exhibit a porous structure with intact pore walls, and a closed cellular structure is difficult to be avoided. It would impede the diffusion of seeded cells into the scaffold and prevent nutrient transport. In a previous paper, a phenomenon was observed and reported that cell morphology was poor in some pores, especially in the middle part of a scaffold, but was satisfactory in some other pores on the same scaffold, when human fibroblast cells were cultured in plasma-treated PLGA scaffolds [29]. This was closely related to mass transport issues. Both Harris and Nam had reported biodegradable matrices with open pore structure that were fabricated by combining particle-leaching and gas-foaming method [30,31]. It was suggested that combination of different pore forming techniques might result in a desired porous structure to avoid closed pores.

On the other hand, although synthetic biodegradable scaffolds of aliphatic polylactones have been widely used in tissue engineering, their poor hydrophilicity can significantly affect cell suspension penetrating into the scaffolds. Moreover, there were no cell recognition sites on the surface of these polymeric scaffolds, which will lead to poor cell affinity. To improve the cell affinity, many efforts have been directed to modify their surface properties by adjusting the hydrophilicity/hydrophobicity [32], surface energy [33], surface charge [34] and surface roughness [35]. One of the possible and promising approaches to overcome this problem is to introduce hydrophilic segments into the aliphatic polyester. Many researches on synthesis of block copolymers of polylactone with poly(ethylene glycol) (PEG) and terpolymerization with polyhydroxy compounds had been carried out and indeed succeeded in improving the hydrophilicity of aliphatic polyesters [36–39], but blending natural biodegradable hydrophilic polymers into PLA-like polymers would be an more easy way to ameliorate the cell affinity of synthetic materials. Polysaccharides, such as starch and dextran, are typical examples of natural biodegradable hydrophilic polymers, which show enzymatic degradation behavior and relatively good biocompatibility. Starch-based polymers and composites have been introduced as promising biomaterials for orthopaedic applications [40,41]. These starch-based materials have been generally deemed as non-cytotoxic and could induce a satisfactory tissue response when they were implanted in muscle or bone tissue [42,43].

In this work, a blend of dextran and PLA was prepared by dissolving them in organic solvent after dextran was trimethylsilyl (TMS) protected. Influence of dextran on cell affinity of PLA-based material was

studied. A new method to fabricate cell scaffolds was proposed, that the particle-leaching and phase-separation techniques were applied together.

2. Materials and methods

2.1. Materials

Dextran (Mn = 15,000–20,000) was purchased from ICN Biomedicals Inc. (OH, USA). Chlorotrimethylsilyl-lane (TMS-Cl) was obtained from Beijing No. 2 Chemical Plant (China). L-lactide was purchased from Purac (Netherlands) and was recrystallized twice from ethyl acetate before use. Stannous octoate (Sigma) was used as-received. Benzene, pyridine and other organic solvents were dried over 4 Å molecular sieves and purified by the usual distillation method.

2.2. PLA synthesis

Poly lactide was synthesized by ring opening polymerization at 140°C for 12 h, using hexadecanol as initiator and stannous octoate as catalyst [44]. The raw product was dissolved in chloroform, precipitated into cold ethanol to obtain the final PLA polymer and then was vacuum-dried for use. The weight average molecular weight (Mw) of the final product was 1.3×10^5 , which was measured by gel permeation chromatography (Waters510 with Shodex KF-800 columns) in chloroform using polystyrene as standard.

2.3. Trimethylsilyl-protected dextran (TMSD) preparation

9.72 g dextran (0.06 mol glucose units) was suspended in 10 ml of dried pyridine (0.12 mol), then 13 g TMS-Cl (0.12 mol) was added into the system. The reaction was carried out at 50°C for 20 h. In the course of reaction, 50 ml of dried benzene was added to push the reaction by dissolving the produced TMSD. In a second step, the reaction mixture was washed three times with a saturated NaCl aqueous solution to remove pyridine hydrochloride. Subsequently, the solvent was removed by distillation. After further drying by azeotropic distillations of benzene, 16.2 g TMSD was obtained. The introduction of TMS groups was confirmed by ¹H NMR measurement in D-benzene (Bruker DMX300).

