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PREPARATION AND CHARACTERIZATION OF HYDROPHILIC PLGA/TWEEN 80 FILMS AND POROUS SCAFFOLDS

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Poly(DL-lactic-co-glycolic acid) (PLGA)/Tween 80 films and porous scaffolds were fabricated to improve the hydrophilicity and cell compatibility of the PLGA films and scaffolds for tissue engineering applications. PLGA/Tween 80 films and scaffolds with different Tween 80 compositions up to 20 wt% were fabricated by solvent casting and melt-molding particulate-leaching methods, respectively. It was observed that the 10 wt% addition of Tween 80 to PLGA to fabricate PLGA/Tween 80 film is enough for improving its hydrophilicity and cell adhesiveness as human chondrocytes were cultured on the surface. The hydrophilized PLGA/Tween 80 (10 wt%) porous scaffold was also found to have improved cell compatibility compared to the control hydrophobic PLGA scaffold, which can be directly applied for tissue regeneration without any pre-wetting treatment.

Keywords: biodegradable polymer; cell compatibility; hydrophilicity; poly(lactic-co-glycolic acid); porous scaffolds; Tween 80

INTRODUCTION

Biodegradable polymers have often been utilized to fabricate porous scaffolds for three-dimensional cell culture to regenerate tissue-based artificial organs. Poly(DL-lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable polymers since it is biocompatible and its degradation rate and mechanical properties can be easily controlled by varying the copolymer ratio of lactic to glycolic acid [1]. One of the limitations to the tissue regeneration using PLGA scaffolds is the hydrophobic issue. Because

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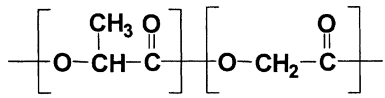
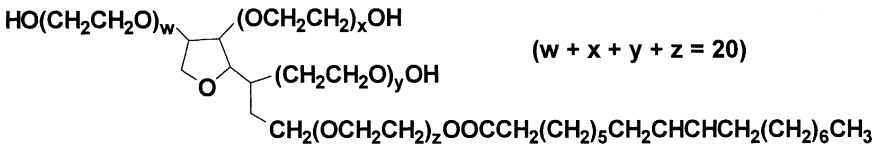
common synthetic biodegradable polymers including PLGA are hydrophobic, the porous scaffolds fabricated with these polymers are floating in cell culture medium. Or when cells in culture medium is plated on top of a porous scaffold or injected into its interior for seeding, the majority of its pores remain empty since the scaffold does not absorb the culture medium. It is important to obtain a uniform distribution of initial seeded cells throughout the scaffold volume for the creation of a tissue with homogeneous cellularity by cell culture.

Some approaches have been carried out to improve hydrophilicity of biodegradable polymer scaffolds and thus to ensure uniform and dense cell seeding. They include the treatments of the scaffolds by prewetting with ethanol [2], hydrolysis with NaOH [3], oxidation with perchloric acid mixture solution [4], oxygen or ammonia plasma discharge treatment [5,6], coating with some hydrophilic polymers [7,8] or cell-adhesive proteins [4,5,9–11], and blending with hydrophilic polymers such as polyvinyl alcohol, dextran, and polyethylene oxide derivatives [12–14]. The coating or blending with hydrophilic polymers may be a simple way to fabricate hydrophilized scaffolds if they can remain stably in the scaffolds.

In this study, we fabricated PLGA/Tween 80 films and porous scaffolds to improve the hydrophilicity and cell adhesiveness of the PLGA films and scaffolds. Tween 80, which has three short polyethylene glycol (PEG) chains and a relatively long alkyl chain (Fig. 1), is widely used in oral, parenteral, and topical pharmaceutical formulations and is generally regarded as a nontoxic and nonirritant nonionic surfactant [15]. One merit to utilize Tween 80 as a hydrophilic additive to fabricate hydrophilic PLGA



PLGA



Tween 80

FIGURE 1 Chemical structures of PLGA and Tween 80.

films or scaffolds is that the Tween 80 in the PLGA matrix is not easily leached out into water or cell culture medium owing to hydrophobic interactions between a relatively long alkyl chain in Tween 80 and hydrophobic PLGA chain. Short PEG chains in Tween 80 may also enhance cell adhesion on the surfaces [16]. PLGA/Tween 80 films and scaffolds with different Tween 80 compositions up to 20 wt% were fabricated by solvent casting and melt-molding particulate-leaching methods, respectively. The *in vitro* cell compatibilities as well as surface and bulk properties of the prepared PLGA/Tween 80 films and scaffolds were characterized in terms of added Tween 80 compositions.

