Surface modification of interconnected porous scaffolds

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Abstract: Surface properties of scaffolds play an important role in cell adhesion and growth. Biodegradable poly(α-hydroxy acids) have been widely used as scaffolding materials for tissue engineering; however, the lack of functional groups is a limitation. In this work, gelatin was successfully immobilized onto the surface of poly(α-hydroxy acids) films and porous scaffolds by a new entrapment process. The surface composition and properties were examined using attenuated total reflection–Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectra (XPS), and contact angle measurements. Control over the amount of entrapped gelatin was achieved by varying the solvent composition, the duration of soaking, the concentration of gelatin in solution, and chemical crosslinking. The amount of entrapped gelatin increased with the ratio of dioxane/water in the solvent mixture used. Chemical crosslinking after physical entrapment considerably increased the amount of retained gelatin on the surface of poly(α-hydroxy acids). Osteoblasts were cultured on these films and scaffolds. The surface modification significantly improved cell attachment and proliferation. Cell numbers on the surface-modified films and scaffolds were significantly higher than those on controls 4 h and 1 day after cell seeding. The osteoblasts showed higher proliferation on surface-modified scaffolds than on the control during 4 weeks of *in vitro* cultivation. More collagen fibers and other cell secretions were deposited on the surface-modified scaffolds than on the control scaffolds. This novel surface treatment strategy provides a convenient and universal way to modify the surface properties of three-dimensional scaffolds and thus promote cell adhesion and proliferation for tissue engineering. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 74A: 84–91, 2005

Key words: entrapment; gelatin; PLGA; PLLA; scaffold; surface; tissue engineering

INTRODUCTION

The surface properties of biomaterials are a key aspect in determining compatibility with the biological environment and cellular responses.[1–6] Ideally, scaffolding materials should be designed to be bioactive so that they can receive and respond to specific biological signals, which direct and promote cell attachment, proliferation, differentiation, and tissue regeneration.[7–10]

Poly(α-hydroxy acids), which include poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymers (PLGA), have been extensively studied as scaffolding materials for tissue regeneration.[11–17] They have the advantage of FDA approval for certain clinical applications. They also possess good mechanical properties, low immunogenicity and toxicity, and predictable biodegradation kinetics. However, the lack of available functional groups in the polymeric chains to conjugate specific cell-recognizable signal molecules has limited their scope in tissue engineering applications.

Several approaches have been developed to circumvent this disadvantage. Simple surface coating is a general approach to adsorb the required surface-modifying species onto the polymer surface.[18] However, this method requires that the surface-modifying solution spreads well on the substrate to ensure coating efficiency. The interactions between the bulk polymer and the surface-modifying species are usually weak, and thus the surface is unstable. There are more-complex coating methods involving chemical reactions.[19–21] For example, a biomimetic method has been developed to grow bonelike mineral on three-dimensional (3D) porous polymer materials as scaffolds for bone tissue engineering.[20–22] Another strategy is to

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introduce functional groups in the polymer chains.[23,24] For example, poly(L-lactide-*co*-lysine) was synthesized to provide reactive amine groups in the PLA backbone for the conjugation of cell adhesion peptide arginine-glycine-aspartic acid (RGD).[24] These functionalized polymers, aiming at chemical modification of surface properties, alter their bulk properties as well,[7] which may result in certain undesirable effects. Plasma exposure is effective for two-dimensional (2D) film surface etching.[23,25] This technique can be used to introduce the desired groups or chains onto the surface of a material but is limited for a 3D scaffold with designated morphology and structure.

In this study, we introduce a simple but effective surface modification method. Gelatin was immobilized onto the surface of poly(α-hydroxy acids) by a new entrapment procedure. Gelatin is made up of high-molecular-weight polypeptides derived from collagen, the primary protein component of connective tissues.[26] Gelatin is formed by breaking the natural triple-helix structure of collagen into single-strand molecules and has been used in many tissue engineering applications because of its biocompatibility and ease of gelation.[27–29] Further chemical modification of gelatin is also convenient because it contains abundant amino and carboxyl groups in the polymeric chains. The surface-modified poly(L-lactic acid) (PLLA) films and scaffolds were examined for cell adhesion and proliferation.