2.4. PLA-dextran blend films and scaffolds preparation

To prepare polymeric films, PLA or a mixture of PLA and TMSD was dissolved in a mixed solvent of dichloromethane (DCM) and benzene (v/v = 6/4), and then the polymeric solution was cast onto a glass plate. After most of the solvent had been air-dried at

room temperature, the obtained films were removed from glass plates and further vacuum-dried thoroughly to constant weight. In comparison, PLA films were also prepared by only using DCM as casting solvent. The thickness of all the films was about 0.1 mm.

To prepare scaffolds, an established solvent-casting and particle-leaching method was applied. Briefly, PLA or a mixture of PLA and TMSD was dissolved in a mixed solvent of DCM and benzene (v/v = 6/4). Then presieved NaCl particles (125–200 μm) were added into the solution, and the dispersion was cast into a polytetrafluoroethylene (PTFE) mold. After being air-dried for 48 h and subsequently vacuum-dried for 24 h to remove any remaining solvent, the resulting polymer/salt composites were then immersed in distilled water for 24 h to leach out the salt and sponge-like foams were obtained, whose thickness was about 2 mm. The porosity of all these foams was about 90% as determined according to literature [45]. In comparison, PLA scaffolds were also fabricated by this technique but only DCM was used as solvent.

2.5. TMSD de-protection and confirmation

The above PLA–dextran blend films and foams were suspended in methanol and stirred continuously for 24–48 h to remove the TMS groups. Then they were washed with petrol ether to remove any remaining TMS groups that adhered onto the films or foams surface. The TMS de-protection could be confirmed by X-ray Photoelectron Spectra (XPS). XPS spectra of the de-protected samples were acquired on a VG Escalab 220i-xl spectrometer using AlK_{α} radiation at a power of 300 W under vacuum (2×10^{-7} Pa). A take-off angle of 90° with respect to sample surface was used.

2.6. Characterization of PLA and PLA–dextran blend films and scaffolds

Surface and cross-section morphology of the produced films and scaffolds were observed under Scanning Electron Microscope (SEM, Hitachi S-530) after sputter-coated with gold. Mechanical properties of the films were determined by measuring their tensile strength (σ) on a Shinkch Testing Machine with a tensile speed of 100 mm/min at room temperature. Contact angle of the films to water were measured on air-surface of the samples and performed on a FACE CA-D Contact Angle Meter (Kyowa Kaimenkagaku Co., Japan). Five independent determinations at different sites were averaged. Water absorption of the polymeric films were measured by immersing the films in distilled water for a predetermined time span, then the films were taken out and dried by removing the free water on the surface with filter paper and weighed (W_1). Then the samples were thoroughly vacuum-dried and

weighed again (W_2). The water absorption could be calculated as follows and three specimens were averaged.

$$\text{Water absorption (\%)} = (W_1 - W_2)/W_2 \times 100.$$

2.7. Cell culture on PLA–dextran blend films and scaffolds

Mouse 3T3 fibroblasts were cultured in 50 ml cell culture flasks with 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. 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 get cells dispersion which was used after counting the cells.

PLA and PLA–dextran blend films were cut into small disks (15 mm in diameter) with the aid of cork borer in order to locate the disks into 24 well tissue culture plates. All the samples were prewetted and sterilized in 75% ethanol for 0.5 h, and then ethanol was exchanged with an excess amount of phosphate buffered saline (PBS) [46]. After the PBS in disks was removed by a pipette, mouse 3T3 fibroblasts suspension (1 ml) with a density of $1-2 \times 10^5$ cells/ml was seeded evenly into the wells with a pipette. The cell-seeded disks were maintained at 37°C under 5% CO₂ condition for 5 h, and then the culture medium was removed. Subsequently, any of the residual culture medium and unattached cells were removed by washing the disks three times with PBS. 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.

