Electrospun Poly(D,L)-Lactide Nonwoven Mats for Biomedical Application: Surface Area Shrinkage and Surface Entrapment

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ABSTRACT: Nanofibrous poly(D,L)-lactide mats prepared by electrospinning are useful for numerous biomedical applications. However, it was observed that these mats tend to shrink under physiological conditions. In this research, a physical entrapment method to modify the polymer surface with poly(ethylene glycol) was developed to ensure dimensional stability and to increase the hydrophilicity of the surface of the mats. Nanofiber morphology was characterized by scanning electron microscopy. Surface element analysis was performed by high resolution X-ray photoelectron spectroscopy. Water contact angles were determined to identify surface properties before and after surface entrapment. Canine fibroblasts were prepared and seeded onto the poly(D,L)-lactide mats, followed by cell morphology study by SEM and cell viability tests by MTT assay, which confirmed the improvement of biocompatibility by surface modification. Taking the results into account, hydrophilic and area-stable nanofibrous nonwoven mats were successfully produced, with potential applications as *in vivo* biomedical material. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1219–1225, 2011

Key words: electrospinning; poly(D,L)-lactide; surface modification; tissue engineering

INTRODUCTION

In recent years, electrospinning has been studied intensively as an easy, effective and versatile technique to fabricate nonwoven materials with fiber diameters in the range of a few nanometers to several microns.[1,2] Ultra-fine polymer fibers from almost any kind of polymer solution can be produced by using the electrostatic force between a needle spinneret and a grounded collector.[3,4] Electrospun nonwoven mats with a very large surface area to volume ratio have been developed targeting a wide range of applications, such as filters,[5] catalysts,[6] sensors,[7] and especially biomedical materials in tissue engineering, wound healing, and drug release.[8–10]

For any practical applications of electrospun nanofibers, surface properties are most important. Especially for biomedical materials, surface properties are critical to get the desired biological interactions.[11,12] Surface modifications can be approached during the electrospinning process by design of the set-up,[13,14] or after electrospinning. Postspin chemical modification is the major method studied by researchers, and hydrolysis,[15] air plasma treatment,[16,17] and surface grafting[18] have been reported. Electrospun nanofibers were also coated by fluorescent proteins[19] and conducting polymers.[20] In regard to tissue engineering applications, cell attachment and cell proliferation have been improved by a variety of surface modification techniques,[15,17,18] leading to enhanced biocompatibility.

To incorporate electrospun nonwoven mats into *in vivo* scaffolds or to use as drug carriers, aliphatic polyesters[21,22] were probably the most commonly investigated of all available biodegradable and biocompatible polymers. However, in our former study[23] and other researchers' work,[24] surface area shrinkage was observed with electrospun PLA fiber mats when the fiber mats were immersed in physiological buffer solution at 37°C. For *in vivo* incorporation, the dimensional stability of the scaffold is critical.[25] Among other attempts, Lee et al.[26] used thermal treatments to maintain the area stability and biomechanical properties. A further problem is that poly(D,L-lactide) (PDLLA) is a hydrophobic material, and electrospun into fibers, could be even more hydrophobic,[23] while hydrophilicity is required for tissue engineering scaffolds.[27] Thus, the purpose of this study was to generate nonwoven PDLLA nanofiber mats that retain a stable area at human physiological conditions, and further to devise a method

