Growth Behavior of Endothelial Cells According to Electrospun poly(D,L-Lactic-Co-Glycolic Acid) Fiber Diameter as a Tissue Engineering Scaffold

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Investigating the effect of electrospun fiber diameter on endothelial cell proliferation provides an important guidance for the design of a fabric scaffold. In this study, we prepared biodegradable poly(D,L-lactic-co-glycolic acid) (PLGA) fibrous nonwoven mats with different fiber diameters ranged from 200 nm to 5 μ m using the electrospinning technique. To control the fiber diameters of PLGA mats, 4 mixture solvents [hexafluoro-2-propanol, 2,2,2-trifluoroethanol:dimethylformamide (9:1), 2,2,2-trifluoroethanol:hexafluoro-2-propanol (9:1), chloroform] were used. Average diameters were 200 nm, 600 nm, 1.5 μ m, and 5.0 μ m, respectively. Stereoscopic structure and spatial characterization of fibrous PLGA mats were analyzed using atomic force microscopy and a porosimeter. The mechanical properties of PLGA mats were analyzed using a universal testing machine. The spreading behavior and infiltration of endothelial cells on PLGA mats were visualized by field emission scanning electron microscopy and hematoxylin and eosin staining. Cell proliferation on different PLGA fibers with different diameters was quantified using the MTT assay. Cells on 200 nm diameter PLGA mats showed rapid attachment and spreading. However, the cells did not penetrate the PLGA mat. Cells cultured on 600 nm and 1.5 μ m diameter fibers could infiltrate the pores and cell proliferation was dramatically increased after 14 days. Secreted prostacyclin from endothelial cells on each mat was measured to examine the ability to inhibit platelet activation. This basic study on cell proliferation and fiber diameter with physical characterization provides a foundation for studies examining nonwoven fibrous PLGA mats as a tissue engineering scaffold.

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INTRODUCTION

Organ and tissue loss from injury or other types of damage has been increasing in proportion to the population each year. Tissue transplantation is a standard therapy for substituting damaged tissues in patients, but the number of donors is limited [1]. To overcome this limitation, tissue engineering has been introduced as a promising alternative for treating malfunctioning or lost organs and tissues [2]. Tissue engineering involves the expansion of cells from a small biopsy using growth factors, followed by cultivation of the cells in temporary three-dimen-

sional scaffolds to form engineered tissue or organs. Scaffolds are essential factors because they serve as attachment substrates for cell proliferation and provide physical support to maintain structural guidance of the specific organs [3,4].

The most commonly used porous scaffolds are constructed on the polymeric materials, which can be classified into two different types. The first type consists of synthetic biodegradable polymers such as aliphatic polyester, poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer poly(D,L-lactic-co-glycolic acid) (PLGA) [5-10]. The other types include naturally derived polymers such as collagen, chitosan, and hyaluronic acid. Natural polymers show good biocompatibility compared to synthetic polymers. In this study, we focused on the use of synthetic polymers as a scaffold because their molecular weight, degradation rate, and other properties are more easily modified. PGA, PLA, and PLGA are biocompatible and biodegradable and have been approved by the Food and Drug Administra-

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tion for specific human clinical applications, such as surgical sutures and some implantable devices. These materials can be easily formed into scaffolds and used to support the specific shape and structure of tissue until the new tissue is formed. The degradation period of synthetic polymers can be controlled by matching the rate of tissue regeneration [2,11,12].

Numerous methods have been developed to prepare porous scaffolds composed of PLGA, including phase separation, salt-leaching, emulsion freeze-drying, and gas foaming. In this study, electrospinning was employed to fabricate substrate scaffolds for cell culture [13]. Electrospinning is an efficient method used to create fibers on micro- and nanoscales [14,15]. The advantage of the electrospinning process is that a wide variety of materials can be electrospun and the fiber morphology can be easily controlled by altering parameters such as polymer molecular weight, polymer concentration, solvent properties, electrical field applied, and solution feed rate [16-21]. Particularly, in the tissue engineering field, electrospinning has the great advantage of producing nanofibrous scaffolds similar in fiber diameter and specific surface area to the natural extracellular matrix [22,23]. Cell adhesion and proliferation are greater on nanofibers than on microfibers. Furthermore, the three-dimensional structure of electrospun scaffolds allows for full cell differentiation with improved biological activity compared to in two-dimensional scaffold environments.