PLA and PLA–dextran blend scaffolds were cut into small disks, located into 96 well tissue culture plates and sterilized as described above, then 20 μl of mouse 3T3 fibroblasts suspension with a density of $7-8 \times 10^6$ cells/ml was seeded evenly into the wells with a pipette. The cell-seeded disks were maintained at 37°C under 5% CO₂ condition for 2, 4 and 6 days, respectively. The culture medium was refreshed everyday. At the pre-determined intervals, 10 μl of MTT solution (5 mg/ml) was added into the wells. Then the culture medium was removed after further cultured at 37°C under 5% CO₂ for 4 h. Subsequently, the intracellular formazan was solubilized by adding 200 μl of 0.04 mol/l HCl/isopropanol to each well and the absorbance of produced formazan was measured at 570 nm with a microplate reader (Tecan, Australia).

2.8. Cell morphology observation

PLA and PLA–dextran blend scaffolds were cultured for 3 days and then fixed with 3% glutaraldehyde in PBS for 24 h at 4°C. After thoroughly washed with fresh PBS, the samples were dehydrated sequentially in 50%, 70%, 95% and 100% ethanol for 2×10 min, respectively. Then the fixed samples were freeze-dried, sputter-coated with gold and observed under SEM (Hitachi S-530).

3. Results and discussion

3.1. TMSD preparation

Polysaccharides are widely existing natural biodegradable hydrophilic polymers. It can be used to hybridize with hydrophobic synthetic polymers to improve biocompatibility, biodegradability, hydrophilicity and cell affinity of polymeric materials. A simple technique to fabricate a uniform blend was solvent-

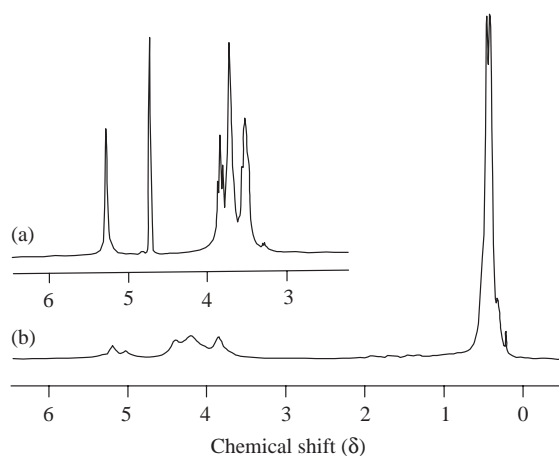


Fig. 1. Confirmation of the introduction of TMS groups into dextran by ^1H NMR: (a) the spectrum of pure dextran measured in D_2O ; (b) the spectrum of trimethylsilyl dextran measured in D -benzene.

casting method, however, polysaccharides are insoluble in common organic solvents. Using protection technique via TMS groups reacting with hydroxyls, dextran derivative became soluble in organic solvents, such as benzene and tetrahydrofuran (THF). It had been found that dextran became soluble in organic solvents when its degree of trimethylsilylation was above 40%. The introduction of TMS groups was confirmed by the methyl proton signal at 0.1 ppm besides the broad methylene and methenyl proton signals of dextran at 3.5–5.5 ppm in the ^1H NMR spectrum (Fig. 1). Degree of trimethylsilylation was estimated to be 63.8%/hydroxyl group by ^1H NMR, and the yield could be calculated to be 90%.

3.2. PLA and PLA–dextran blend films and scaffolds fabrication

Although dichloromethane (DCM) was usually used to fabricate PLA films and scaffolds for its good solubility to PLA-family polymers and its fast evaporation rate, benzene was found having better solubility for TMSD, but it was not a good solvent for PLA. In this study, a mixed solvent of DCM and benzene ($v/v = 6/4$) was tested as a suitable solvent for both PLA and TMSD.

Instead of dense films, micropores with sizes about 5–10 μm were detected and well distributed throughout PLA films, as shown in Fig. 2, when DCM was replaced by the mixed solvent. This phenomenon was considered as a result of phase separation occurring in the course of film formation. After the mixed polymeric solution was cast into a mold, DCM should evaporate faster than benzene for its lower boiling point and benzene would still remain in the films. Since PLA had poor solubility in benzene, it could be envisioned that a phase rich in PLA and another phase rich in benzene would be formed. Subsequently, the evaporation of benzene would result in a microporous structure. This inference could be further confirmed by the morphology and mechanical properties of PLA–dextran blend films containing different dextran content.