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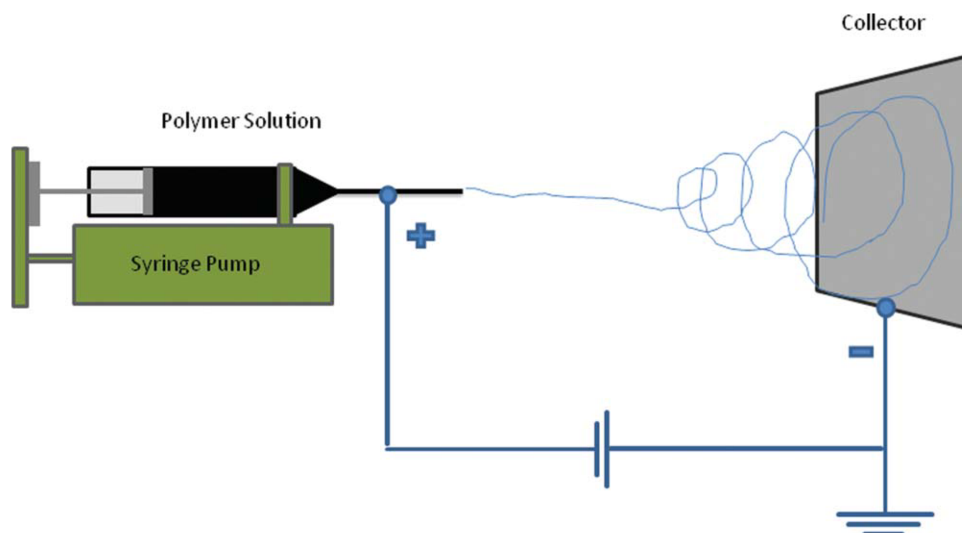

Scheme 1 Experimental set-up of the electrospinning process. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that would increase their hydrophilicity. We developed a simple physical surface entrapment method to introduce hydrophilic poly(ethylene glycol) (PEG) onto the surface of the nanofibers. The surface area stabilization and PEG introduction were conducted in a one-step process. Both of these two modifications could be critical for electrospun nonwovens as tissue engineering materials in the future. Cell viability tests were performed using canine fibroblasts on the modified mats.

EXPERIMENTAL

Materials

Poly(D,L-lactide) (PDLLA, Mw = 75,000–120,000), poly(ethylene glycol) (PEG, Mw = 14,000), 2,2,2-trifluoroethanol (TFE) and benzyltriethylammonium chloride (BTEAC) were obtained from Sigma-Aldrich and used as received. Chloroform (analytical grade) was obtained from Fisher Scientific. Tris buffer solution (0.05*M*) was prepared from tris(hydroxymethyl)aminomethane hydrochloride (Trizma^{TM} HCl; Sigma-Aldrich) and adjusted to pH 7.35. The MTT cell proliferation assay kit (V-13154) was purchased from Invitrogen and used by following the instructions of the manufacturer.

Electrospinning and surface modification

For electrospinning, 7 wt % PDLLA were dissolved in chloroform by gently stirring at room temperature for at least 12 h. Ten milligrams of BTEAC was added to the solution to improve the spinnability. For the electrospinning process, a horizontal experimental setup was used, consisting of a syringe, an 18-gauge needle, an aluminum collecting board, and a high voltage supply. A syringe pump connected to the syringe controlled the flow rate to 1 mL/h. PDLLA solution was electrospun at a voltage of 18 kV with a tip-to-collector distance of 15 cm. The experimental set-up of the electrospinning process is shown in Scheme 1.

The surface entrapment modification was conducted according to the work of Quirk et al.[28] A solution containing 50 wt % PEG, 10 wt % TFE, and 40 wt % deionized water was prepared first. PDLLA nanofiber mats were immersed in the solution at 42°C for 120 min, followed by washing with a large excess of water to remove the un-trapped PEG.

Characterization

The morphology of the electrospun fibers was investigated with a Zeiss DMS 940 scanning electron microscope (SEM) at 15 kV. Electrospun mats were sputter-coated with gold for 2 min to minimize charging effects. The diameters of the fibers were estimated from SEM images.

For surface area shrinkage measurements (*in vitro*) electrospun nonwoven mats were first cut into squares of 20 mm × 20 mm. Then PDLLA nonwoven pieces were immersed in Tris buffer at different set temperatures. For the surface area measurement, electrospun mats were considered as films. At each time interval, their dimensions were rapidly measured by a caliber. After the surface entrapment, the fibrous mats were cut into 10 mm × 10 mm and immersed into Tris buffer at 37°C to test the surface area stability.