In order to form a specific three-dimensional structure of growing cells, the infiltration of cells into the scaffold must be considered. Therefore, the pore size and porosity of a scaffold are critical features and the cavity must be sufficiently large to allow for cell migration. For cell infiltration, the pore size must be larger than 10 μm , and 100 μm of extra space is necessary for further cell proliferation and migration [24-31]. Pore sizes and fiber diameters have been shown to affect cell development, such as differentiation and matrix production [28,32-34]. High porosity and large pore sizes are beneficial in many aspects; however, if the distances between the fibers are too large, the cells may not be able to cover the gap, negatively affecting cell growth. Since different cells having various requirement, being able to control porosity would be a great advantage and may even determine whether the process is successful. The size ranges of nanofibers are an attractive target for tissue engineering. However, small fiber diameters have small pore sizes and low porosities of the matrices, which is one of the limitations of using nanofibrous scaffolds [35]. Using electrospinning, higher polymer concentrations can be used to generate larger fiber diameters, which in turn increase the porosity of the structure but may negatively affect cell adherence and proliferation.

In this study, PLGA with different fiber diameters was fabricated using various mixtures of solvent and other parameters

were controlled using the electrospinning technique. Endothelial cell behavior and adhesion was monitored in each PLGA scaffold. Particularly, we compared cell adhesion, proliferation behavior, and prostacyclin secretion as a function of fiber diameter.

MATERIALS AND METHODS

Poly(D,L-lactic-co-glycolic acid) (molar ratio of lactic to glycolic acid 50:50, Mw 40000–75000) was purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP), 2,2,2-trifluoroethanol (TFE), N,N-dimethylformamide (DMF), and chloroform were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dielectric constant and electric conductivity of HFIP, TFE, DMF, and chloroform are 28.4, 26.1, 36.7, and 1.2 at 25°C and 4020, 3900, 5, and 1 ($\times 10^{-8}$ Ω/m), respectively. All solvents were used without further purification.

Preparation of nonwoven PLGA mats with different fiber diameters

To fabricate the series of fibrous nonwoven PLGA mats, the electrospinning method was used. PLGA fiber diameters were set to 200, 600, 1500, and 5000 nm. The average diameters of electrospun fiber strands can be controlled based on solvent properties (dielectric property, electric conductivity) and electrospinning parameters [concentration, applied electric potential, tip-to-collector distance (TCD), flow rate]. To fabricate 200 nm diameter PLGA fibers, electrospinning parameters were set to 12 wt% PLGA-HFIP solution, 0.75 kV/cm voltage/TCD, and 1 mL/h flow rate by preliminary repeat experiments. For 600 nm fibers, electrospinning parameters were set to 19 wt% PLGA-TFE:DMF (9:1), 0.4 kV/cm voltage/TCD, and 0.5 mL/h flow rate. For 1500 nm fibers, electrospinning parameters were set to 21 wt% PLGA-TFE:HFIP (9:1), 0.75 kV/cm voltage/TCD, and 0.5 mL/h flow rate. For 5000 nm, electrospinning parameters were set to 21 wt% PLGA-chloroform, 1 kV/cm voltage/TCD, and 1 mL/h flow rate. The electrospun fibrous mats were dried under vacuum (24 h, 25°C) to eliminate residual solvents. PLGA mats were stored in a desiccator until use.

Observation of mat morphology

The morphology of electrospun PLGA fibers was observed using a field emission scanning electron microscope (FE-SEM, S-4300, HITACHI, Tokyo, Japan). All samples were sputter-coated with gold before observation. The average fiber diameters and distributions were determined by measurements of SEM images using an image analyzer (TDISE, TECHSAN Co., Ltd., Seoul, Korea). The three-dimensional topography of each PLGA mat surface was visualized using an atomic force mi-

croscope (AFM, Nanoscope IIIa, Digital Instruments Inc., Tonawanda, NY, USA). The PLGA mats were scanned at a scan size of 30 μm , data scale of 10 μm , and scan rate of 3.3 Hz under dry conditions.