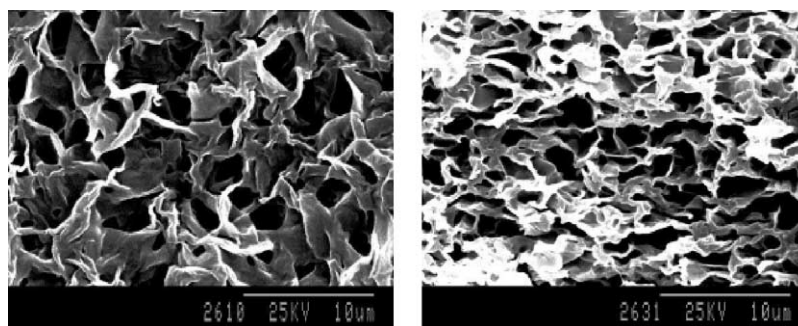


Fig. 2. SEM photographs of the microporous structure of PLA film that was fabricated by using mixed solvent of DCM and benzene: left—surface, right—cross-section.

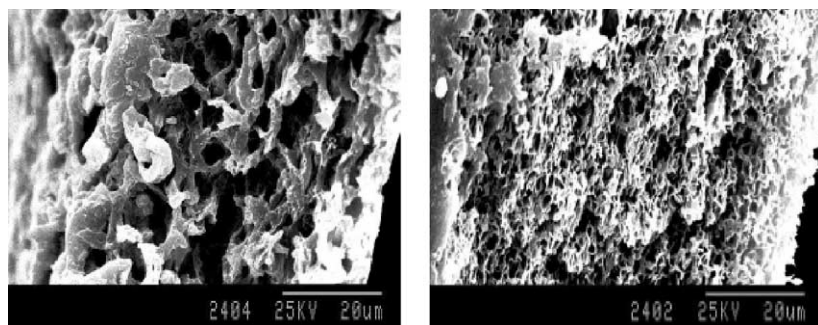


Fig. 3. SEM photographs of the microporous structure of PLA–dextran blend films that were fabricated by using mixed solvent of DCM and benzene: left—containing 10 wt% of dextran; right—containing 30 wt% of dextran.

Two kinds of PLA–dextran blend films were fabricated, which had 10 and 30 wt% of TMSD, respectively. Similar to PLA films, the microporous structure was formed throughout the films, as shown in Fig. 3, and their pore size decreased with the amount of TMSD increasing, which were also ascribed to phase separation during the films formation. A phase rich of TMSD dissolving in benzene would be formed and separated from another phase rich of PLA with the fast evaporation of DCM. It was easily understood that the continuous phase was mainly made up of PLA since PLA was the dominant component in the blend (90 and 70 wt%, respectively). After the benzene was thoroughly removed by air- and vacuum-drying, microporous structure was formed and most of the TMSD should remain inside the micropores. Therefore, higher TMSD content in the blend resulted in smaller pore sizes.

These PLA and PLA–dextran blend films were subjected to the measurement of tensile strength, and the results are presented in Table 1. The mechanical properties of PLA–dextran blends were sure to be lowered in comparison with that of pure PLA because of the incompatibility between hydrophobic PLA and hydrophilic dextran. So the tensile strength of PLA could reach 39 MPa, whereas those of PLA–TMSD blends were only about 13 MPa, but they did not vary with the dextran content. As elucidated above, PLA was the continuous phase and most of dextran existed inside the micropores as separated phase. Therefore, the mechanical properties of the PLA–dextran blends were mainly provided by the PLA and it would change little with the dextran content being increased from 10 to 30 wt%. In fact, these results also further confirmed that phase separation indeed have occurred during the formation of PLA or PLA–dextran blend films when a mixed solvent of DCM and benzene was used.