Glass transition temperature (T_g) was analyzed by a differential scanning calorimeter (DSC, TA Instrument Q2000) at a ramp rate of 10°C/min with a

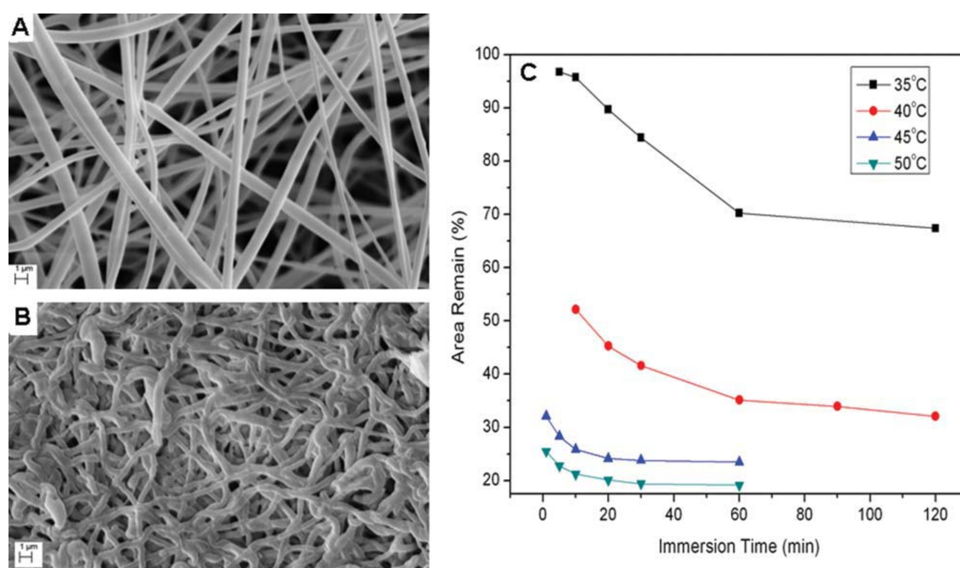

Figure 1 (A) SEM image of as-spun PDLLA fibers, (B) SEM image of shrunk PDLLA fibrous mat after 2 h immersion in Tris buffer at 37°C, (C) surface area shrinkage of PDLLA mats in Tris buffer at different solution temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

sample size of 3 mg each. A DCA-322 (Cahn Instruments) was used to determine the contact angle of the electrospun fiber mats to water based on the Wilhelmy plate method. Fiber mats were first cut into squares of 10 mm × 10 mm width. To avoid effects caused by fiber swelling, the advancing distance was set to 2 mm with a speed of 80 μm/s and all tests were conducted at room temperature. The tests were done in triplicate and results averaged.

X-ray Photoelectron Spectroscopy (XPS) was utilized to characterize the surface element composition before and after PEG entrapment. XPS is a technique which offers the possibility of element identification and quantification, as well as information on the chemical bonding. The specimens were attached to the AES sample holder by pressing into double-sided sticky tape for high resolution XPS spectra over the C1s peak. A binding energy of 285.0 eV was assigned to the C1s of saturated hydrocarbon. The C1s spectrum was subsequently fitted by XPSPeak 4.1 software with a linear background and Gaussian peak shape.

Canine fibroblasts were cultured in a 75 cm^2 flask L-15 media (Gibco) with antibiotics (Sigma) and 10% fetal bovine serum (Hyclone) essentially as previously described.[29,30] The cells were grown at 100% humidity and 37°C with 5% CO_2. Cells were originally isolated from a biopsy fragment of fascia from the abdominal wall of a normal beagle, placed on a plate, allowed to attach for approximately 5 min and then 2 mL of media was added to the well. Fibroblasts were allowed to grow from the biopsy fragment for several days until a monolayer had formed. The media was changed every 3 days. The cells were harvested by trypsin digestion.[29,30] The cell number was halved, unused cells frozen for future use, and the cell passage numbers recorded. After 20 passages the fibroblasts ceased to grow and a new vial was started.