Porosity and mechanical properties

The porosity, specific surface area of PLGA nanofibrous mats were measured using a Mercury porosimeter (Autopore IV 9500 V1.03, Micrometrics, Norcross, GA, USA) and then analyzed using Brunauer-Emmet-Teller (BET) method. The total surface area (S_{total}) and specific surface area (S_{BET}) are given by following equations.

$$S_{\text{total}} = \frac{(v_m N s)}{V} \quad (1)$$

$$S_{\text{BET}} = \frac{S_{\text{total}}}{a} \quad (2)$$

Where, v_m is the monolayer adsorbed gas quantity, N is Avogadro's number, s is the adsorption cross section of the adsorbing species, V is the molar volume of the adsorbate gas, and a is the mass of the solid sample [36].

The pore size distribution of nonwoven PLGA mat was calculated by the equivalent area diameter equation for the measurement of polygonal pores using an image analysis software (TDISE, TECHSAN Co. Ltd., Seoul, Korea). The equivalent area diameter (D) was calculated using the following formula: $D^2 = 4A/\pi$, where A is the pore area and π is the ratio of the circumference of a circle to its diameter. Three representative images of each specimen from three replicates were used for measuring pore diameters with more than 50 pores.

The mechanical properties were investigated using a Universal Testing Machine (4467, Instron, Canton, MA, USA). Tensile tests were carried out using the Standard Test Method for Tensile Properties of Plastics (ASTM D638). Specimen dimensions were $90 \times 5 \times 0.2$ mm and the gauge length was 70 mm. The load cell and extension speed were 100 N and 5 mm/min, respectively. The average values were obtained from 10 specimens.

Cell culture, cell proliferation, and histology

The mouse pancreatic endothelial cell line (MILE SVEN 1, CRL-2279) was obtained from American Type Culture Collection (Manassas, VA, USA). The endothelial cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) and 1% penicillin G-streptomycin (Gibco, Grand Island, NY, USA) until the cells reached 80% confluence. The cell culture was maintained in an incubator equilibrated with 5% CO_2 at 37°C. The endothelial cells were collected and suspended in culture medium at a concen-

tration of 5×10^4 cells/mL. For cell culture, fibrous mats were detached from an aluminum collector and immersed in ethanol for sterilization. Then, the PLGA mat was rinsed with phosphate buffered saline solution. The suspension (1 mL) of endothelial cells was dropped onto the electrospun PLGA mat, which had been placed in a culture dish and cultured for 1 day. Unattached cells were collected and removed using medium and fresh culture medium was supplied after 1 day of cell seeding. The cell-scaffold constructs were cultured for 28 days, and the medium was exchanged twice each week. After 1, 3, 5, 7, 9, 14, and 28 days of incubation, the morphology of endothelial cells on the PLGA mat scaffolds was investigated using a phase contrast microscope and FE-SEM. The quantitative proliferation behavior of endothelial cells was evaluated using an MTT assay. Briefly, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/mL, phosphate-buffered saline) was added and incubated for 4 h at 37°C. The supernatant was removed and dimethyl sulfoxide was added to each well to dissolve any resulting formazan crystals for 10 min. The absorbance was measured at 540 nm using an ELISA reader (F-2500, Hitachi).

For conventional histology, the PLGA mat within tissue constructs were fixed in formalin (4°C overnight) after 7 and 14 days of seeding, dehydrated in series of aqueous ethanol solutions, embedded in paraffin, sectioned to 10 μm thick, and deparaffinized. Finally, tissue sections were stained with hematoxylin and eosin. Tissue sections were observed under an optical microscope (Eclipse TS100-E, Nikon, Tokyo, Japan) equipped with a digital camera (Coolpix 4500, Nikon, Tokyo, Japan).

Cell functionality assessment

Prostacyclin (prostaglandin I₂ or PGI₂) is a potent vasodilator and inhibitor of platelet aggregation. To analyze endothelial cell metabolism, prostacyclin was quantified. Prostacyclin has a half-life of 1 h *in vivo*, but is broken down into prostaglandin F_{1 α} in 42 s *in vitro*. Thus, the function of endothelial cells could be quantified by measuring 6-keto-prostaglandin F_{1 α} rather than prostacyclin. The Enzyme Immunoassay Kit (Assay Designs, Ann Arbor, MI, USA) was used to measure the amount of prostacyclin according to manufacturer's instructions. After the immunoreaction, absorbance (405 nm) of the solution was measured using a microplate reader.

Statistical analysis

Statistical analysis was performed using data analysis software (KyPlot version 2.0, KyensLab Inc., Tokyo, Japan). Each value represented mean $\pm$ standard deviation. Significance levels were determined by parametric Student's t-test and one-way analysis of variance. Statistical significance was considered

as $p < 0.05$.