EXPERIMENTAL

PLGA (lactic to glycolic acid mol ratio, 50:50) was purchased from Boehringer Ingelheim (Germany). Weight average molecular weight of PLGA determined by gel permeation chromatography (GPC; Waters Model SP8810, USA) using polystyrene standards (Polysciences, USA) was 110,000. Tween 80 (polysorobate 80) as a hydrophilic additive was purchased from Yakuri Pure Chemicals (Japan) and used without further purification. Their chemical structures are shown in Figure 1. All other chemicals were analytical grade and were used as received.

Methylene chloride (MC; Junsei, Japan) was used as a cosolvent for PLGA and Tween 80. PLGA was dissolved in MC to a 20 wt% solution. To the PLGA solutions were added 0 to 20 wt% of Tween 80 (based on dry PLGA). PLGA/Tween 80 films were prepared by solvent casting from the PLGA and Tween 80 mixture solutions (10 mL) on clean glass Petri dishes (diameter 10 cm). The solvent was slowly evaporated at room temperature for 3 days in a desiccator cabinet followed by vacuum drying overnight at 40°C to eliminate the residual solvent. The prepared film thickness was about 200 μm.

The prepared PLGA and PLGA/Tween 80 film surfaces were characterized by measuring water contact angles. The water contact angle, which is an indicator of the wettability or hydrophilicity of surfaces, was measured by a Sessile drop method using an optical bench-type contact angle goniometer (Model 100-0, Rame-Hart, USA). Drops of purified water (2 μL) were deposited onto the film surfaces and direct microscopic measurement of the contact angles was done with the goniometer. The contact angles were measured at several different positions on each film surface. The mechanical properties of PLGA and PLGA/Tween 80 films were measured by an ultimate tensile test machine (AG-5000G, Shimadzu, Japan) equipped with a 10 kg_f load cell at a crosshead displacement speed of 50 mm/min. To examine the stability of the Tween 80 entrapped in PLGA/Tween 80 films,

the films (about 0.5 g) were weighed after thorough drying (W_{dry}) and immersed in water for 1 and 7 days with continuous shaking. After that, the films were taken out of the water, rinsed with fresh water, dried to constant mass in a vacuum oven, and then weighed again ($W_{dry,final}$). The extraction of Tween 80 from the films was determined as follows:

$$\text{Tween 80 extraction}(\%) = (W_{dry} - W_{dry,final}) \times 100/W_{dry}$$

Human chondrocytes (immortalized human costal chondrocyte cell line C-28/I2, kindly supported by Dr. M. Goldring, Havard Medical School, USA) were used to compare cell adhesion and growth behavior on the control PLGA and PLGA/Tween 80 film surfaces. The cells routinely cultured in tissue culture polystyrene (PS) flasks (Corning, USA) at 37°C under 5% CO_2 atmosphere were harvested after the treatment with 0.25% trypsin (Gibco, USA). The films were cut into discs with 22 mm in diameter, sterilized by ethylene oxide (EO) gas, and placed on the bottom of 12-well PS tissue culture dishes (Corning). The films were equilibrated with prewarmed (37°C) Dulbecco's phosphate buffered saline (PBS, pH 7.3–7.4, Gibco) for 30 min. After the PBS solution was removed from the dish wells by pippetting, the cells ($4 \times 10^4/cm^2$) were seeded to the film surfaces. The culture medium used was Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Gibco), 0.1% gentamicin sulfate (Sigma, USA), and 1% penicillin G (Sigma). The cell culture on the film surfaces was carried out for up to 4 days at 37°C under 5% CO_2 atmosphere. The culture medium was changed into the fresh one everyday during the cell culture periods. After incubation for given times, the cells attached on the film surfaces were rinsed with PBS and trypsinized. The cell density on the surfaces was estimated by counting the number of detached cells with a haemocytometer.

PLGA/Tween 80 porous scaffolds were fabricated by a melt-molding particulate-leaching method designed by our laboratory [17]. The scaffolds were fabricated using PLGA/Tween 80 films prepared by solvent casting, as discussed above. The films were cut into discs with 16 mm in diameter. The PLGA/Tween 80 disc was placed into a brass mold (18 mm diameter and 2.5 mm thickness) covered with 0.9 g sodium chloride salt particles (sieved to sizes between 200 and 300 μm) at the bottom and was covered at the top again with 0.9 g salt particles. Then the mold was thermally compressed under $20 \sim 30$ MPa at 180°C for 1 min and then the following compression under $50 \sim 60$ MPa at 180°C for 1 min using a compression molding press. After taking out from the mold, the salt-containing disc scaffold was immersed in water for one day with mild shaking (the water was changed every $2 \sim 4$ hrs) to leach out salts from the scaffold. Then the scaffold was freeze-dried and stored in a vacuum oven until use to avoid degradation.