For each assay, cells were washed with 1× Hanks (Sigma) and trypsinized. Initial fibroblast concentration was determined by flow cytometry (Accuri C6) and plated on a piece of sterile gauze in a 24-well plate (50,864 cells/well).

Cell morphology on electrospun scaffolds were observed by SEM. Cells were seeded on PDLLA nanofibers in 24-well tissue culture plates (TCPS). First, all samples were sterilized by ethanol solution (70 vol %) for 30 min under a Geneva GAH-4F-B horizontal laminar flow hood with UV light before being placed on scaffolds. After 4 days incubation at 37°C, cells and scaffolds were rinsed with buffer solution and fixed by 3% glutaraldehyde solution for 4 h. After drying by solvent exchange with a series of ethanol solutions, the morphology was observed by SEM.

Cell viability on electrospun PDLLA before and after surface modification was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT, Invitrogen). Fibroblasts were placed on each well with medium being changed every 2 days. After 1, 3, and 7 days cell seeding, the medium was carefully removed and replaced with 100 μL fresh medium. Then 10 μL of 12 m*M* MTT solution was added to each well, followed by 4 h incubation at 37°C. Hundred microliters of SDS-HCl solution was added after the incubation, followed by another 10 h incubation. Then the solution in each

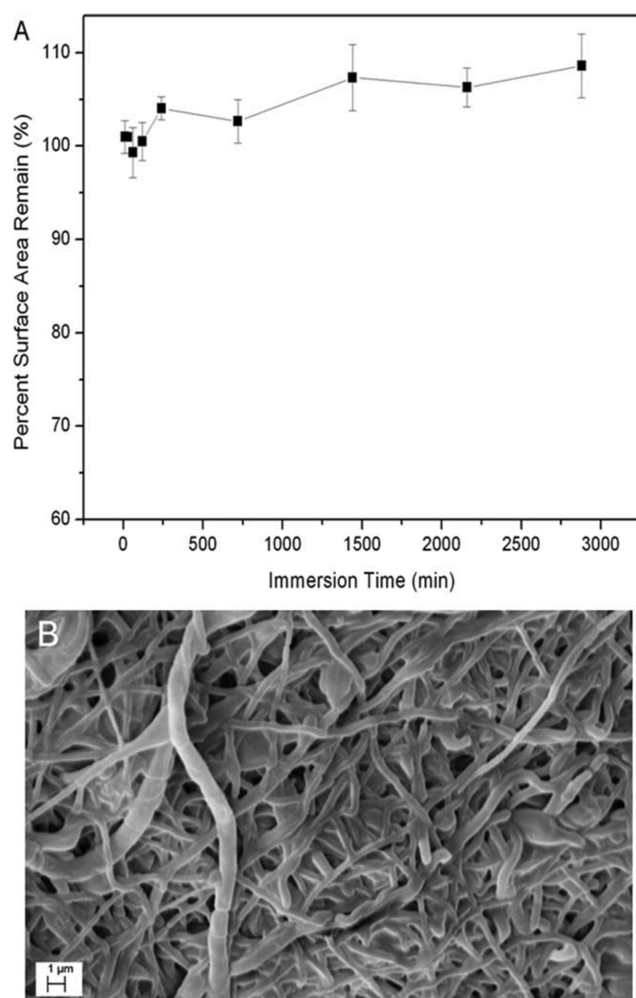

Figure 2 (A) Surface area stability of electrospun PDLLA fibrous mat after 2 h PEG entrapment in TFE/water solution at 42°C, (B) SEM image of electrospun PDLLA after PEG entrapment.

well was transferred to a 96-well plate and the absorbance read at 570 nm.

RESULTS AND DISCUSSION

The morphology of as-spun PDLLA nanofibers is shown in Figure 1(A). Electrospun PDLLA showed a well-formed nonwoven structure with average fiber diameters of 750 ± 320 nm.

