Evaluation of Electrospun (1,3)-(1,6)- β -D-Glucans/ Biodegradable Polymer as Artificial Skin for Full-Thickness Wound Healing

Hye-Lee Kim, M.S.,^{1,2} Jeong-Hyun Lee, M.S.,^{1,2} Mi Hee Lee, Ph.D.,¹
Byeong Ju Kwon, M.S.,^{1,2} and Jong-Chul Park, Ph.D.^{1,2}

(1,3)-(1,6)- β -D-glucan (BG), a natural product of glucose polymers, has immune stimulatory activity that is especially effective in wound healing. In this study, poly(lactic-co-glycolic acid) (PLGA) membranes containing BGs (BG/PLGA membranes) were investigated for their wound-healing effects. The growth rate of human dermal fibroblasts was enhanced in BG/PLGA membranes. Their growth rates were improved with the increase of BG concentration in the membranes. The PLGA membranes with and without BGs were treated in full-thickness skin wound using male BALB/c nude mice ($n=6$ for each group). According to the animal study, BG/PLGA membranes enhanced the interaction with the surrounding cells in wound sites. In the wound site treated BG/PLGA, the positive of the Ki-67 (a proliferation cell marker) and the CD 31 (an endothelial cell marker) were $77.2\% \pm 5.6\%$ and 34 ± 8.6 capillaries. In the wound site treated PLGA, the Ki-67 positive cells were $51.3\% \pm 7.0\%$, and the positive-stained capillaries of CD 31 were 22.7 ± 8.6 . The wound site treated with BG/PLGA membranes was stronger stained of them in the wound site than those of the wound sites treated with PLGA membranes. BG/PLGA membranes accelerated wound healing by improving the interaction, proliferation of cells, and angiogenesis. BG/PLGA membranes can be useful as a skin substitute for enhancing wound healing.

Introduction

WOUND SITES MAY be treated with ointment and coverage with skin substitutes. Treatment of a wound site has been used to protect the wound site, to reduce the pain, and to improve wound healing.¹ β -glucans, which are natural products of glucose polymers,² have been found to promote immune stimulatory activity. Particularly in wound sites, β -glucans enhance wound healing by increasing infiltration of macrophages, stimulating tissue granulation, collagen deposition, and re-epithelialization.³⁻⁶ In previous studies, the wound-healing effects of water-soluble (1,3)-(1,6)- β -D-glucans (BGs) have been demonstrated using cells such as adult human dermal fibroblasts (HDFs) and adipose tissue-derived mesenchymal stem cells (ADSCs). BGs accelerated the proliferation and migration of HDFs and ADSCs. The concentration of collagen gels formed by fibroblasts was accelerated after BG treatment.⁷ Therefore, BGs have a possibility of an additive the skin substitute.

Skin substitutes have been investigated to accelerate wound healing and to control pain. These skin substitutes sometimes have the mimic structure of a natural extracellular

matrix (ECM), because the skin dermal structure is comprised of ECM.⁸⁻⁹ The structure of a natural ECM is a three-dimensional network composed of multifibers. These kinds of the multifiber structures are easily formed by electrospinning with synthetic and natural polymers.¹⁰⁻¹² The electrospinning method enables users to generate micro-/nanoscale features with polymers, and these structures provide large surface-to-volume ratios and offer a large surface area for cell attachment and growth. Thus, the electrospinning method has the potential for application as a production method for skin substitutes.

In the present study, we aimed to fabricate membranes with BGs and poly(lactic-co-glycolic acid) (PLGA) using electrospinning, and investigated the behavior of fibroblasts in these membranes. Furthermore, the wound-healing effects of the membranes were evaluated in an animal study.

Methods and Materials

Electrospun BG/PLGA membranes

PLGA polymer (PLA/PGA=75/25) was obtained from Lakeshore Biomaterials. Water-soluble BG powder was

¹Department of Medical Engineering, Yonsei University College of Medicine, Shinchon-dong, Seodaemun-gu, Seoul, Republic of Korea.

²Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Shinchon-dong, Seodaemun-gu, Seoul, Republic of Korea.

obtained from Adeka Corp. (from *Aureobasidium pullulans*).¹³ The solvent for polymer solution used was 1,1,1,3,3,3-hexafluoro-2-propanol (Wako Pure Chemical Industries, Ltd.). The PLGA concentration was 20% (w/v) of solvent, and the BG concentrations were 25 wt% and 50 wt% of the total PLGA weight. Only BGs were dispersed in solvent, and the concentration of BGs was 50% (w/v) of the solvent. All solutions were used to manufacture the membranes at 15 kV by an electrospinning system (NNC-ESP200; NanoNC). The solution flow rate was maintained at 2 mL/h using a syringe infusion pump (KDS 781100; KD Scientific, Inc.). After electrospinning, the membranes were dried for 2 days at room temperature, and then sterilized with 25-kGy gamma irradiation (Greenpia Tech. Inc.) at room temperature.

Characterization of electrospun membranes

The surface morphology of the membranes was observed by a field emission-scanning electron microscope (FE-SEM, Hitachi S-4200; Hitachi Ltd.). The membranes were Pt sputter-coated for 120 s before the FE-SEM analysis. The average diameter of the fibers on membranes was measured by analyzing the FE-SEM images. For release kinetics of BGs from the membranes, the membranes were immersed in 2 mL phosphate-buffered saline (PBS; Welgene Inc.). Before the immersion, PBS was boiled at 36°C in incubation, and then the membranes were protected from light for 16 days in a shaking incubator. The weight of the membranes was determined at 2 mg/mL before their immersion in PBS. The BG concentration in PBS at each time point was calculated with a BGSTAR kit (Wako Pure Chemical Industries Ltd.). The samples were conjugated with the detected reagent, and then reacted in 37°C for 30 min. The reacted samples were added the color-developing reagents in numerical order, and detected by the enzyme-linked immunosorbent assay reader.

Viability and proliferation of HDFs on electrospun membranes

HDFs were obtained from Cambrex BioScience Walkersville, Inc., and maintained in the Dulbecco's modified Eagle's medium containing 4.0 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mM sodium pyruvate, 10% fetal bovine serum, and 1% antibiotic solution. After reaching confluence, the cells were washed with PBS and detached using 0.025% trypsin in 0.04 mM ethylenediaminetetraacetic acid (EDTA). All supplements for HDFs were obtained from Welgene, Inc.

Membranes were cut out into circles 12 mm in diameter and put onto 24-well culture plates. HDFs were placed in membranes at a density of 5×10^4 cells per membrane for a proliferation assay. HDFs on membranes were incubated in a humidified atmosphere containing 5% CO₂ at 36°C for 1, 3, 5, and 7 days. The proliferation assays were performed with an ATP bioluminescence assay kit (CLS II; Roche Molecular Biochemicals). At each time point, the membranes were immersed in 0.1% Triton-X in 100 mM Tris and 4 mM EDTA (pH 7.81) during 30 min for the cell lysis. ATP from the cells lysis was collected by a centrifuge at 1000 g for 60 s, and then each supernatant was transferred to each fresh tube. The luciferase reagent was added to the supernatant, and the ATP concentration was quantified using a microplate luminometer (Centro XS3 LB960; Berthold Technologies GmbH & Co.). To calculate the cell numbers using ATP

concentrations, the ATP standard curve was plotted using serial dilutions of the cells and the ATP concentrations.

The morphology of cells incubated for 5 days in membranes was observed by an FE-SEM. The cells in the membranes were fixed in PBS included 2% glutaraldehyde (Merck KGaA), 2% paraformaldehyde (Merck KGaA), and 0.5% CaCl₂ for 6 h. Then, the membranes were treated with 1% OsO₄ (Polysciences, Inc.) in 0.1 M PBS for 2 h, and washed twice with 0.1 M PBS. The fixed cells in the membranes were dehydrated with absolute alcohol (Merck KGaA). The dehydrated cells were treated with isoamylacetate (Merck KGaA) and dried. Finally, the cells in the membranes were coated with gold by an ion coater (Eiko IB-3; Eiko Engineering) and observed with an FE-SEM (Hitachi S-800; Hitachi Ltd.).