Surface and cross-section morphologies (porous structures) of the porous PLGA and PLGA/Tween 80 scaffolds were observed by a scanning electron microscope (SEM; Model 2250 N, Hitachi, Japan) operated at an accelerating voltage of 15 kV. The cross-sectional samples were prepared by fracturing the scaffolds after being frozen in liquid nitrogen. Before morphology observations, the scaffold samples were coated with platinum using a sputter coater (SC 500 K, Emscope, UK) under argon atmosphere. The porosity of the scaffolds was measured by a mercury intrusion porosimetry (PMI 30K-A-A, Porous Materials, USA). To determine the porosities, it was assumed that the shape of the pores is a cylinder, the contact angle of mercury is 140°, and the surface tension of mercury is 480 dyne/cm [4,18]. The PLGA/Tween 80 scaffolds were compared with the control PLGA scaffold for their wettability or absorbability of cell culture medium, which is an essential factor for cell culture and homogeneous tissue regeneration. For this, cell culture medium (DMEM) was dropped on top of the scaffold and the time required for complete absorption of the medium into the scaffold was measured.

The control PLGA and PLGA/Tween 80 (10 wt%) scaffolds (diameter ~16 mm, thickness ~2 mm) sterilized by EO gas were placed into the 12-well PS tissue culture dishes. For cell seeding, the control PLGA scaffolds were prewetted by two-step immersion in ethanol and culture medium (DMEM) as described by Mikos et al. [2] because they are hydrophobic and can not be wetted in cell culture medium. For the PLGA/Tween 80 scaffolds, the cells could be directly seeded without any prewetting treatment owing to their hydrophilic character. A suspension of human chondrocytes in 10% FBS-containing DMEM was seeded to the each scaffold (cell density, 2.0×10^5 cells/scaffold). The cell-seeded scaffolds were maintained for 2 hr at 37°C in an incubator for cell adhesion to the scaffolds. Then the scaffolds were transferred to new 12-well PS tissue culture dishes, the culture medium was added to the culture plate (5.0 mL/well), and the cells in the scaffolds were cultured up to 4 weeks with mild shaking (about 50 rpm). The culture medium was changed into the fresh one everyday during the cell culture periods. The viable cell numbers in the scaffolds after cell cultures for 0, 1, 2, and 4 weeks were estimated by MTT assay method [12]. Data were collected and averaged from the five different sample scaffolds per each condition.

RESULTS AND DISCUSSION

Tween 80 used as a hydrophilic additive for PLGA in this study is an oligomer-type nonionic surfactant with three short PEG chains and a relatively long alkyl chain. The hydrophobic alkyl chain may provide homogeneous

distribution and stable entrapment of Tween 80 in PLGA matrix through hydrophobic interactions. The short PEG chains in Tween 80 may provide the hydrophilicity and enhanced cell adhesion on the PLGA surface [16,19].

To evaluate whether the hydrophilicity of the PLGA films was improved by the addition of Tween 80 or not, the water contact angles of control PLGA and PLGA/Tween 80 films with different Tween 80 compositions up to 20 wt% were measured. The control PLGA film surface showed a high water contact angle (about 70°), indicating that the surface is hydrophobic (Fig. 2). The water contact angles sharply decreased on the PLGA/Tween 80 film surfaces with Tween 80 compositions more than 5 wt%. The decrease in contact angles (and thus the increase in hydrophilicity) on the surfaces may be owing to the hydrophilic property of PEG chains in Tween 80 exposed onto the surfaces. To investigate the mechanical properties of the films, stress-strain curves from the PLGA/Tween 80 films with different Tween 80 compositions were obtained (Fig. 3). The control PLGA film showed low tensile strength and elongation at break, indicating that the PLGA film is somewhat brittle. The films showed increased elongation at break (i.e., increased elasticity) with increasing Tween 80 compositions, particularly more than 5 wt%. This may be interpreted as a plasticizing effect of Tween 80 in PLGA. The stability of Tween 80 entrapped in the PLGA films was examined by measuring weight changes of the films after immersion in water for 1 and 7 days. As seen in Figure 4, all PLGA/Tween Tween 80 films demonstrated some extraction of Tween 80, but the