Solvent-casting and particle-leaching methods was known as a convenient way to obtain sponge-like scaffolds with macroporous structure, but a porous structure with intact pore walls was usually found in the

Table 1
Mechanical properties of blend of PLA with dextran in comparison with pure PLA

Sample	Content of dextran (wt%)	σ (MPa)	Elongation (%)
PLA	0	39.2 ± 1.6	23.2 ± 6.4
Blend-1	10	13.4 ± 1.1	9.8 ± 1.3
Blend-1a ^a	10	13.5 ± 1.2	9.9 ± 1.7
Blend-2	30	13.0 ± 1.3	8.3 ± 0.4
Blend-2a ^a	30	13.4 ± 1.4	8.3 ± 1.2

^aThese sample films were de-protected by being treated with methanol for 48 h at room temperature to remove the TMS groups.

center of the scaffolds, as shown in Fig. 4 (left). This structure would hinder the mass transport and cell diffusion. By replacing DCM with a mixture of benzene and DCM, a phase separation would occur during the formation of polymer/salt composite as elucidated above. It caused the formation of micropores (pore size around 5–10 μm) on macroporous walls (pore size around 100–200 μm) and made the macropores interconnected as demonstrated in Fig. 4 (middle and right). Porosity of the scaffold increased from $93.9 \pm 2.0\%$ to $95.5 \pm 1.6\%$, when the mixed solvent was used instead of DCM. And if dextran was blended into the scaffolds, the smooth macropore walls of PLA scaffold had changed into rough structures (Fig. 4, right). This phenomenon was caused by the phase separation between hydrophilic dextran and hydrophobic PLA, and the dextran should act as a coating on PLA due to the formation mechanism of micropores as elucidated above. Since the micropores on the macroporous walls had a dimension around 5–10 μm , they would facilitate the nutrient transportation inside the scaffold and be suitable for cell culture [7]. Therefore, this kind of PLA–dextran blend scaffold, which had high hydrophilicity, high porosity and interconnected porous structure, should be a promising cell scaffold for tissue engineering.

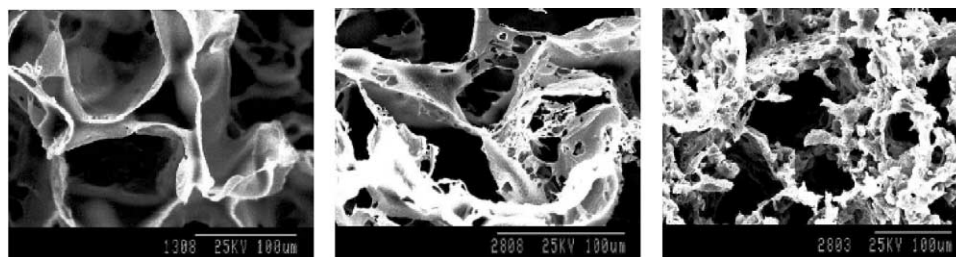


Fig. 4. SEM photographs of the cross-section of foams fabricated by combining the phase-separation and particle-leaching techniques: left—PLA foam fabricated only using DCM as solvent; middle—PLA foam fabricated using mixed solvent of DCM and benzene; right—PLA–dextran blend foam (containing 30 wt% of dextran) fabricated using mixed solvent of DCM and benzene.

3.3. De-protection of trimethylsilyl dextran and hydrophilicity of PLA–dextran blends

To fabricate PLA–dextran blend films or scaffolds by solvent-casting method, TMSD was synthesized to make dextran soluble in organic solvent. After the films or scaffolds were prepared, the TMSD should be de-protected by removing TMS groups to regain its hydroxyls and hydrophilicity thereof. The de-protection was a simple process by treating the blend films or scaffolds with methanol for 48 h, and it could be confirmed by the disappearance of Si_{2S} and Si_{2P} signals around 100–150 eV in XPS spectrum (Fig. 5). If the de-protected products had not been further washed with petrol ether after methanol treatment, the Si signals still could be observed, as shown in Fig. 5b, although they were much weaker than those of the untreated samples. This was due to some of the produced trimethylsilane that was absorbed on the surface of matrices, because trimethylsilane could not dissolve in methanol and be removed completely.

To evaluate hydrophilicity of PLA–dextran blends, their surface contact angle to water and water absorption were measured in comparison with PLA. The initial contact angle of pure PLA was about 83° and it decreased slowly with time (Fig. 6b). The untreated PLA–dextran blends showed higher contact angles than PLA because of the existence of TMS groups on their surfaces as indicated by the Si signals in XPS spectrum. The contact angles were 90° and 108° for PLA–dextran blends containing 10 and 30 wt% of TMSD, respectively (Fig. 6a and c). They also decreased slowly with time, but a little faster than that of PLA. In contrast, the de-protected PLA–dextran blends showed much smaller contact angles (72° and 60°, respectively) than both PLA and untreated blends, and the values decreased with the dextran content increasing (Fig. 6d and e). Contact angles of the de-protected blends decreased fast with time and could reach a value as low as 30° within half an hour. It resulted from the regained hydroxyls after TMS groups elimination, which obviously depended on the dextran content in the blends.