MATERIALS AND METHODS

Materials

Poly(L-lactic acid) and poly(lactide-*co*-glycolide) (85/15) (PLGA85/15) with an inherent viscosity of approximately 1.6 and 0.5–0.6, respectively, were purchased from Boehringer Ingelheim (Ingelheim, Germany) and used without further purification. Gelatin (type B, from bovine skin, approx 225 bloom), 1,4-dioxane, glutaraldehyde, osmium tetraoxide, hexamethyldisilazane (HMDS), β-glycerophosphate, neutral-buffered formalin, trypan blue, and DNA quantitation kit were purchased from Sigma Chemical Co. (St. Louis, MO). N-Hydroxy-succinimide (97%) (NHS) and {2-[N-morpholino] ethanesulfonic acid} hydrate (MES) were purchased from Aldrich Chemical (Milwaukee, WI). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) was purchased from Pierce Biotechnology (Rockford, IL). Fetal bovine serum, α-minimum essential medium (α-MEM), penicillin-streptomycin, Dulbecco's phosphate-buffered saline (PBS), and trypsin–edetic acid (EDTA) were purchased from Gibco BRL Products, Life Technologies (Grand Island, NY). Ascorbic acid was purchased from Fisher Scientific (Pittsburgh, PA). Ethylene oxide was purchased from H.W. Anderson Products (Chapel Hill, NC).

Fabrication of poly(α-hydroxy acids) films and scaffolds

The PLLA (0.5 g) was dissolved in 1,4-dioxane (10 mL) at 50°C, and the PLLA solution was cast onto a 10 cm × 10 cm Teflon mold. After solvent evaporation in a fume hood at room temperature for 24 h, the films were removed from the mold and dried under vacuum for another 24 h. The thickness of the film was controlled to be approximately 40 μm by adjusting the concentration of PLLA solution and coverage area.

Dried PLLA films were first washed with hexane to remove possible surface contaminants and then wetted in ethanol. The ethanol-treated films were soaked in distilled water for 30 min. Water was changed three times to ensure that ethanol was completely replaced by water.

Poly(α-hydroxy acids) 3D scaffolds were fabricated by using paraffin as porogen as reported earlier.[30] Briefly, paraffin spheres (diameter 250–420 μm) were added to Teflon molds, and the top surface was leveled. The molds were then preheated at 37°C for 20 min to ensure that paraffin spheres were interconnected. A 10% (w/v) PLLA/dioxane solution was cast onto paraffin sphere assemblies and vacuum-treated in a vacuum oven to remove air trapped inside the paraffin sphere assemblies. The polymer/paraffin composite was air- and vacuum-dried for 3 days, and then hexane was added to leach paraffin from the composite. Cyclohexane was exchanged with hexane. Polymer scaffolds were lyophilized and cut to samples with 7.2 mm diameter and 2.0 mm thickness. The poly(α-hydroxy acids) scaffolds were washed with hexane followed by ethanol pretreatment before surface modification.

Surface physical entrapment of gelatin

Gelatin was dissolved in dioxane/water solvent mixture at around 50°C. The pretreated poly(α-hydroxy acids) films or scaffolds were immersed in the gelatin solution for a designated time, then taken out and quickly put into 200 mL ice/water mixture for 10 min. The above-treated films or scaffolds were rinsed in water at 40°C for 12 h. Water was changed every 3 h to remove nonentrapped gelatin from the poly(α-hydroxy acids) surface. The films or scaffolds were dried at room temperature for 24 h and vacuum-dried for another 24 h.

Simple coating of poly(α-hydroxy acids) films or scaffolds was also performed as described above, except that distilled water instead of dioxane/water mixture was used as solvent for gelatin.

Chemical crosslinking after physical entrapment

Chemical crosslinking was performed after the surface treated poly(α-hydroxy acids) films or scaffolds were dipped in an ice/water mixture. Crosslinking of gelatin with EDC and NHS was carried out in MES buffer (pH 5.3, 0.05*M*) at 4°C for 24 h. The films or scaffolds were then

washed three times with distilled water at 4°C, followed by rinsing at 40°C for 12 h (water was changed every 3 h). The chemically crosslinked films or scaffolds were then air- and vacuum-dried for 2 days.