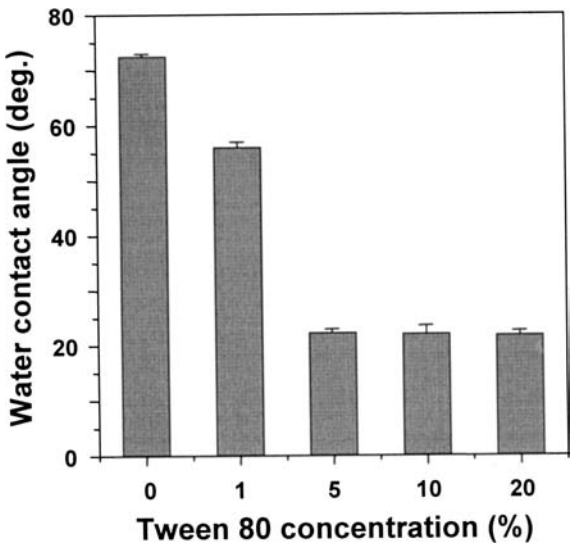


FIGURE 2 Water contact angles of PLGA/Tween 80 film surfaces (n = 5 ∼ 7).

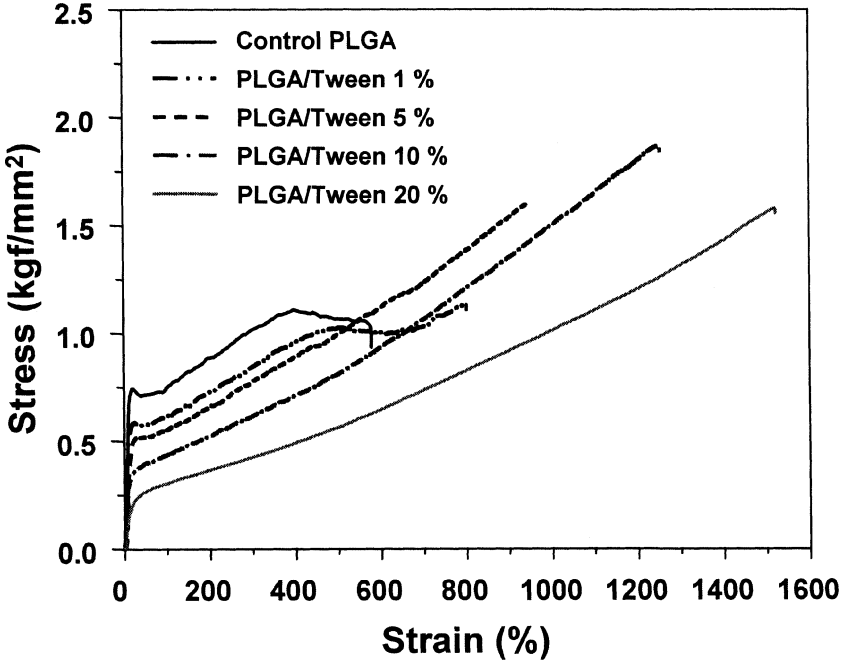


FIGURE 3 Stress-strain curves of PLGA/Tween 80 films.

extraction extent was not much even after 7 days immersion in water. The low extraction of Tween 80 from PLGA/Tween 80 films may be owing to the hydrophobic interactions between a relatively long alkyl chain in Tween 80 and hydrophobic PLGA chain, as discussed earlier.

In vitro cell adhesion and growth behavior on the control PLGA and PLGA/Tween 80 film surfaces were compared to see the effect of added Tween 80 in PLGA. For this, human chondrocytes were seeded ($4 \times 10^4/cm^2$) and cultured on the film surfaces for up to 4 days and the number of cells attached on the film surfaces were counted. Figure 5 shows the cell adhesion (after 1 day) and growth (after 2 and 4 days) behavior on the film surfaces. As the Tween 80 composition in PLGA films increased, the cells attached on the surfaces increased (Tween 80 compositions up to 10 wt%) and then decreased. The increased cell adhesion and growth on the film surfaces may be closely related with the surface hydrophilicity. The preferential adsorption of some serum proteins like fibronectin and vitronectin from culture medium onto the moderately hydrophilic surfaces may be a reason for better cell adhesion and growth [20]. It is not clear yet the reason of decreased cell adhesion and growth on the PLGA/Tween 80 (20 wt%) film surface. The possible explanations may be cell detachment