RESULTS

Morphology and topography of fibrous PLGA mats

Four different fibrous PLGA nonwoven mats with different

diameters were successfully fabricated using 4 solvents (A: 12wt% HFIP, B: 19 wt% TFE/DMF (9:1), C: 21 wt% TFE/HFIP (9:1), D: 21 wt% chloroform) using the electrospinning method (Fig. 1A-D). The average diameters of the PLGA fibers were 200 nm (PLGA200), 600 nm (PLGA600), 1.5 μm (PLGA1500), and 5.0 μm (PLGA5000), respectively (Fig. 1E). Diameter dis-

Figure 1. SEM micrographs of electrospun fibers using PLGA solutions as a function of solvent [A: HFIP, B: TFE/DMF (9:1), C: TFE/HFIP (9:1), D: chloroform] and diameter distribution (E). SEM: scanning electron microscope, PLGA: poly(D,L-lactic-co-glycolic acid), HFIP: hexafluoro-2-propanol, TFE: trifluoroethanol, DMF: dimethylformamide.

tribution of each group was increased according to increasing average diameters of PLGA fibers. Each PLGA nanofiber showed a randomly aligned homogeneous fiber structure.

The topography of each fibrous mat was observed by AFM (Fig. 2). The surface roughness of PLGA nonwoven mats was dramatically increased with increasing fiber diameter. The surface of PLGA200 was flat and dense, while PLGA600 showed a rough surface with a compact network branch structure. The fibrous structure of PLGA1500 was coarse and appeared as stacks of cylindrical branches.

Porosity, pore size, specific surface area, and mechanical properties

As shown in Table 1, the pore size and porosity of PLGA mats were increased as a function of the average fiber diameter. The porosity of each PLGA mat was not significantly different. The mean pore size of the PLGA200 (1.4 μm) mat was very small compared to those of PLGA600 (8.5 μm), PLGA1500 (10.9 μm), and PLGA5000 (27.3 μm). The mechanical properties of electrospun PLGA mats are shown in Table 2. The thickness of

PLGA mats was 200 μm in diameter. The electrospun PLGA mat with a 200 nm fiber diameter showed the lowest tensile strength and elongation at break.

Effect of fiber diameter on cell spreading, penetration, and proliferation

To investigate the effect of fiber diameter on initial cell spreading, penetration, and proliferation behavior, endothelial cells were cultured on each electrospun PLGA mat for 14 days and cell spreading were observed after 1, 3, 5, and 7 days (Fig. 3). In general, cells should be distributed over the entire scaffold area, not just on the outer surface. To clarify cell penetration in the PLGA mat, cross-sections of the cell-scaffold constructs were observed by SEM and hematoxylin and eosin staining. Figure 4 shows the cross-sections of cell-scaffold constructs after 14 days of culture. The cells did not infiltrate the inner part of the PLGA200 mat (Fig. 4A and B). The small growth area limited cell proliferation and apoptosis (Fig. 5). A large number of cells with a dense extracellular matrix was observed in PLGA600, PLGA1500, and PLGA5000 mats after 2 weeks.

Figure 2. AFM images of fibrous nonwoven mats of PLGA200 (A), PLGA600 (B), and PLGA1500 (C). Roughness of surface morphology increased with average fiber diameter. AFM: atomic force microscope, PLGA: poly(D,L-lactic-co-glycolic acid).

Table 1. Spatial characteristics of PLGA nonwoven fabrics on different fiber diameters

Fiber diameters	Specific surface area (m^2/g)	Porosity (%)	Pore size (μm)
200 nm	24.5	80.9	1.4 $\pm$ 0.4
600 nm	20.7	85.2	8.5 $\pm$ 0.9
1.5 μm	12.9	87.3	10.9 $\pm$ 1.1
5.0 μm	9.7	89.8	27.3 $\pm$ 0.8

PLGA: poly(D,L-lactic-co-glycolic acid)

Table 2. Mechanical properties of PLGA nonwoven mats (n=10)

Fiber diameters	Young's modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)
200 nm	235 $\pm$ 10	2.4 $\pm$ 0.3	9.6 $\pm$ 0.6
600 nm	314 $\pm$ 12	3.3 $\pm$ 0.5	12.8 $\pm$ 0.8
1.5 μm	319 $\pm$ 9	3.8 $\pm$ 0.3	16.2 $\pm$ 0.6
5.0 μm	576 $\pm$ 16	5.5 $\pm$ 0.5	20.4 $\pm$ 0.9

PLGA: poly(D,L-lactic-co-glycolic acid)

Figure 3. FE-SEM photomicrographs of cultured endothelial cells on electrospun PLGA mats of PLGA200 (A), PLGA600 (B), PLGA1500 (C), and PLGA5000 (D). Cells spread fastest on the dense PLGA200 surface. FE-SEM: field emission scanning electron microscope, PLGA: poly(D,L-lactic-co-glycolic acid).