Animals and surgical manipulations

Male BALB/c nude mice (7-week old) were used for *in vivo*. This animal experiment was performed in accordance with the Guidelines for the Care and Use of Laboratory Animals, and the protocol was approved by the Animal Care and Use Committee of Yonsei University College of Medicine. A single full-thickness skin wound (diameter of 12 mm) was created on the upper back of each mouse. There were two study groups ($n=6$): PLGA membrane and PLGA membrane which was including 50 wt% BG. Each membrane was placed into the wound site, and then the membranes were attached to the surrounding tissue by suturing. The sutured wound sites were covered with Tegaderm[®] (3M Co.). At 7, 14, and 28 days after the membranes were transplanted, the mice were euthanized using zoletile (120 mg/kg; Boehringer Ingelheim Agroveter) and rompun (40 mg/kg; Bayer). The transplanted membranes and the surrounding tissues were isolated, and fixed with 10% formaldehyde for 2 days. After fixation, they were embedded in paraffin for the histological and the immunohistochemical evaluation.

Histological and immunohistochemical analyses

The paraffin-embedded blocks were sectioned into 4-μm thicknesses. The sections were deparaffinized and rehydrated using xylene (Merck KGaA), absolute alcohol (Merck KGaA) and distilled water. Some rehydrated sections were stained with hematoxylin and eosin (H&E). Other rehydrated sections were put into a target retrieval solution (0.01 M citrate buffer; Sigma-Aldrich Corp.). The sections into a target retrieval solution were heated using microwave during 7 min for three times. Then, the sections were incubated with rat monoclonal anti-mouse Ki-67 (Dakocytomation, Inc.) and mouse monoclonal antibody CD 31 (PECAM-1; Novacastra). The sections treated with Ki-67 were incubated for 30 min at room temperature and washed with PBS. Next, the sections were incubated with polyclonal rabbit anti-rat Ig/HRP (Dakocytomation, Inc.) for 30 min at room temperature, followed by treatment with α-rabbit EnVision HRP solution (Dakocytomation, Inc.) for 30 min. The sections treated with CD 31 were first blocked with 1% bovine serum albumin in Tris-buffered saline for 10 min at nonspecific binding sites. The blocked sections were then incubated with CD 31 at 4°C overnight and washed with PBS. Both antibody-binding sites were visualized after incubation with DAB (Dakocytomation Inc.). The sections were counterstained for 3 min with hematoxylin (Dakocytomation Inc.)

and then dehydrated with sequential ethanol solutions for sealing and microscopic observation.

The H&E-stained sections were prepared for examination of the epithelial regeneration and interaction with original tissues. The H&E-stained sections were scanned with a digital virtual microscope (dotSlide; Olympus) with the OlyVIA 6771 program (Olympus). The scanned images were measured by an image viewer program (OlyVIA 6771; Olympus). The sections expressing Ki-67 and CD 31 were used to determine the amount of proliferated cells and capillaries. The wound sections stained with Ki-67 and CD 31 were observed under $200\times$ magnifications using a microscope (BX40F-3; Olympus) with a test grid eyepiece. The number of the Ki-67-positive cells and the Ki-67-negative cells was counted at both sides of basement epidermis. The Ki-67-positive cells were expressed as percentages of the total cell numbers on basement epidermis. The positive stained capillaries of CD 31 were measured in three different areas (at $200\times$ magnifications): both of the end sides and the middle of the wound sites, which was applied.

Statistical analysis

Cells proliferation assays were conducted in three independent cultures for each experiment. Quantitative data are expressed as means $\pm$ SDs. Statistical comparisons were carried out using the Student's *t*-test (one-way). A *p*-value < 0.05 was considered statistically significant.

Animal studies were conducted with six mice per group. The results are reported as means $\pm$ SDs and were compared to PLGA membranes. Statistical analyses were performed using analysis of variance, followed by the Tukey's HSD test (one-way) using SPSS software (12.0 KO for windows). A *p*-value < 0.05 was considered statistically significant.

Results

Membrane surface morphology

Influence of BG concentration in membranes on their surface morphology is shown in Figure 1. The fibers made of PLGA

had an average fiber diameter of $1.96 \pm 0.09 \mu\text{m}$. The average fiber diameter of the PLGA membranes with 25 wt% BGs (25BG/PLGA membranes) was $1.42 \pm 0.34 \mu\text{m}$. In the PLGA membranes with 50 wt% BGs (50BG/PLGA membranes), their fiber diameter ranged from 1.25 to $0.52 \mu\text{m}$, with an average fiber diameter of $0.89 \pm 0.37 \mu\text{m}$. The fiber diameters were reduced with increased BG concentration on the membranes. While the solution containing a mixture of PLGA and BGs was electrospinning, several beads were deposited on the collector (Fig. 1C, G). The bead number on the membranes increased with the increase of the BG percentage in the polymer solutions. The BGs in solvent without PLGA were deposited as several sizes of the particles on the collector during electrospinning (Fig. 1D, H), because BGs were insoluble in the solvent.