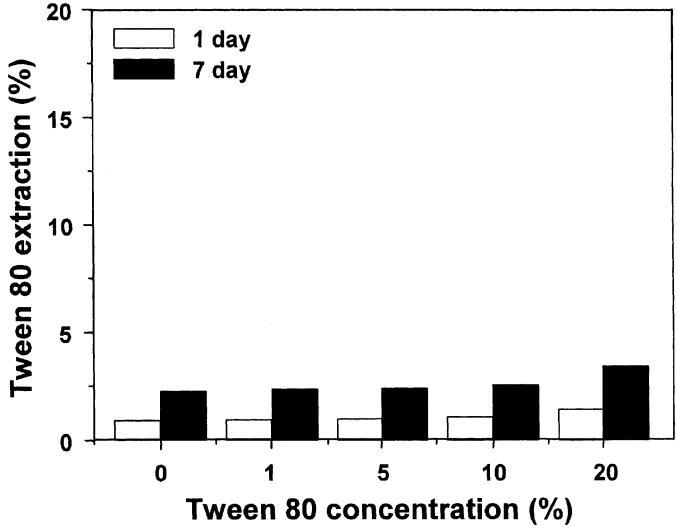


FIGURE 4 Extraction of Tween 80 from PLGA/Tween 80 films after immersion in water for 1 and 7 days.

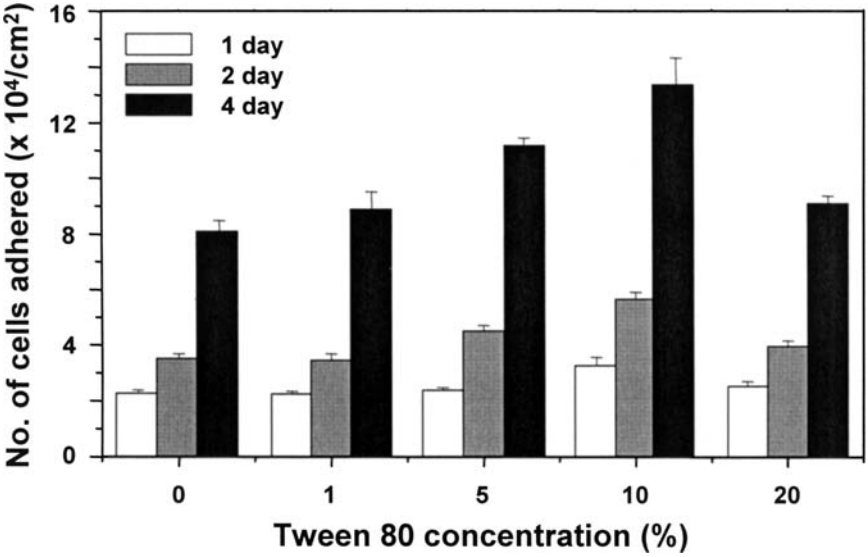


FIGURE 5 Number of chondrocytes adhered and grown on PLGA/Tween 80 film surfaces (n = 3).

during some extraction of Tween 80 existed on the film surface region or some cell membrane injury by extracted Tween 80 even though this possibility is not high.

From above study using PLGA/Tween 80 films, about 10 wt% addition of Tween 80 into PLGA to fabricate PLGA/Tween 80 porous scaffolds seemed optimal at the point of view for their hydrophilicity, mechanical properties and cell compatibility, and thus we fabricated hydrophilic PLGA/Tween 80 (10 wt%) scaffolds for the study of *in vitro* cell compatibility and compared the result with PLGA scaffold as a control.

The PLGA and PLGA/Tween 80 (10 wt%) porous scaffolds were fabricated by a melt-molding particulate-leaching method designed by our laboratory [17]. There have been several methods to fabricate porous biodegradable polymer scaffolds, including solvent casting/particulate leaching, phase separation, emulsion freeze-drying, gas foaming, gel casting, fiber bonding, and 3-D printing [12]. The solvent casting/particulate leaching method has been extensively utilized for the fabrication of porous scaffolds, however the scaffolds prepared by this method often exhibited a dense surface skin layer which is usually formed during the solvent evaporation step. The skin layer causes negative effects for *in vitro* cell seeding and culture or *in vivo* tissue regeneration into the scaffolds. The PLGA and PLGA/Tween 80 scaffolds prepared by a melt-molding particulate-leaching method exhibited highly porous and open-cellular pore structures with almost same surface and interior porosities (Fig. 6). This indicates that the salt particles were homogeneously distributed in the melted polymer matrix and the skin layer was not formed since the solvent was not used during the scaffold fabrication steps. The pore sizes in the scaffolds were almost the same as those of added salt particles (sizes range from 200 to 300 μm). The porosity of the scaffolds determined by a mercury intrusion porosimetry was about 90% for both The PLGA and PLGA/Tween 80 scaffolds. It was reported that the porosity of scaffold should be at least 90% in order to provide a surface area for cell-polymer interactions, sufficient space for extracellular matrix regeneration, and minimal diffusion constraints during *in vitro* cell culture [21,22]. Figure 7 compares the PLGA and PLGA/Tween 80 (10 wt%) scaffolds for their wettability in cell culture medium (DMEM). When the culture medium was dropped to the scaffolds, the medium was completely wetted into the hydrophilized PLGA/Tween 80 scaffold within a few seconds, however it was not wetted at all into the control PLGA scaffold even after 1 hr owing to the hydrophobic character of the scaffold. The fast wetting of the PLGA/Tween 80 scaffold into cell culture medium are highly desirable for tissue engineering applications because the cells can be directly seeded and cultured in this hydrophilized scaffold without any prewetting treatments. If we want to culture cells in the PLGA scaffold, the prewetting treatment is absolutely necessary.