Cell functionality with prostacyclin

To examine the metabolic activity of endothelial cells on each PLGA mat, the secretion of prostacyclin, an inhibitor of platelet aggregation, was measured (Table 3). This value was calculated in comparison with a standard curve of prostacyclin combined with the MTT assay results. These results revealed the amount of prostacyclin secreted per day from single cultured endothelial cells in each PLGA mat. Total prostacyclin secretion was increased in proportion to the cell proliferation behavior in each PLGA mat.

DISCUSSION

The diameters and morphological characteristics of fiber

strands are important factors governing the physical properties of fibrous nonwoven scaffolds [14–18]. In this study, we used 4 fabrication parameters for electrospinning to prepare PLGA nonwoven mats with average diameters of approximately 200 nm (PLGA200), 600 nm (PLGA600), 1.5 μ m (PLGA1500), and 5.0 μ m (PLGA5000). The average diameter was controlled by adjusting the solvent concentration and electrospinning parameters such as voltage/TCD and flow rate. Particularly, fiber diameters were increased in proportion to the dielectric constant of the solvent, except for chloroform, and decreased in proportion to the electric conductivity of the solvent (HFIP, TFE, DMF, chloroform). Figure 1 shows photomicrographs and the diameter distribution of electrospun PLGA fibers prepared using different solvents and parameters. PLGA was dissolved in the

solvents HFIP, TFE/DMF (9:1), TFE/HFIP (9:1), and chloroform. Differences in nanofiber diameter were related to the dielectric constant, volatility, conductivity, and permittivity of solvents. Thus, we adjusted fiber diameter by altering solvent system.

The stereoscopic topography of each mat became increas-

Figure 4. Cross-sectional photomicrographs and hematoxylin and eosin staining of cultured endothelial cells in PLGA mats with different diameters at 14 days of culture. PLGA200 (A and B), PLGA600 (C and D), PLGA1500 (E and F), and PLGA5000 (G and H). Scale bars are 10 μ m. PLGA: poly(D,L-lactic-co-glycolic acid).

ingly rough and deep with increasing fiber diameter (Fig. 2). The topography of PLGA5000 could not be obtained because of the limited cantilever capacity. The topography of nanofibrous scaffolds affects the initial spreading and later proliferation of cells [23,31]. A dense topography may accelerate rapid cell spreading, while a rough surface facilitates cell penetration. Tensile strength and elongation at break of the PLGA mats were increased with increasing fiber diameters. A previous study reported that increased fiber diameters decrease tensile strength and modulus [37]. However, in this case, the mechanical properties were increased as a function of PLGA fiber diameter. We assumed that the randomly oriented fibers were aligned in one direction and resisted tensile stress through extension of the elastic nonwoven PLGA mat.

We investigated fiber diameter, morphology, and surface topography of fibrous PLGA mats as a scaffold. Optimizing the specific surface area, porosity, and pore size of a three-dimensional scaffold is critical in tissue engineering. Cells require a sufficiently large attachment area for spreading and the interconnection of pore channels for nutrient delivery and transport-

Figure 5. MTT assay of cultured endothelial cells on PLGA mats with different diameters. PLGA600 showed significant cell proliferation after 7 days. Data are mean $\pm$ SD for $n=5$ specimens. Statistical analysis was performed at 7 and 14 days. *** $p<0.001$, ** $p<0.01$, * $p<0.05$ to the PLGA 200 mat. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, PLGA: poly(D,L-lactic-co-glycolic acid).