Characterization of surface modification

X-ray photoelectron spectroscopy

The XPS analyses were performed on a PerkinElmer model PHI 5400 XPS instrument (Physical Electronics Inc., Chanhassen, MN) with a monochromatized Al anode operated at 300 W, 15 kV, and 20 mA. Samples were attached to the aluminum sample platform with double-sided tapes. The take-off angle was 30° with respect to sample plane. The pressure during analysis was maintained at about 10^{-9} Torr. Survey spectra were obtained using a passing energy of 89.45 eV, and high-resolution spectra were obtained using a passing energy of 17.90 eV. All binding energies were referenced to carbon CHx peaks at 285.0 eV. Quantitative analyses were performed using peak areas and elemental sensitivity factors. Element surface composition was expressed in atomic percent (at%), and nitrogen (N) was used to characterize the amount of entrapped gelatin on the surface of poly(α-hydroxy acids).

Contact angle measurements

Contact angles were measured at room temperature with an image analyzing system (CAM100; KSV Instruments Ltd., Helsinki, Finland) using the sessile drop technique. Double-distilled water was used in the measurement. Each determination was obtained by averaging the results of at least eight droplets placed at different positions on the sample surface.

ATR-FTIR analysis

The ATR-FTIR spectra of films were obtained with a Perkin Elmer 1800 FTIR spectroscopy, in the region from 500 to 4000 cm^{-1}. During the measurement, the sample chamber was purged with nitrogen gas to reduce moisture content. We determined the average for 1024 scans at a resolution of 4 cm^{-1}.

Cell culture

The thawed MC3T3-E1 osteoblasts (clone 26) were cultured in a supplemented α-MEM and 10% fetal bovine serum (FBS) containing 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator at 37°C with 5% CO_2. The medium was changed every other day. The cells of passages 3 and 4 were seeded onto the 2D PLLA films and 3D PLLA scaffolds (control and surface-modified groups). The viability of cells before seeding was higher than 90%, as determined by the Trypan blue exclusion assay.

Cell attachment on 2D films

Four types of surface-modified PLLA films, namely, DI00, DI20, DI40, and DI60, were prepared in dioxane/water solvent mixture with a dioxane volume percent of 0%, 20%, 40%, and 60% in the solvent mixture, respectively, and used for cell attachment experiments. These films were assembled on the bottoms of custom-made 12-well Teflon plates with a well diameter of 10 mm. The film-containing culture plates were sterilized with ethylene oxide for 24 h. The sterilized films were soaked in ethanol for 30 min, exchanged with 1% PBS solution for 30 min, and then washed twice with α-MEM (with 10% FBS and 1% antibiotics). Each film was seeded with 1×10^4 cells and cultured at 37°C with 5% CO_2. The cells were detached by adding 0.05% trypsin and 0.53 mM EDTA 4 h after cell seeding and were counted using a hemocytometer.

Cell adhesion and proliferation on gelatin-modified scaffolds

Three types of scaffolds, namely, PLLA scaffolds (control), surface-modified PLLA scaffolds by physical entrapment, and surface-modified PLLA scaffolds by chemical crosslinking after physical entrapment, were prepared for cell attachment and proliferation. The scaffold-containing culture flasks were sterilized with ethylene oxide for 24 h. The scaffolds were soaked in ethanol for 30 min and then exchanged three times with PBS. The scaffolds were washed twice with a complete medium (α-MEM, 10% FBS, 1% antibiotics, and additional 50 mg/L of L-ascorbic acid and 80 mM β-glycerophosphate). Cells (5×10^5) suspended in 2 mL of complete medium were seeded on each scaffold. They were cultured in 25-cm^2 tissue culture flasks on an orbital shaker (Model 3520; Lab-Line Instrument, Melrose Park, IL) at 100 rpm in a humidified incubator at 37°C with 5% CO_2. The cell-scaffold constructs were removed from the tissue culture flasks and transferred into six-well tissue culture plates 24 h after cell seeding. The constructs were cultured with the complete medium on the orbital shaker at 50 rpm in the humidified incubator at 37°C with 5% CO_2. Four milliliters of medium was used for each construct, and the medium was changed every other day. Total DNA amounts were measured 1 day and 4 weeks after cell seeding.

DNA assay

Cell numbers on the scaffold were determined using a DNA assay. The cell-scaffold constructs were homogenized using a Polytron homogenizer (Brinkmann Easycare Generator; Polytron-Aggregate, Switzerland) for 30 s at top speed (VI) three times before DNA isolation. Using fluorescence

assay with Hoechst 33258 dye, DNA assays were performed 1 day and 4 weeks after cell seeding.