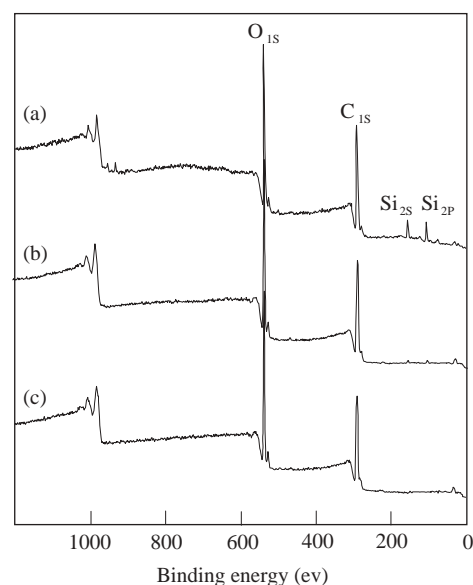


Fig. 5. XPS spectra of the PLA–dextran blend films before and after de-protection in methanol by removing TMS groups: (a) before de-protection; (b) after de-protection without being washed by petrol ether; (c) after de-protection with being washed by petrol ether.

On the other hand, the water absorption of these samples also changed regularly with their compositions (Fig. 7). The de-protected PLA–dextran blends exhibited higher water absorption than their corresponding untreated counterparts, respectively. It could be seen that the water absorption increased with the dextran content increasing and all the blends had higher water absorption than PLA, which was caused by the hydrophilic nature of dextran. Besides, the water absorption of de-protected PLA–dextran blends could level off within 1 day, while their corresponding untreated samples still showed an increase in water absorption after being immersed in distilled water for 2 days. This difference was due to that the untreated blends would lose some of their TMS groups by interacting with water and their hydrophilicity increased thereof.

By the way, mechanical properties of PLA–dextran blends changed little before and after de-protection, as

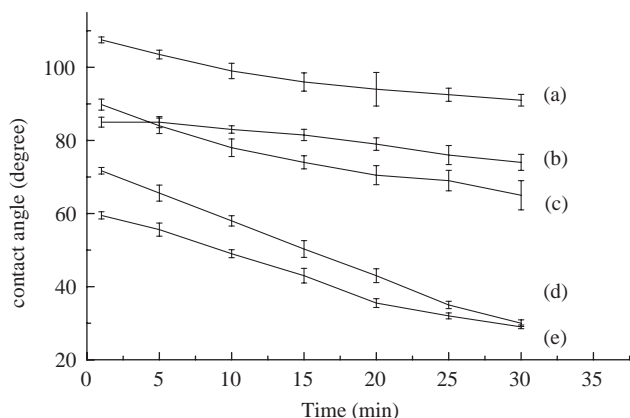


Fig. 6. Changes of surface contact angle to water of various PLA–dextran blends in comparison with PLA as function of time: (a) PLA–dextran blend containing 30 wt% of dextran before de-protection; (b) pure PLA; (c) PLA–dextran blend containing 10 wt% of dextran before de-protection; (d) PLA–dextran blend containing 10 wt% of dextran after de-protection; (e) PLA–dextran blend containing 30 wt% of dextran after de-protection.

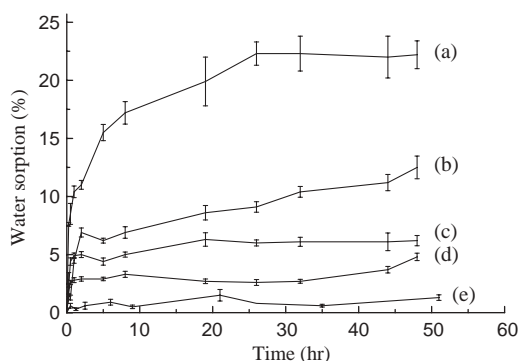


Fig. 7. Change of water sorption of various PLA–dextran blends in comparison with PLA as function of time: (a) PLA–dextran blend containing 30 wt% of dextran after de-protection; (b) PLA–dextran blend containing 30 wt% of dextran before de-protection; (c) PLA–dextran blend containing 10 wt% of dextran after de-protection; (d) PLA–dextran blend containing 10 wt% of dextran before de-protection; (e) pure PLA.

data presented in Table 1. The reason for this is that the de-protection only took place on TMSD and had minor influence on PLA, and TMSD mainly existed inside the micropores as stated above.