BG release pattern from BG/PLGA membranes

Figure 2 illustrates the cumulative release of BGs at a 16-day period from the membranes. After incubation for 4 days in PBS, the cumulative amount of BGs released from the 25BG/PLGA membranes was $0.41 \pm 0.5 \text{ mg}$, which was $51.86\% \pm 6.52\%$ of the total BG concentration on the membranes. The BGs on the 25BG/PLGA membranes were released in a logarithmic manner for up to 6 days, after which the release rate decreased with time. In the 50BG/PLGA membranes, $0.81 \pm 0.09 \text{ mg}$ of BGs had been released in 4 days, which was $61.12\% \pm 7.12\%$ of the total BG concentration on the membranes. The amount of BGs released from the 50BG/PLGA membranes decreased after 4 days. As shown in Figure 2, the release profile of BGs from the 50BG/PLGA membranes was an initial burst release within 4 days upon contact with PBS. Figure 3 shows the surface morphology of the membranes incubated for 7 days. Some beads on the 50BG/PLGA membranes specifically had holes in their surfaces.

Viability and proliferation of cells on membranes

The viability and growth rate of HDFs on membranes were measured using an ATP assay and a FE-SEM (Figs. 4 and 5). The same amounts of cells were seeded onto all

FIG. 1. Scanning electron microscope images of (A, E) the poly(lactic-co-glycolic acid) (PLGA) membrane, (B, F) 25BG/PLGA membrane, and (C, G) 50BG/PLGA membrane. Electrospun β -D-glucans (BGs) without PLGA were on (D, H).

FIG. 2. Cumulative release of BGs from electrospun PLGA membranes made with 25 and 50 wt% BGs into PBS at 37°C during 16 days. The BG concentration in PBS at each time point was calculated with a BGSTAR kit.

membranes. For the 5 days after cell seeding, there were no distinct differences in the growth rate associated with the BG concentrations in the membranes. The cell density significantly ($p < 0.05$) increased in the 50BG/PLGA membranes after 7 days. The 50BG/PLGA membranes were considered a significant effect on the growth rate of HDFs after 7 days. The morphology of HDFs on membranes incubated for 5 days at 36°C is shown in Figure 5. On both membranes, HDFs exhibited the polygonal shape that is typical of normal cell morphology. The growth rate of the HDFs was improved

FIG. 4. Influence of human dermal fibroblast (HDF) proliferation on PLGA membranes containing different amounts of BGs. After incubation for 1, 3, 5, and 7 days, the cell viability was detected by an ATP assay. (* $p < 0.05$ versus PLGA membranes at the same time, as analyzed by a t -test with $n = 5$).

in the 50BG/PLGA membranes, and the morphology of the HDFs in both membranes showed the normal shapes.

Wound-healing and histological examination

The full-thickness skin wounds were made on the back of each mouse for a wound-healing test. Figures 6 and 7 show the appearance of the wounds after the operations. At 14

FIG. 3. Scanning electron microscope images of (A, C) the PLGA membrane and (B, D) 50BG/PLGA membrane incubated for 7 days at 37°C.

FIG. 5. Scanning electron microscope images of HDFs incubated for 5 days at 37°C on the (A, C) PLGA membrane and the (B, D) 50BG/PLGA membrane.

FIG. 6. Appearance of the wounds in mice at 0, 1, 2, and 4 weeks after treatment with PLGA membranes and 50BG/PLGA membranes. Color images available online at www.liebertpub.com/tea

FIG. 7. (A, B) Histological morphology with hematoxylin and eosin of wound sites treated with PLGA membranes and 50BG/PLGA membranes. The half images of wound sites showed in (A). At 14 days after the operations, the part of wound sites showed in (B). Color images available online at www.liebertpub.com/tea

FIG. 8. Immunohistochemical observation of wounds treated with PLGA membranes with and without BGs and stained with anti-Ki-67 and anti-CD 31 antibody at 14 days. **(A)** Microscopic observation of immunohistochemical results; **(B)** quantitative analyses of cells showing immunopositivity for cells Ki-67 or CD 31 (* $p < 0.05$, analyzed by one-way (analysis of variance), followed by the Tukey HSD test). Color images available online at www.liebertpub.com/tea

days after treatment, the connective tissues had slightly regenerated with some local infection. Microscopic findings did not reveal any significant differences between the PLGA and the 50BG/PLGA membranes with respect to wound contraction at 14 days (Fig. 6). However, the 50BG/PLGA membranes significantly enhanced the infiltration of the surrounding cells (Fig. 7B). PLGA membranes can be clearly distinguished from the tissue 14 days after the operations. In contrast, the boundary line between the 50BGs/PLGA membrane and the tissue was barely distinguishable in the wound site, and cells were abundantly evident inside the 50BG/PLGA membranes.