Cell morphology

After having been cultured for 4 weeks, the scaffolds were taken out of the culture plates and washed three times with PBS. The samples were fixed with 3% glutaraldehyde in PBS at 4°C for 24 h. After being thoroughly washed with PBS, the samples were treated with 1% osmium tetraoxide in 0.1*M* cacodylate buffer for 1 h and then washed with PBS. The samples were dehydrated sequentially in 50%, 60%, 70%, 80%, 90%, and 100% ethanol for 30 min each and then critical-point–dried with HMDS. The dried samples were sliced evenly into two 1-mm-thick pieces. The sliced side of the scaffolds was coated with gold and analyzed with SEM. A domain around 1.0 mm from the perimeter of the cylindrical sample was selected for SEM observation.

Statistical analysis

All data were presented as means ± standard deviations (SD). To test the significance of observed differences between the study groups, an unpaired Student's t test (two-tail) was applied. A value of $p < 0.05$ was considered to be statistically significant.

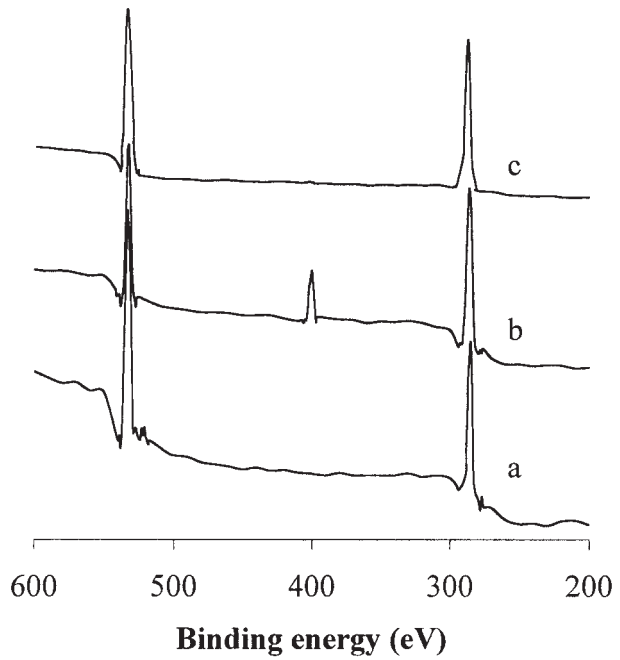

Figure 1. The XPS survey scan spectrum of simply coated PLLA surface: (a) control; (b) immersed in aqueous gelatin solution (3.0 g/L) for 10 min; (c) immersed in aqueous gelatin solution (3.0 g/L) for 10 min, then rinsed in water at 40°C for 12 h.

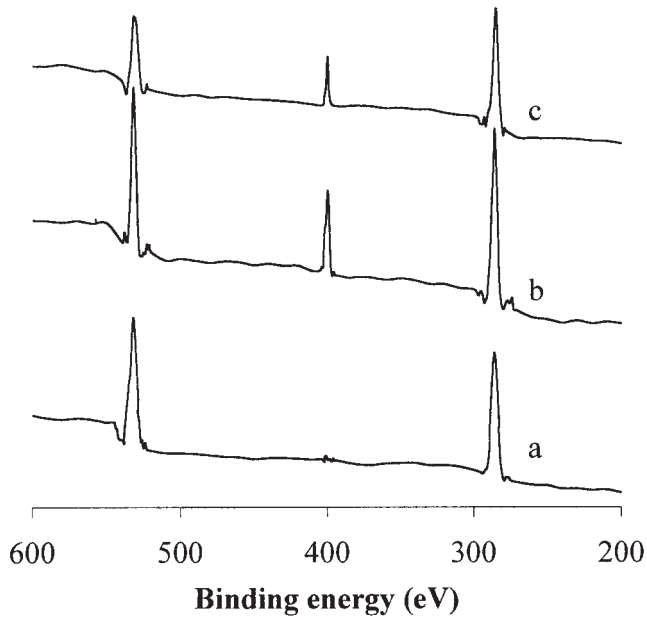

Figure 2. The XPS survey scan spectrum of surface entrapped PLLA films: (a) control; (b) immersed in 3.0 g/L gelatin solution (dioxane/water = 60/40) for 10 min; (c) immersed in 3.0 g/L gelatin solution (dioxane/water = 60/40) for 10 min, then rinsed in water at 40°C for 12 h.