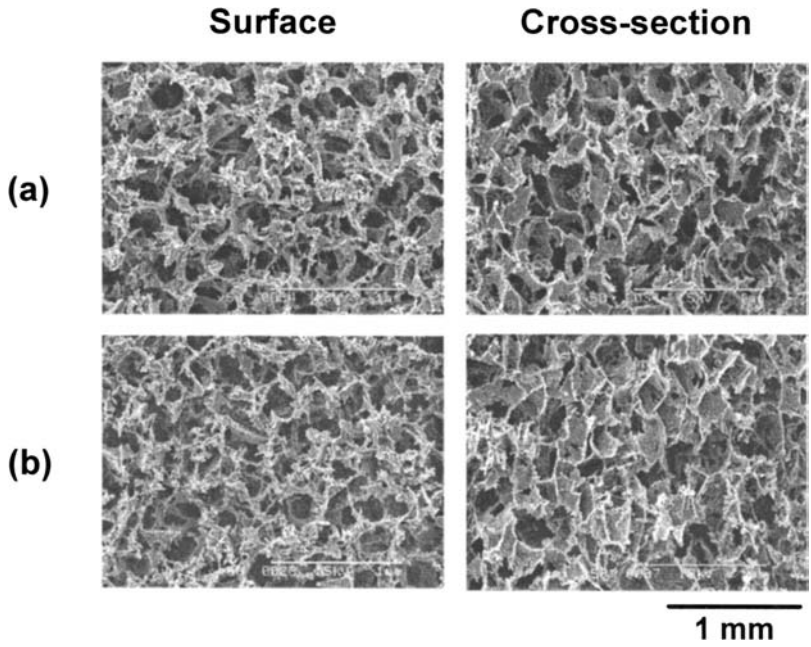


FIGURE 6 SEM photographs of (a) PLGA scaffold and (b) PLGA/Tween 80 (10 wt%) scaffold.

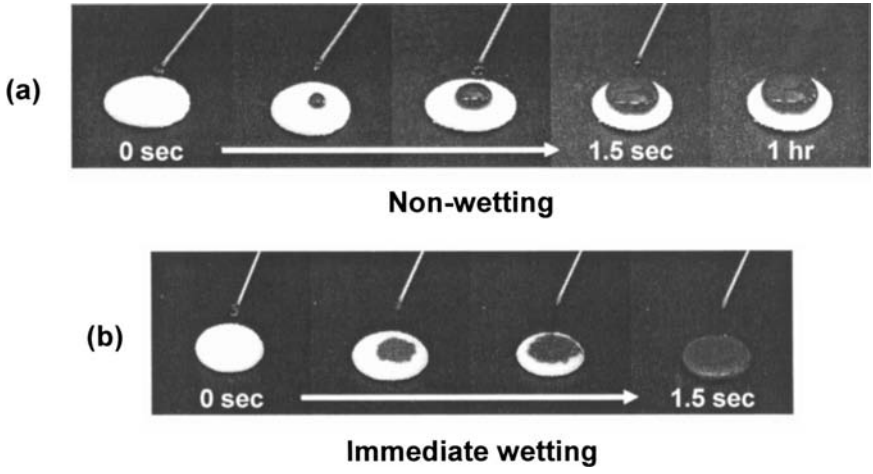


FIGURE 7 Photographs demonstrating the wettability of cell culture medium into (a) control PLGA scaffold and (b) hydrophilized PLGA/Tween 80 (10 wt%) scaffold.