Thermal shrinkage was found in PLA[31] and nonwovens from other polymers[32,33] as reported in literature. Normally, postspin thermal treatment has been utilized as an effective technique to improve stability.[34] To study the surface area shrinkage of electrospun fibrous mats, *in vitro* immersion tests in Tris buffer were first applied [Fig. 1(B)]. As can be seen in Figure 1(C), the surface area of PDLLA nonwovens obviously decreased considerably when immersed in buffer solution of a temperature close to the T_g of PDLLA. From DSC tests, the observed T_g of electrospun PDLLA was 50.95°C, while the shrunk sample showed a T_g at 54.17°C. This result indicated that PDLLA chains relaxed during the surface area shrinkage. Hyperbolic-shaped curves were observed when the final area was graphed versus the shrinkage rate. The results significantly depended on the solution temperature. Further, the morphology of nanofibrous mats dramatically changed. Figure 1(B) demonstrates that the originally straight fibers had obviously lost stiffness and elongation due to exposure to buffer at 37°C. It was concluded that to create electrospun nonwovens that are dimensionally stable at human body temperature, the fiber mats needed to be pretreated at 37°C or higher. Thus, for the subsequent surface entrapment process, a temperature of 42°C was chosen and a treatment time of 120 min to ensure dimensional stability of the PDLLA nonwovens.

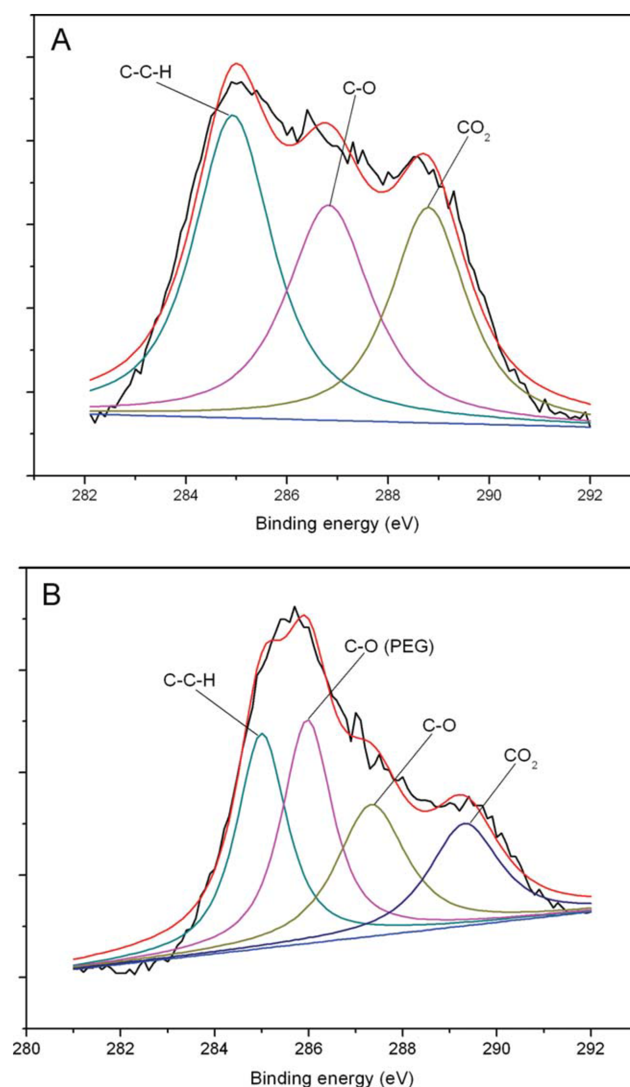

Figure 3 XPS C1s scans of PDLLA (A) as-spun, (B) after entrapment of PEG. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE I
Curve Fitting Data from XPS C1s Peaks of Electrospun PDLLA Before and After Entrapment Modification

	% Peak area				
Samples	C-C-H	C-O	CO_2	C-O (PEG)	% PEG Surface coverage
As-spun PDLLA	40.70	31.20	28.10		
After entrapment	28.13	23.75	18.72	29.40	40.3