RESULTS

An aqueous gelatin solution was used to examine the effect of simple physical adsorption or deposition of gelatin on PLLA film surface. The XPS measurement revealed that a visible new peak at binding energy of 400.0 eV appeared after simply immersing PLLA films in an aqueous gelatin solution (Fig. 1). The peak at binding energy of 400.0 eV corresponds to nitrogen element of gelatin molecules. However, the peak disappeared after the sample was rinsed in distilled water at 40°C, indicating that the adsorbed gelatin left the surface of PLLA films and dissolved in water.

Nitrogen peak at binding energy of 400.0 eV appeared after the PLLA film was immersed in gelatin solution [dioxane/water = 60/40 (v/v); Fig. 2]. The peak remained after the gelatin-entrapped PLLA film was rinsed in distilled water at 40°C for 12 h, although the peak strength decreased. The decrease of peak strength resulted from the dissolution of simply deposited gelatin molecules on the PLLA film surface. In the ATR-FTIR spectra, two new vibration peaks at 1528 and 1636 cm^{-1} appeared after the PLLA film was immersed in gelatin solution (Fig. 3). These two peaks were the amide bond absorption of gelatin, and their strength decreased when the PLLA film was rinsed in water at 40°C. Both results indicated that gelatin was successfully entrapped onto the PLLA film surface.

The amount of nitrogen on the surface of gelatin-

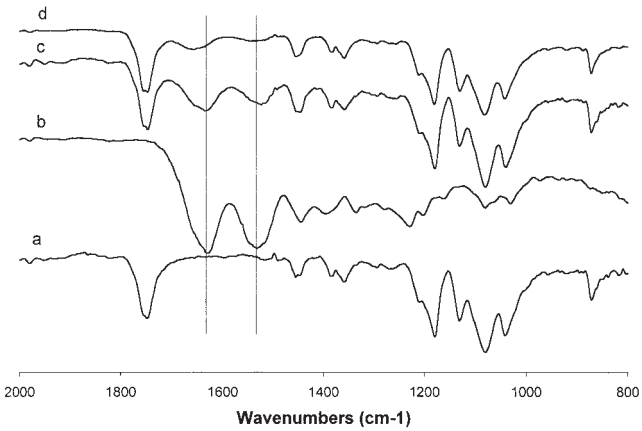

Figure 3. The ATR-FTIR spectra of surface-modified PLLA films: (a) PLLA film; (b) gelatin; (c) immersed in 3.0 g/L gelatin solution (dioxane/water = 60/40) for 10 min; (d) immersed in 3.0 g/L gelatin solution (dioxane/water = 60/40) for 10 min, then rinsed in water at 40°C for 12 h.

modified PLLA film increased with the ratio of dioxane/water used (Table I). For surface-modified PLLA films with chemical crosslinking after physical entrapment, the amount of entrapped gelatin on the surface was significantly increased (Table II). The amount of nitrogen reached 12.4% for the PLLA film treated with chemical crosslinking after physical entrapment in 60/40 (v/v) dioxane/water solvent mixture, whereas the nitrogen amount was 6.3% when only physical entrapment was applied. The amounts of gelatin entrapped on PLGA films were always higher than that on PLLA films when the same gelatin solution was used. This seemed to indicate that an amorphous polymer (PLGA) allowed more entangles to form than did a semicrystalline polymer (PLLA).

The amount of nitrogen on the PLLA film surface increased from 5.2% to 12.4% as immersing time increased from 1 min to 10 min (Table III). Further increasing the immersing time had little effect on the amount of entrapped gelatin.

The surface wettability changed as the poly(α-hydroxy acids) surfaces were modified with gelatin (Fig. 4). The contact angle decreased when PLLA films were

TABLE I
Effect of Solvent Composition on the Amount of Entrapped Gelatin on PLLA Films[a]

Polymer	Dioxane/H_2O (v/v)	N (at%)
PLLA	0/100	1.1
	20/80	2.7
	40/60	5.1
	60/40	6.3

[a] The PLLA films were immersed in 3.0 g/L gelatin solution for 10 min, followed by rinsing in water at 40°C for 12 h.

TABLE II
Amount of Gelatin on Polymer Films (PLLA and PLGA) After Physical Entrapment (Immersed in 3.0 g/L Gelatin Solution for 10 min) and Chemical Crosslinking

Polymers	Dioxane/H_2O (v/v)	N (at%)
PLLA	0/100	1.2
	20/80	4.5
	40/60	8.9
	60/40	12.4
PLGA85	10/90	6.8
	20/80	8.1
	30/70	10.2

entrapped with gelatin, indicating the enhanced hydrophilicity of surface-modified PLLA films.