Figure 8 shows the representative immunolocalization of Ki-67 and CD 31 after 14 days in a wound treated with PLGA or 50BG/PLGA membranes. Ki-67 positive cells were counted at the basal layers of the epidermis (Fig. 8A). The positive cells that were stained Ki-67 were $77.2\% \pm 5.6\%$ in 50BG/PLGA membranes and $51.3\% \pm 7.0\%$ in PLGA membranes. The strongest positive staining for Ki-67 was evident after 14 days in the wound treated with the 50BGs/PLGA membrane. Moreover, the wounds treated with 50BG/PLGA membranes showed significantly higher positive staining for CD 31 (34 ± 8.6 capillaries) than PLGA membranes (22.7 ± 8.6 capillaries).

Discussion

β -glucans have been demonstrated to have immune stimulatory activity, such as the increasing of macrophage infiltration,

the tissue granulation stimulating, and the collagen deposition.³⁻⁶ Kwon *et al.*¹⁴ investigated that *Sparassis crispa*, a medicinal mushroom that has more than 40% of β -glucans, could significantly improve the wound healing in patients with diabetes by oral administration. According to these reports, it can be suggested that BGs has a potentially effective skin substitute.

The skin substitutes were manufactured with the membranes composed with PLGA and BGs using electrospinning (Fig. 1). The beads in membranes can be expected BG particles, because the beads showed the holes after incubation for 7 days (Fig. 3). BG particles suggested the disruption of the polymer chain entanglement. The polymer chain entanglement causes the fiber formation, and the high concentration of the entanglement induces the increase of the fiber thickness.¹⁵ It suggested that BGs dispersed in the solution and then disrupted the entanglements. Therefore, the increase of BGs concentration induced the increase of the bead numbers and the decrease of the fiber thickness. However, BG/PLGA membranes were able to be manufactured using electrospinning, and BGs can be supplied in the wound site using BG/PLGA membranes.

HDFs are the main cell type involved in the regulation of ECM protein production in wound sites, and the formation of ECM promotes the granulation tissue. Improvement of the HDF activity induces the acceleration of ECM production, and the HDF activity is reflected by their growth rate.¹⁶⁻¹⁸ Researchers demonstrated that the attachment area and the growth rate of HDFs have been reduced dramatically by the

decrease in fiber diameters.^{16,19} The fiber diameter average of the 50BG/PLGA membranes was 45% of the PLGA membranes' (Fig. 1). According to the researchers, the 50BG/PLGA membranes cannot support sufficient areas for the attachment and growth of HDFs as compared with PLGA membranes. However, HDFs showed normal shapes on both membranes (Fig. 5). The growth rate of HDFs was improved in 50BG/PLGA membranes after incubating for 7 days (Fig. 4). In previous studies, BGs dose dependently enhanced the proliferation of HDFs on well plates for 5 days.^{6,20} According to the release profile (Fig. 2), the BGs continually released from the membranes during 16 days. The released BGs from the membranes can accelerate the HDF activity by the improvement of the attachment area and the growth rate of HDFs on membranes. Therefore, the 50BG/PLGA membranes could accelerate wound healing by improving the HDF activity.

Clinically, it is an important factor to wound repair as fast as possible. For the acceleration of wound healing, the skin substitutes required the interaction with the surrounding cells and the excellent infiltration of them.²¹ Their interaction should directly influence cellular activity such as the adhesion, proliferation, and migration of cells.^{17,22} BGs have been demonstrated to stimulate the immune system and to enhance macrophage infiltration into injury sites.²³ In the present study, 50BG/PLGA membranes significantly improved the interaction with the surrounding cells and the infiltration of them (Fig. 7). It suggested that the BGs in the wound site can improve the wound healing by the induction of the interaction with the surrounding cells and the stimulation of inflammatory.