3.4. Cell affinity of the PLA–dextran blend

The surface and bulk hydrophilicity of PLA have been significantly improved by addition of dextran, which was thought as valuable features to facilitate cell attachment and proliferation.

The results of cell attachment efficiency measurement are profiled in Fig. 8. In a period of 5 h, the attachment efficiency for pure PLA films was only $65.4 \pm 1.4\%$,

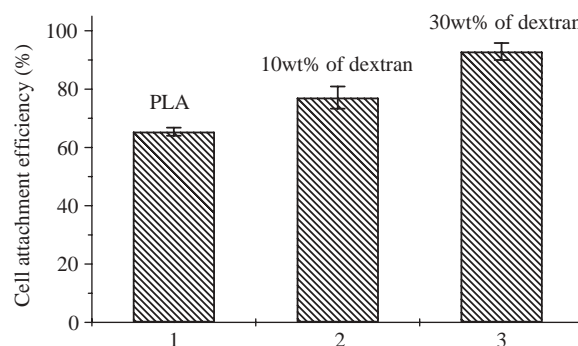


Fig. 8. Attaching efficiency of mouse 3T3 fibroblasts on films fabricated from different materials using mixed solvent of DCM and benzene.

whereas it was significantly enhanced on the PLA–dextran blend substrates. The cell attachment efficiency had increased with dextran content in the blend increasing. It was $77.1 \pm 3.8\%$ when 10 wt% dextran was contained, while it reached $92.9 \pm 2.9\%$ if 30 wt% dextran was added. As elucidated above, the contact angle of PLA–dextran blend films containing 10–30 wt% of dextran was about $60\text{--}70^\circ$ after de-protection. It would facilitate cell attachment according to the literature report that cell adhesion appeared to be maximized on surfaces with intermediate wettability [47]. On the other hand, the PLA–dextran blend films had uneven surfaces, which were caused by both the phase separation during film formation and the incompatibility between hydrophilic dextran and hydrophobic PLA. It had been reported that the surface roughness would also affect the cells attachment and growth [48]. Therefore, we could conclude that the cell attachment to the blend films should be significantly accelerated due to their mediate hydrophilicity and surface roughness.

The morphology of cells cultured on scaffolds was observed under SEM and is shown in Fig. 9. After 3 days cell culturing, the SEM pictures confirmed that the cells on the surface and cross-section (Fig. 9b and d) of blend scaffolds spread well and their morphology was satisfactory. However, the cells on the surface of PLA scaffold (Fig. 9a) tended to aggregate together and the cells distribution was not even. And few cells could be found on the cross-section of PLA scaffolds because of its hydrophobicity impeding the cells to migrate into the internal pores of the scaffold (Fig. 9c).

MTT assays showed that higher absorbance was obtained when the PLLA–dextran scaffolds were applied in comparison with PLA scaffolds (Fig. 10). There was statistical difference ($P < 0.05$) between the two sets of data determined every one day. The introduction of dextran into PLA could significantly enhance the cell growth. In addition, during the cell seeding, the culture medium was found penetrating into