To investigate cell compatibility, human chondrocytes were seeded into EO-sterilized PLGA and PLGA/Tween 80 (10 wt%) scaffolds with the cell density of 2.0×10^5 cells/scaffold. For cell seeding, the PLGA scaffold was prewetted with ethanol because they are hydrophobic and can not be directly wetted in the cell culture medium, as discussed earlier. For the PLGA/Tween 80 scaffold, the cells could be seeded without any prewetting treatments. The cells were cultured in the scaffolds for given periods (0, 1, 2 and 4 weeks) and the viable cell numbers in each scaffold were estimated by MTT assay method, as shown in Figure 8. The seeding density of the cells (data for 0 week) was obtained after the cell-seeded scaffolds were maintained for 2 hr at 37°C in a CO_2 incubator for cell adhesion to the scaffolds. Both scaffolds, PLGA scaffold with cell seeding after ethanol prewetting treatment and PLGA/Tween 80 scaffold with direct cell seeding, showed over 90% cell seeding efficiencies, indicating that both scaffolds fabricated by the melt-molding particulate-leaching method satisfied the requirements for cell scaffolds, i.e., highly porous and interconnected pore structures with almost same exterior and interior porosities. The cells were grown in both scaffolds with time, however the PLGA/Tween 80 scaffold showed better cell growth than the PLGA scaffold. This suggests that the hydrophilized scaffold provided better conditions for efficient transports of oxygen and nutrients to the seeded cells. In addition, even though the PLGA scaffold is wetted with the cell culture medium by the prewetting treatment, the pore surfaces are still hydrophobic and thus may not be a

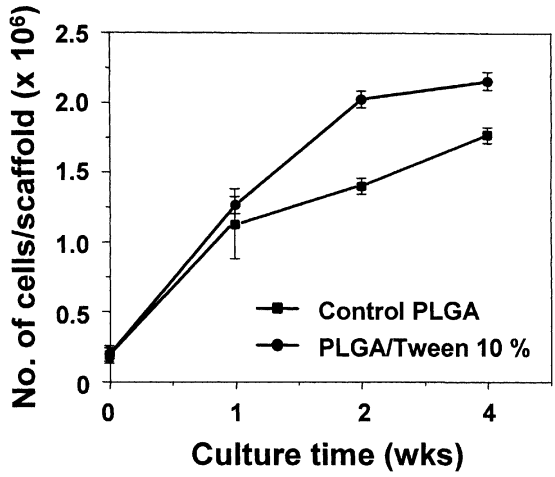


FIGURE 8 Number of chondrocytes adhered and grown in control PLGA and hydrophilized PLGA/Tween 80 (10 wt%) scaffolds. The cell numbers were determined from the MTT assay calibration curve (n = 3).

very good surface condition for cell adhesion and growth compared to the hydrophilized PLGA/Tween 80 scaffold.

CONCLUSIONS

We prepared PLGA/Tween 80 films and scaffolds by solvent casting and a melt-molding particulate-leaching method, relatively. The prepared PLGA /Tween 80 films showed the improved hydrophilicity, elasticity and cell compatibility by the addition of Tween 80. The PLGA/Tween 80 scaffolds exhibited highly porous (about 90% porosity) and open-cellular pore structures with almost same exterior and interior porosities. The PLGA /Tween 80 (10 wt%) scaffold was easily wetted (within a few seconds) in cell culture medium and thus cells could be not only seeded uniformly into the scaffold without any further treatments, but also provides to optimum environment (hydrophilic surface, efficient transports of oxygen and nutrients) for cell adhesion and growth, which are highly desirable for tissue engineering applications. The hydrophilized PLGA/Tween 80 scaffold showed better cell adhesion and growth than the control PLGA scaffold.

REFERENCES

[1] Wu, X. S. (1995). Synthesis and properties of biodegradable lactic/glycolic acid polymers. In: *Encyclopedic Handbook of Biomaterials and Bioengineering*, Wise, D. L., Trantolo, D. J., Altobelli, D. E., Yaszemski, M. J., Gresser, J. D., & Schwartz, E. R. (Eds.), Part A. Marcel Dekker: New York, Vol. 2, 1015–1054.

[2] Mikos, A. G., Lyman, M. D., Freed, L. E., & Langer, R. (1994). Wetting of poly(L-lactic acid) and poly(DL-lactic-co-glycolic acid) foams for tissue culture. *Biomaterials*, *15*, 55–58.

[3] Gao, J., Niklason, N., & Langer, R. (1998). Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells. *J. Biomed. Mater. Res.*, *42*, 417–424.

[4] Ju, Y. M., Oh, S. H., Lee, K. H., Choi, S. W., Cho, C. S., & Lee, J. H. (2000). A study on biodegradable polymer scaffolds with uniform 3-dimensional porosity for artificial cartilage. *Biomater. Res.*, *4*, 52–59.

[5] Hasirci, V., Berthiaume, F., Bondre, S. P., Gresser, J. D., Trantolo, D. J., Toner, M., & Wise, D. L. (2001). Expression of liver-specific functions by rat hepatocytes seeded in treated poly(lactic-co-glycolic) acid biodegradable foams. *Tissue Eng.*, *7*, 385–394.