Surface entrapment techniques are easy and effective to improve the surface properties, especially for various biocompatible polymers, both in form of films,[28] fibers,[35] and particles.[36] Generally, a solvent/nonsolvent system is applied to modify the polymer surfaces through reversible gelation. Most commonly, PEG,[28,35] PEG derivatives,[37] or polylysine[36,38] were used to modify polymer surfaces. In this study, TFE/water was selected as the solvent/nonsolvent mixture for PDLLA. In this system, TFE assists the surface swelling of PDLLA so that PEG is able to diffuse into fiber surface. After the treatment, the fibers are washed with an excess of nonsolvent (water) which causes the swollen structure to collapse. This method can also be applied for surface engineering of other polymers, for example, polypropylene[39] and polyurethane.[40,41]

After surface entrapment at 42°C, the PDLLA nanofiber mats shrunk to approximately 25% of their original size. Then the nonwoven mats were cut into 10 mm × 10 mm specimens and immersed in Tris buffer at 37°C. Their size was measured at each time interval. As can be determined from Figure 2(A), the surface area of PEG coated ultrafine fiber mats remained stable for the selected time interval of 12 h. After that, the specimens slightly increased due to the swelling of the PDLLA. The T_g of the PEG coated samples (55.90°C) remained similar to that of shrunk PDLLA, indicating that the entrapment process did not affect the bulk morphology of PDLLA. Thus, surface entrapment showed to be an effective process to generate surface stable electrospun PDLLA at 37°C.

The morphology of nonwoven fibers after surface entrapment is displayed in Figure 2(B). There was no obvious difference of the electrospun fiber mats before [Fig. 1(B)] and after the surface entrapment process when exposed to Tris buffer. Since the modification was conducted at 42°C, the PEG entrapment occurred along with the surface shrinkage and therefore the fibers remained stable at 37°C.

High resolution XPS C1s scans were used to determine the surface components of electrospun PDLLA. As shown in Figure 3(A), three peaks were identified from the spectrum of as-spun PDLLA fibers. The C-C-H, C-O, and CO_2 groups can be assigned to the peaks of 285, 287, and 289 eV, respectively,.[28] After PEG entrapment [Fig. 3(B)], one more peak appears at about 286 eV, which can be assigned to the C-O ether group of PEG. This result suggests that PEG was successfully coated onto the PDLLA nanofiber surface.

Percent peak areas from curve fitting of C1s data are shown in Table I. From XPS data, the PEG/PLA monomer ratio can be calculated, which is necessary to estimate the percentage of PEG surface coverage. After 120 min exposure in PEG/water/TFE mixture, 40.3% of the nonwoven mat was covered by PEG.

Accordingly, the water contact angle tests confirmed the effect of the surface modification. The as-spun PDLLA mats showed a contact angle of 125.6 ± 4.0°, while that of PEG coated nonwovens was 34.1 ± 1.9°. The dramatic decrease in contact angle suggests that hydrophilic PEG was indeed coated on the surface of the nanofibers. Compared with other surface modification methods, the result was better than plasma treatment (around 50°)[42] and surface grafting with acrylic acid (40–45°).[43] The resultant hydrophilicity is crucial for future applications as biomedical materials.

Canine fibroblasts were seeded on electrospun nanofibers; after 4 days, their morphology was studied by SEM (Fig. 4). The cells were spread on TCPS in a mono-layer pattern and leaf-like shape [Fig. 4(A)]. On electrospun PDLLA nanofibers, the cells adhered and grew in a discontinuous and irregular pattern [Fig. 4(B)]. However, the SEM images