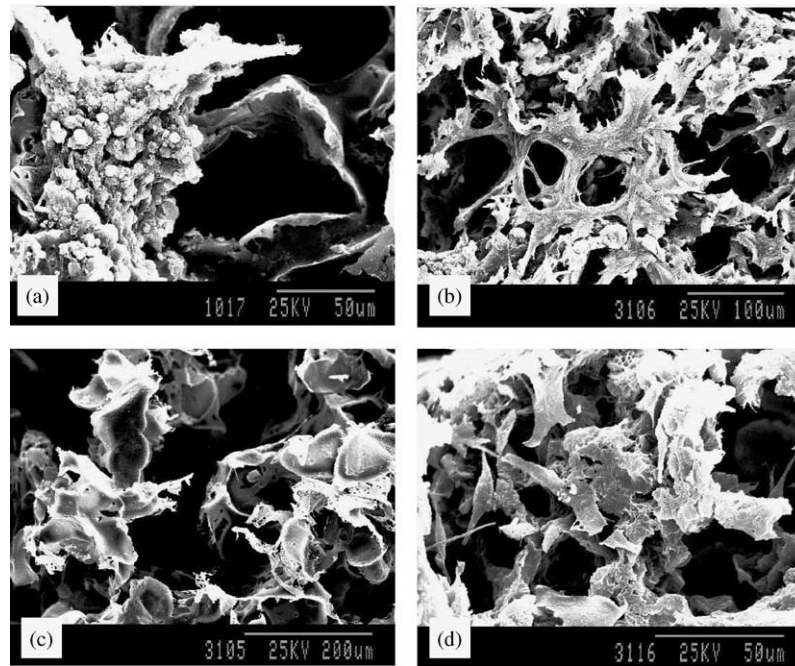


Fig. 9. Morphology of mouse 3T3 fibroblasts on the surface (a and b) and cross-section (c and d) of PLA (a and c) and PLA–dextran blend scaffold (b and d) observed under SEM after being cultured 3 days and fixed by glutaraldehyde.

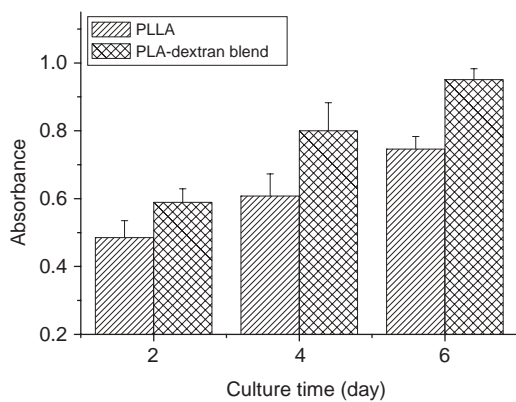


Fig. 10. MTT-tetrazolium assay after mouse 3T3 fibroblasts being cultured on PLA and PLA–dextran blend scaffold. Formazan absorbance expressed as a function of culture time. The statistic difference between the two sets of data determined every one day was below 0.05 ($p < 0.05$).

the blend scaffolds quickly, whereas it was hard to enter the hydrophobic PLA scaffolds. As a result, the cells proliferation mainly took place on the surface of PLA scaffold, in contrast, the PLA–dextran blend scaffolds could facilitate cells migration into the pores and provide enough space for cell proliferation, as illustrated in Fig. 9. Besides, microporous structure between macropores could be formed in the blend scaffolds by this fabrication technique and it was supposed to benefit the nutrient transportation.

In a word, it was hopeful to develop the PLA–dextran blend foam to be a potential scaffold for cells transplantation because of its good cell affinity.

4. Conclusion

In this study, a kind of PLA–dextran blend was prepared by a simple solvent-casting method. Dextran was blended into PLA by dissolving both of them in a mixture of DCM and benzene after dextran was TMS-protected. The hydroxyls of dextran could be easily regained by removing TMS groups in methanol. By replacing DCM with the mixed solvent of DCM and benzene, films with microporous structure could be formed. It was caused by phase separation occurring in the course of films formation, which was due to the different solubility of PLA and TMSD in DCM and benzene. By this technique, PLA and PLA–dextran blend scaffolds was fabricated in combination of particle-leaching method. As a result, micropores with size of 5–10 µm appeared on the macroporous walls. This led the scaffold having higher porosity and an open porous structure. In comparison with pure PLA, the hydrophilicity of PLA–dextran blend was significantly improved. It had good cell affinity and biocompatibility that it enhanced cell attaching efficiency, facilitated cells penetrating into scaffold and cells proliferation. It would be a kind of potential and promising cell scaffolds to be used in tissue engineering.

Acknowledgements

The authors are indebted to Basic Science Research and Development Grants (973) (Project No. G1999054305 and G1999054306) for financial supports.

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