[6] Yang, J., Shi, G., Bei, J., Wang, S., Cao, Y., Shang, Q., & Yang, G. (2002). Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic-co-glycolic acid) (70/30) cell scaffolds for human skin fibroblast cell culture. *J. Biomed. Mater. Res.*, *62*, 438–446.

[7] Mooney, D. J., Park, S., Kaufmann, P. M., Sano, K., McNamara, K., Vacanti, J. P., & Langer, R. (1995). Biodegradable sponges for hepatocyte transplantation. *J. Biomed. Mater. Res.*, *29*, 959–965.

[8] Maquet, V., Martin, D., Malgrange, B., Franzen, R., Schoenen, J., Moonen, G., & Jerome, R. (2000). Peripheral nerve regeneration using bioresorbable macroporous polylactide scaffolds. *J. Biomed. Mater. Res.*, *52*, 639–651.

[9] Aframian, D. J., Cukierman, E., Nikolovski, J., Mooney, D. J., Yamada, K. M., & Baum, B. J. (2000). The growth and morphological behavior of salivary epithelial cells on matrix protein-coated biodegradable substrate. *Tissue Eng.*, *6*, 209–216.

[10] Nikolovski, J. & Mooney, D. J. (2000). Smooth muscle cell adhesion to tissue engineering scaffolds. *Biomaterials*, *21*, 2025–2032.

[11] Bhati, R. S., Mukherjee, D. P., McCarthy, K. J., Rogers, S. H., Smith, D. F., & Shalaby, S. W. (2001). The growth of chondrocytes into a fibronection-coated biodegradable scaffold. *J. Biomed. Mater. Res.*, *56*, 74–82.

[12] Oh, S. H., Kang, S. G., Kim, E. S., Cho, S. H., & Lee, J. H. (2003). Fabrication and characterization of hydrophilic poly(lactic-co-glycolic acid)/poly(vinyl alcohol) blend cell scaffolds by melt-molding particulate-leaching method. *Biomaterials*, *24*, 4011–4021.

[13] Cai, Q., Yang, J., Bei, J., & Wang, S. (2002). A novel porous cells scaffold made of poly lactide-dextran blend by combining phase-separation and particle-leaching techniques. *Biomaterials*, *23*, 4483–4492.

[14] Maquet, V., Martin, D., Scholtes, F., Franzen, R., Schoenen, J., Moonen, G., & Jerome, R. (2001). Poly(D,L-lactide) foams modified by poly(ethylene oxide)-block-poly(D,L-lactide) copolymers and a-FGF: *in vitro* and *in vivo* evaluation for spinal cord regeneration. *Biomaterials*, *22*, 1137–1146.

[15] Kibbe, A. H. (2000). *Handbook of Pharmaceutical Excipients*, 3rd ed. Pharmaceutical Press: London.

[16] Lee, J. H., Ju, Y. M., Lee, W. K., Park, K. D., & Kim, Y. H. (1998). Platelet adhesion onto segmented polyurethane surfaces modified by PEO- and sulfonated PEO-containing block copolymer additives. *J. Biomed. Mater. Res.*, *40*, 314–323.

[17] Lee, K. H., Oh, S. H., Choi, S. W., & Lee, J. H. (2001). *In-situ* mechanical behavior of porous biodegradable polymer scaffolds fabricated by melt molding compression method. *Biomater. Res.*, *5*, 17–22.

[18] Ritter, H. L. & Drake, L. C. (1945). Pore-size distribution in porous materials. I. Pressure porosimeter and determination of complete macropore-size distribution. *Ind. Eng. Chem.*, *17*, 782–786.

[19] Lee, J. H., Lee, H. B., & Andrade, J. D. (1995). Blood compatibility of polyethylene oxide surfaces. *Prog. Polym. Sci.*, *20*, 1043–1079.

[20] Lee, J. H., Khang, G., Lee, J. W., & Lee, H. B. (1998). Interaction of different types of cells on polymer surfaces with wettability gradient. *J. Colloid Interface Sci.*, *205*, 323–330.

[21] Park, A., Wu, B., & Griffith, L. G. (1998). Integration of surface modification and 3 D fabrication techniques to prepare patterned poly(L-lactide) substrates allowing regionally selective cell adhesion. *J. Biomater. Sci. Polym. Edn.*, *9*, 89–110.

[22] Freed, L. E., Marquis, J. C., Nohria, A., Emmanual, J., Mikos, A. G., & Langer, R. (1993). Neocartilage formation *in vitro* and *in vivo* using cells cultured on synthetic biodegradable polymers. *J. Biomed. Mater. Res.*, *27*, 11–23.