BGs have been demonstrated to increase the expression of proinflammatory factors such as interleukin-6 (IL-6) and interferon- γ in splenocytes.^{23,24} These factors are secreted by macrophages and T cells to stimulate an immune response and promote proliferation, migration, and activation.²² As a result, proliferated cells (the positive cells stained Ki-67) were obtained at high levels in the wound site treated with 50BG/PLGA membranes as compared with those treated with PLGA membranes (Fig. 8). The stronger staining of Ki-67 was considered that the keratinocytes were proliferated on the basement layer. Improvement of keratinocytes proliferation can enhance the re-epithelialization, because keratinocyte is a main cell of the epidermal layer.¹⁰ Thus, 50BG/PLGA membranes were regarded to improve the re-epithelialization by the increase of keratinocyte proliferation on the basement layer. IL-6 had been reported to have multiple functions in angiogenesis and vascular remodeling. Fan *et al.* had concluded that IL-6 significantly improved the proliferation of endothelial cells.²⁵ Therefore, 50BG/PLGA membranes may enhance angiogenesis because of the promotion of IL-6 expression by BGs.

Conclusions

BGs have been demonstrated to enhance the cellular responses, proliferation, and migration of HDFs. In this study, we investigated the possible roles of electrospun PLGA membranes, including BGs for the acceleration of skin wound healing. The proliferation of HDFs improved as the ratio of BGs increased in the PLGA membranes. At 14 days after membranes application, the 50BG/PLGA membranes

had enhanced the interaction of the wound with surrounding cells, proliferation, and angiogenesis as compared with the PLGA membranes. Therefore, BG/PLGA membranes may be applied as a skin substitute for accelerated wound healing.

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Disclosure Statement

No competing financial interests exist.

References

- Peter, K., and Angela, M. Wounds-from physiology to wound dressing. *Dtsch Arztebl Int* **105**, 239, 2008.
- Ruiz, H.J. Biosynthesis of beta-glucans in fungi. *Antonie Van Leeuwenhoek* **60**, 72, 1991.
- Portera, C.A., Love, E.J., Memore, L., Zhang, L., Muller, A., Browder, W., and Williams, D.L. Effect of macrophage stimulation on collagen biosynthesis in the healing wound. *Am Surg* **63**, 125, 1997.
- Delatte, S.J., Evans, J., Hebra, A., Adamson, W., Othersen, H.B., and Tagge, E.P. Effectiveness of beta glucan collagen for treatment of partial thickness burns in children. *J Pediatr Surg* **36**, 113, 2001.
- Rose, G.D., Vervicka, V., Yan, J., Siz, Y., and Vetvickova, J. Therapeutic intervention with complement and beta-glucan in cancer. *Immunopharmacology* **42**, 61, 1999.
- Kougias, P., Wei, D., Rice, P.J., Ensley, H.E., Kalbfleisch, J., Williams, D.L., and Browder, I.W. Normal human fibroblasts express pattern recognition receptors for fungal (1 $\rightarrow$ 3), (1 $\rightarrow$ 6)-glucan. *Mycol Res* **69**, 3933, 2001.
- Woo, Y.I., Park, B.J., Kim, H.-L., Lee, M.H., Kim, J., Yang, Y.I., Kim, J.K., Tsubaki, K., Han, D.-W., and Park, J.C. The biological activities of (1 $\rightarrow$ 3), (1 $\rightarrow$ 6)- β -D-glucans and porous electrospun PLGA membranes containing β -glucan in human dermal fibroblasts and adipose tissue-derived stem cells. *Biomed Mater* **5**, 044109, 2010.
- Rovert, M.N., and Athanassions, S. Tissue engineering: form biology to biological substitutes. *Tissue Eng* **1**, 3, 1995.
- Joshua, S.B., Kerr, H.M., Howard, N.E.S., and Gillian, M.E. Wound healing dressings and drug delivery systems: a review. *J Pharm Sci* **97**, 2892, 2008.
- Rho, K.S., Jeong, L., Lee, G., Seo, B.-M., Park, Y.J., Hong, S.-D., Roh, S., Cho, J.J., Park, W.H., and Min, B.-M. Electrospinning of collagen nanofibers: effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterial* **27**, 1452, 2006.
- Chen, J.-P., Chang, G.-Y., and Chen, J.-K. Electrospun collagen/chitosan nanofibrous membrane as wound dressing. *Colloids Surface A: Physicochem Eng Aspects* **313**, 183, 2008.
- Duan, B., Yuan, X., Zhu, Y., Zhang, Y., Li, X., Zhang, Y., and Yao, K. A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning. *Eur Polymer J* **42**, 2013, 2006.
- Tada, R., Yoshikawa, M., Kuge, T., Tanioka, A., Ishibashi, K.-I., Adachi, Y., Tsubaki, K., and Ohno, H. A highly branched 1,3- β -D-glucan extracted from *Aureobasidium Pullulans* induces cytokine production in DBA/2 mouse-derived splenocytes. *Int Immunopharm* **1431**, 9, 2009.