Table 3. Secreted prostacyclin from endothelial cells cultured on PLGA nonwoven mats on different fiber diameters ($n=3$, SD of all value are less than ± 0.01)

Fiber diameters	Secreted prostacyclin (ng/ 10^5 cells/day)			
	Day 1	Day 3	Day 5	Day 7-14
200 nm	0.48	0.47	0.48	0.50
600 nm	0.47	0.51	0.51	0.51
1.5 μ m	0.48	0.52	0.52	0.52
5.0 μ m	0.50	0.51	0.51	0.51

PLGA: poly(D,L-lactic-co-glycolic acid)

tation of metabolic wastes. Scaffolds should also have appropriate surfaces and inner pore sizes to facilitate cell penetration. Pore sizes less than 50 μm may prohibit cell penetration. Epithelial cells are approximately 50 μm in diameter [38]. The specific surface areas of PLGA200 and PLGA600 were 24.5 and 20.7 m^2/g , respectively. Those of PLGA1500, PLGA5000 were dramatically lower at 12.9 and 9.7 m^2/g , respectively. The specific surface area is inversely proportional to the size of the material. Reducing the fiber diameter of fibrous PLGA mats the specific surface area were increased and pore size and porosity were decreased. A large specific surface area can provide many spreading sites for cells, and a large pore size with high porosity facilitates cell penetration into the scaffold as well as transportation of nutrient and metabolic wastes.

As shown in Figure 3, few cells were observed on the PLGA5000 mat compared to the other PLGA mats after 1 and 3 days. The surface pore sizes of the other PLGA mats were less than 10 μm , while the mean pore size of the PLGA5000 mat was 27.3 μm . Endothelial cells are very flat, and 10–20 μm in diameter. Large pores enable direct penetration of spherical endothelial cell pellets into the PLGA5000 mat during the seeding procedure. In contrast, large numbers of cells were observed on the surfaces of the other PLGA mats (Fig. 4). Despite the large surface pores, the endothelial cells had nearly covered the PLGA5000 mat after 5 days. This indicates that the initial cell spreading behavior was directly affected by the surface pore size of fibrous PLGA mats. Tissue engineering scaffolds should facilitate not only cell spreading, but also homogeneous cell penetration. All PLGA mats except the PLGA200 mat showed cell penetration into the inner part of the mats after 7 days (data not shown). This may be because the cells adhered onto the inside of mats with large pores (8.5 μm). Based on the cross-sectional photomicrographs on 14 day, a large number of endothelial cells penetrated and adhered to the inside of PLGA mats through the fibrous pore structure. After 14 days of cultivation, the initial nanofibrous pore structure of the PLGA200 mat was deformed, appearing similar to a film. Therefore, seeded endothelial cells remained on the upper surface without further proliferation.

Cell proliferation in all PLGA mats was not significantly increased until 5 days of cultivation and the PLGA5000 mats showed the highest proliferation rate after 7 days. A high specific surface area promotes degradation reactions. In the PLGA200 mat, the fibrous surface began degrading when the cells were spread to the outside of the mat. The PLGA600 mat began degrading when the cells were distributed both on the outside and in the inner part of the mat. Degradation created an empty area inside the mat and promoted cell proliferation in the PLGA600 mat. The PLGA600 mat showed dramatic cell

proliferation after 14 days (Fig. 5). This occurred because of the higher specific surface area and effect of numerous focal contacts on the nanofibrous PLGA 600 mat.

Prostacyclin is an index of functionality of endothelial cells. Prostacyclin secretion was similar in the different fibrous mats, indicating that cell function was unrelated to mat diameter. The secretion of prostacyclin was similar after 7 days of culture, indicating that cell function of a single endothelial cell was not suppressed by the varying fiber diameters of fibrous PLGA scaffolds.

In summary, four fibrous PLGA nonwoven mats with different diameters were successfully fabricated using 4 different solvents with the electrospinning method. The average diameters of PLGA fibers were 200 nm, 600 nm, 1.5 μm , and 5.0 μm . Stereoscopic topography revealed that the mats became rougher and deeper with increasing fiber diameter. Tensile strength and elongation at break of the PLGA mats were increased with increasing fiber diameter. Porosity and pore size were increased with increasing fiber diameter. Endothelial cells spread fastest on the 200 nm PLGA fibrous mat, but did not show infiltration into the mat. Endothelial cells homogeneously proliferated into PLGA mat scaffolds more than 600 nm in diameter. Cell proliferation was dramatically increased on the 600 nm mat after 14 days of culture because of the rapid degradation of nanofibers. Secretion of prostacyclin was similar in the different fibrous mats. These results reveal that the 600 nm diameter electrospun nanofibrous PLGA scaffold may be useful for tissue engineering applications.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

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