The cell numbers on the gelatin-modified PLLA films (DI40 and DI60) were significantly higher than that on the control (DI00) (Fig. 5). Increasing the amount of entrapped gelatin on the film surface improved cell adhesion.

The DNA content of the cell-scaffold constructs was measured to quantify the osteoblast adhesion and proliferation on the surface-modified scaffolds and control scaffolds (Fig. 6). The DNA amount on the surface-modified PLLA scaffold (physical entrapment followed by chemical crosslinking) was significantly higher than that on the control scaffold after 1 day of cell seeding ($p < 0.05$). The DNA amount on the surface-modified scaffolds (including both physical entrapment and chemical crosslinking after physical entrapment) was significantly greater than that on the PLLA control 28 days after cell seeding. In addition, the DNA amount on the surface-modified scaffold with only physical entrapment was significantly lower than that on the surface-modified scaffold with physical entrapment followed by chemical crosslinking ($p < 0.05$).

The SEM images of osteoblasts cultured on the surface-modified PLLA scaffolds and control scaffolds were taken 4 weeks after cell seeding (Fig. 7). More collagen fibers and other cell secretions were observed on the surface-modified scaffolds than on the control. In surface-modified scaffolds with chemical crosslinking, secreted collagens were bound together to form

TABLE III
Effect of Entrapment Time on the Amount of Entrapped Gelatin on PLLA Films[a]

Entrapment time (min)	N (at%)
1	5.2
5	9.7
10	12.4
60	12.8

[a] The PLLA films were immersed in 3.0 g/L gelatin solution (dioxane/water = 60/40 (v/v)) followed by chemical crosslinking.

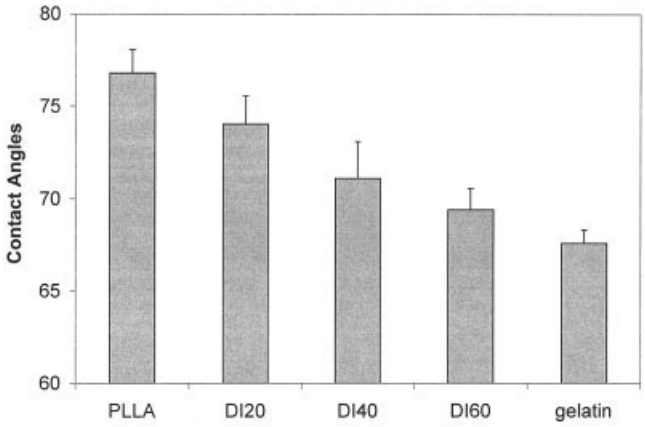

Figure 4. The effect of solvent composition on contact angles of surface-modified PLLA films. The PLLA films were immersed in 3.0 g/L gelatin solution for 10 min, followed by rinsing at 40°C for 12 h.

large collagen bundles as a result of increased fiber density [Fig. 7(c,d)].

DISCUSSION

Simple coating is an uncomplicated and commonly used surface modification method. However, the main shortcomings of this method are its low efficiency and surface instability. Although gelatin molecules could deposit on poly(α-hydroxy acids) surface when a poly(α-hydroxy acids) film or scaffold was immersed in an aqueous gelatin solution, the adsorption of gelatin was not stable. When rinsed in distilled water above the gelation temperature, the adsorbed gelatin molecules on the film or scaffold surface were dissolved, leaving behind an uncoated surface.

The main difference of surface modification by entrapment from simple coating was the use of a solvent mixture. For the poly(α-hydroxy acids)-gelatin system, a mixture of dioxane and water was selected as the solvent system. The solvent mixture composition was chosen such that gelatin was soluble in the solvent mixture, while the poly(α-hydroxy acids) swelled but did not dissolve in the solvent mixture. When a poly(α-hydroxy acids) film or scaffold was immersed in the gelatin/dioxane/water mixture, gelatin molecules diffused onto the swollen surface of the polymer and were entangled with polymer molecules on the surface. As the surface-swollen polymer was removed from gelatin solution and quickly dipped into cold water, a nonsolvent for the polymer, the surface rapidly shrank and gelatin molecules on the polymer surface were entrapped and immobilized. The entrapped gelatin was stable and did not dissolve when rinsed in water at 40°C. This is a simple and universally effective method for surface modification. No functional groups in polymer chains are needed for this surface modification method. In contrast to the method of incorporating modifying groups by copolymerization, the entrapment method maintains the bulk properties of materials. As the modifying molecules only gather on the surface of materials, the efficiency is high for surface modification. In addition, the entrapment method can be used for any geometry, morphology, and thickness of 3D scaffold, which is a limitation for some other surface modification methods (for example, surface treatment with plasma). Furthermore, the entrapment method allows us to modify the surface in a controlled fashion because various parameters (solvent ratio, gelatin concentration, immersing time, and chemical crosslinking) can be varied to tailor the process.