14. Kwon, A.-H., Qiu, Z., Hashimoto, M., Yamamoto, K., and Kimura, T. Effects of medicinal mushroom (*Sparassis crispa*) on wound healing in streptozotocin-induced diabetic rats. *Am J Surg* **197**, 503, 2009.
15. Ramakishna, S. An introduction to electrospinning and nanofibers. In: Fujihara, K., Teo, W.-E., Lim, T.-C., Ma, Z., eds. Singapore: World Sci., 2005. pp. 90–98.
16. Tettamanti, G., Grimaldi, A., Rinaldi, L., Arnaboldi, F., Congiu, T., Valvassori, R., and Eguileor, M. The multifunctional role of fibroblasts during wound healing in *Hirudo medicinalis* (Annelida, Hirudinea). *Biology Cell* **96**, 443, 2004.
17. Leong, M.F., Rasheed, M.Z., Lim, T.C., and Chian, K.S. *In vitro* cell infiltration and *in vivo* cell infiltration and vascularization in a fibrous, highly porous poly (D,L-lactide) scaffold fabricated by cryogenic electrospinning technique. *J Biomed Mater Res* **91A**, 231, 2009.
18. Darby, I.A., and Hewitson, T.D. Fibroblast differentiation in wound healing and fibrosis. *Int Rev Cytology* **257**, 143, 2007.
19. Lowery, J.L., Datta, N., and Rutledge, G.C. Effect of fiber diameter, pore size and seeding method on growth of human dermal fibroblasts in electrospun poly(ϵ -caprolactone) fibrous mats. *Biomaterial* **31**, 491, 2010.
20. Woo, Y.I., Son, H.J., Lim, H.R., Lee, M.H., Baek, H.S., Tsubaki, K., and Park, J.-C. Effects of (1 $\rightarrow$ 3), (1 $\rightarrow$ 6)- β -D-glucans behavior in human dermal fibroblast cells under serum starvation. *Key Eng Mater* **342–343**, 401, 2007.
21. Sheterian, A., Borboa, A., Sawada, R., Costantini, T., Potenza, B., Coimbra, R., Baird, A., and Eliceiri, B.P. Real-time analysis of the kinetics of angiogenesis and vascular permeability in an animal model of wound healing. *Burns* **35**, 811, 2009.
22. Gregory, S.S., and Annette, W. Interactions between extracellular matrix and growth factors in wound healing. *Wound Rep Reg* **17**, 153, 2009.
23. Tada, R., Yoshikawa, M., Kuge, T., Tanioka, A., Ishibashi, K.-I., Adachi, Y., Tsubaki, K., and Ohno, H. Granulocyte macrophage colony-stimulating factors is required for cytokine induction by a highly 6 branched 1,3- β -D-glucan from *Aureobasidium Pullulans* in mouse-derived splenocytes. *Immunopharm Immunotox* **1**, 1, 2010.
24. Barrientos, S., Stojadinovic, O., Golinko, M.S., Brem, H., and Carnic, M.T. Growth factors and cytokines in wound healing. *Wound Rep Reg* **16**, 585, 2008.
25. Fan, Y., Ye, J., Shen, F., Zhu, Y., Yeghiazarians, Y., Shu, W., Chen, Y., Lawton, M.T., Young, W.L., and Yang, G.-Y. Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis *in vitro*. *J Cereb Blood Flow Metab* **28**, 90, 2008.

Address correspondence to:

Jong-Chul Park, Ph.D.

Department of Medical Engineering

Yonsei University College of Medicine

134 Shinchon-dong

Seodaemun-gu

Seoul 120-752

Republic of Korea

E-mail: parkjc@yuhs.ac

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