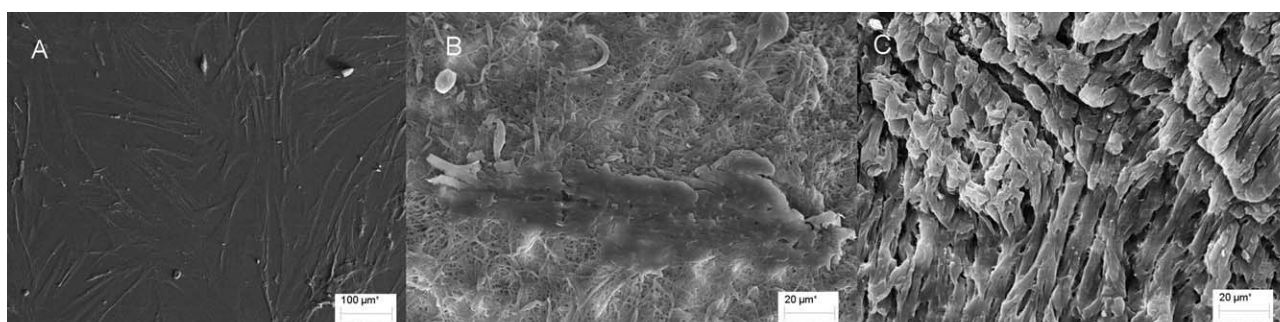

Figure 4 Cell morphology of fibroblasts on (A) TCPS, (B) electrospun PDLLA, (C) PEG-coated electrospun PDLLA.

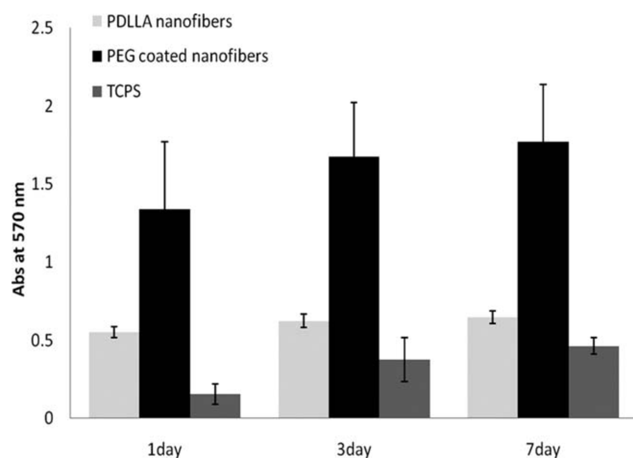

Figure 5 MTT viability on as-spun PDLLA nanofibers, PEG coated nanofibers, and TCPS (24-well tissue culture plates).

showed that canine fibroblasts had fully covered PEG coated PDLLA nanofibers after 4 days incubation [Fig. 4(C)]. The shape of the cells had changed to an elongated spindle-like appearance, indicating the cell adhesion and proliferation were greatly improved by PEG entrapment.

The improvement by surface modification was also investigated by cell viability via MTT assay. The results are summarized in Figure 5, with tissue culture plates (TCPS) as control. Figure 5 shows that with longer incubation period, cell growth gradually increased on all substrates. However, lower absorbance was observed on TCPS due to the formation of a monolayer of cells. Fibroblasts proliferated better on electrospun PDLLA nanofibers due to their porous structure. However, the improvement was limited by the shrinkage of fiber mats. It is clear that after the modification of PEG entrapment, the cell proliferation increased greatly. Thus, surface stabilization and hydrophilicity helped the cell adhesion and growth on PDLLA nanofibrous scaffold.

CONCLUSIONS

In this study, PDLLA ultrafine fibers were created by electrospinning. Surface area shrinkage and temperature dependence of as-spun fiber mats were evaluated. PEG was successfully coated onto PDLLA nanofibers by physical surface entrapment, and characterized by XPS and water contact angle tests. As indicated by XPS analysis, after 120 min treatment, 40.3% of surface was covered by PEG. As expected, electrospun fiber mats changed from hydrophobic to hydrophilic. Simultaneously, since surface entrapment was conducted in 42°C, the final product also showed a stable area at 37°C, which is the human body temperature. Cell morphology and MTT assay confirmed that the cell viability increased by PEG entrapment due to the improvement of dimensional stability and hydrophilicity. Thus, after surface entrapment PEG coated polymeric nanofibers could be considered as potential scaffold material for tissue engineering applications.

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