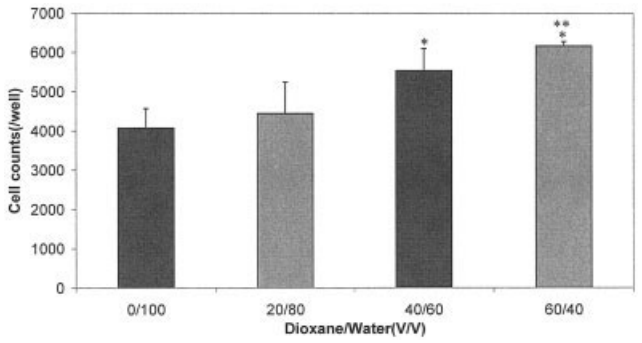

Figure 5. The attached numbers of MC3T3E1 osteoblasts 4 h after cell seeding. Cells (1×10^4) were seeded on each film. Cell numbers were measured using a hemocytometer. (Statistical significance: $^*p < 0.05$ compared with the control group DI00; $^{**}p < 0.05$ compared with DI20.)

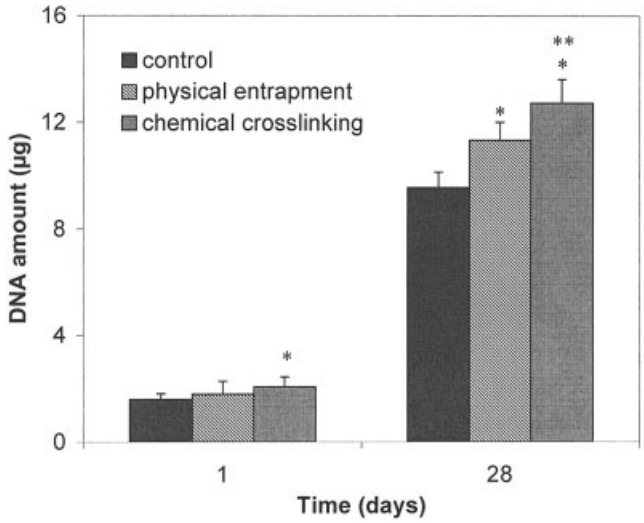

Figure 6. The DNA amounts in the PLLA scaffolds (control group) and gelatin-modified PLLA scaffolds according to cultivating time. Cells (5×10^5) were seeded into each scaffold. (Statistical significance: $^*p < 0.05$ compared with the control group; $^{**}p < 0.05$ compared with the surface physical entrapment group.

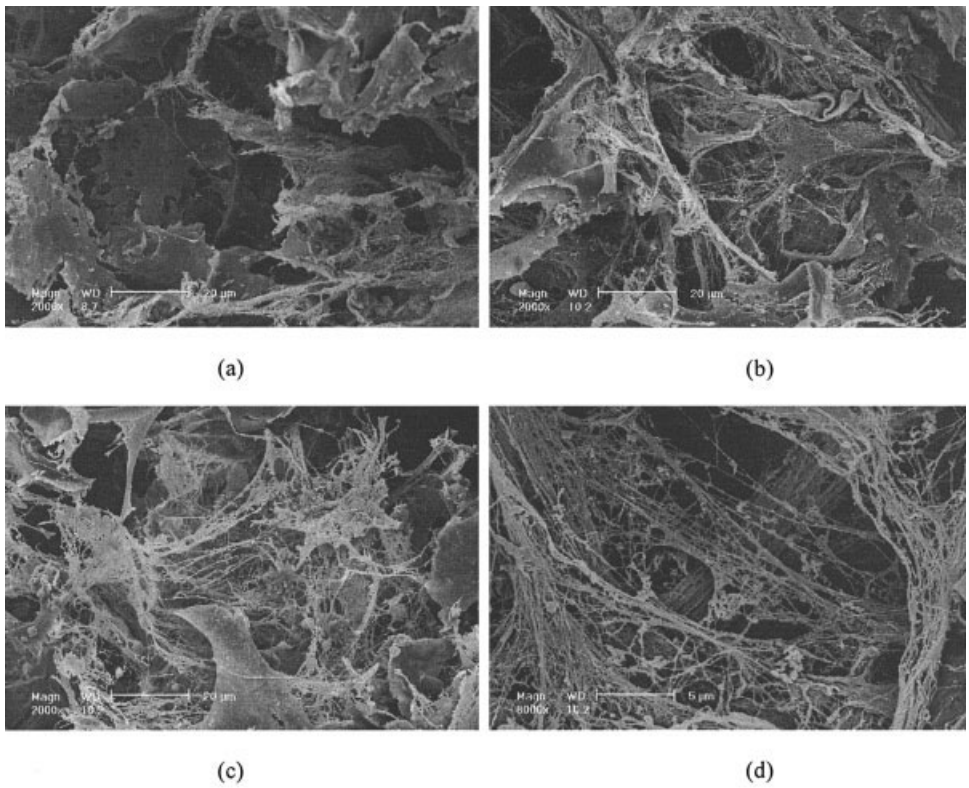

Figure 7. The SEM images of PLLA scaffolds 4 weeks after cell seeding: (a) PLLA control; (b) physical entrapment in 3.0 g/L gelatin solution (dioxane/water = 60/40) for 10 min; (c) chemical crosslinking following the physical entrapment; (d) chemical crosslinking [high magnification of (c)].

Dioxane is a good solvent for PLLA and PLGA, and the interaction between the polymer and solvent mixture increases with the ratio of dioxane in the solvent mixture. The stronger interaction leads to a more swollen surface, thus allowing more gelatin molecules to enter the swollen surface and be entrapped. Therefore, the amount of entrapped gelatin on the polymer surface increased with the ratio of dioxane/water. The gelatin amount on the PLLA surface was highest when the dioxane ratio in solvent composition reached 60%. Further increasing dioxane in the solvent mixture led to the insolubility of gelatin, and the process of entrapment could not be performed.

Chemical crosslinking after physical entrapment was utilized to further increase the amount of gelatin on the surface. When the polymer sample was quenched in cold water, there were some gelatin molecules that were not entrapped on the polymer surface but were entangled with the entrapped gelatin molecules. These gelatin molecules would be washed away when rinsed in warm water. Chemical crosslinking immobilized these gelatin molecules, increasing the amount of gelatin on the polymer surface.

Gelatin is more hydrophilic than is PLLA, thus the decrease of contact angles after surface modification indicated that gelatin molecules were successfully incorporated onto the PLLA surface. The lower contact angle indicated higher gelatin amount on the surface.

The gelatin-modified porous PLLA and PLGA scaffolds combine the advantages of both synthetic and natural materials. Synthetic PLLA provides the mechanical strength and controllable degradation rate. Gelatin is known to promote cell adhesion and proliferation.[26,28] Statistically, more cells attached on the surface-modified 2D films and 3D scaffolds than on the controls (Figs. 5 and 6). The cells attached on the films or scaffolds increased with increasing amount of gelatin. Cell proliferation was higher on the surface-modified scaffolds than on the control scaffolds, and the proliferation rate increased with the amount of gelatin on the scaffold surface. More extracellular matrix (ECM) was secreted by cells in surface-modified scaffolds than in the controls. All these results demonstrated that surface-modified scaffolds are superior to control scaffolds.

CONCLUSIONS

A novel treatment procedure has been developed for surface modification of porous polymer scaffolds. Gelatin was successfully entrapped onto the surface of poly(α-hydroxy acids) films and scaffolds by a procedure that involved soaking polymer films and scaffolds in solutions of gelatin in dioxane/water mix-

tures. Control over the amount of entrapped gelatin was achieved by varying the solvent composition, the duration of soaking, the concentration of gelatin solution, and chemical crosslinking. The hydrophilicity increased with the amount of entrapped gelatin. The surface modification significantly improved cell attachment and proliferation. More collagen fibers and other cell secretions were observed in the surface-modified scaffolds than in the control scaffolds. This novel surface treatment strategy provided a convenient and universal way to modify the surface properties of porous scaffolds and thus promote cell adhesion and proliferation for tissue engineering.

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