

## Migration of Human Dermal Fibroblast is Affected by the Diameter of the Electrospun PLGA Fiber

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Cell migration is an essential activity of the cells in various biological phenomena such as embryonic development, wound healing of damaged tissue, capillary vascularization in angiogenesis and migration of leukocytes to kill the bacteria around the wound site. The properties of nanofibrous surface enhancing cell adhesion, proliferation, migration and differentiation are necessary for application in tissue engineering. Recently, fabricated scaffolds at the nanometer scale are very similar to the architecture of natural human tissue, because of the development of nanofibers. In this study, we observed different cell migration behaviors on PLGA nanofibers with different diameters. 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$  PLGA fibers were fabricated by electrospinning. Adhesion of neonatal human dermal fibroblasts (nHDFs) on the PLGA scaffolds was quantified by MTT assay. Real time observation system was used to analyze the migration of nHDF on the 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$  PLGA scaffolds. There are no significant differences in cell attachment between 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$  PLGA nanofibers. However, the migration was affected by the thickness of the PLGA fiber. The cells were migrated along with the 0.4  $\mu\text{m}$  PLGA fiber but did not cross 1.4  $\mu\text{m}$  PLGA fiber. In this research, it would be evaluated that different diameter of electrospun PLGA fiber effect on the cell migration and proliferation, and it could be applied for the development of the fibrous scaffold in tissue engineering.

**Key words:** Cell migration, Cell adhesion, Electrospinning, Nanofibers

### Introduction

In a variety of biological phenomena, cell migration plays a very important role. In the inflammatory response, leukocytes migrate into the areas where insult has occurred, and then they affect phagocyte and immune functions. Cellular migrations are prominent in morphogenic processes ranging from gastrulation to development of the nervous system in embryogenesis. In normal physiology and pathology, migration remains crucial for the adult organism. Migration of fibroblasts and vascular endothelial cells is essential for wound healing. In metastasis, tumor cells immigrate from the initial tumor mass into the circulatory system, which they subsequently leave and migrate into a new site. Finally, cell migration is crucial to technological applications in tissue engineering and playing an essential role in colonization of biomaterials scaffolding.<sup>1,2)</sup>

As tissue engineering has been developed, therapeutic products have manufactured by combination of matrix scaffolds with cell responsive biomolecules or viable human cell systems,

for the repair, restoration, or regeneration of damaged cells or tissue.<sup>3-5)</sup>

The electrospinning is a very simple method for the preparation of synthetic polymeric fibrous meshes acting as extracellular matrices.<sup>6)</sup> Electrospun fibers have very thin diameter, ranging downward from micrometers to few nanometers. The small diameter of electrospun fibers provides a high surface area, a high length to diameter ratio, and to volume ratio. These characteristics are useful in a variety of applications, such as separation membranes, wound dressing materials, artificial blood vessels, in nano-composites, as a nonwoven fabric, and many other applications.<sup>7)</sup> Electrospun scaffolds help cells to grow by providing adequate mechanical support. In general, the electrospun scaffolds should have biocompatible and biodegradable characteristics for the tissue replacement, and possess good mechanical properties for implantation. In recent years, many polymeric nanofibers have been fabricated by electrospinning method for various applications such as artificial skin,<sup>8)</sup> artificial blood vessels,<sup>9)</sup> bone tissue engineering.<sup>10)</sup> In this study, we investigated the effect of the diameter of the electrospun nanofiber on cell migration for effective control of the cell behaviors.

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## Materials and Methods

### Cell culture

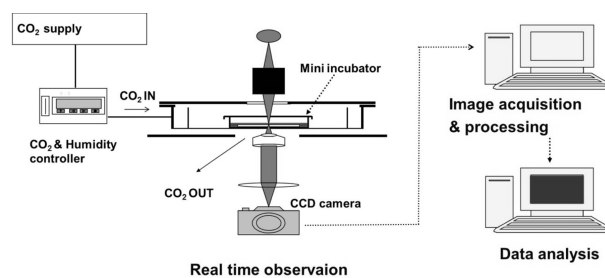
Cell studies were performed using primary cultured neonatal human dermal fibroblasts (nHDFs, Lonza). Cells were maintained in Dulbecco's modified eagle's medium (Welgene, Korea) with 10% fetal bovine serum (FBS, Welgene) and 10% antibiotic solution (Welgene), at 37°C in a 5% CO<sub>2</sub> incubator, and the medium was changed every 2-3 days. Cells were subcultured with 0.25% trypsin/EDTA when they reach 50~70% confluence.

### Preparation of electrospun fiber

Electrospinning method was used to fabricate poly(DL-lactic-co-glycolic acid) (PLGA, Lakeshore Biomaterials, Inc, AL, USA) nanofibers of copolymer ratios of 75:25. The polymer solutions were prepared by dissolving 0.85 g or 2 g of PLGA in each 10 mL of 1,1,1,2,2,2-hexafluoro-2-propanol (HFIP, Sigma, MO, USA), and mixed well for 4 hours using magnetic stirrer (Corning, California, USA). 8.5% (w/v) and 20% (w/v) of PLGA solutions were placed in a 10 mL syringe fitted with 23 G or 30 G needle, respectively. The high voltage power supplies with 18 kV and 20 kV were employed at a distance 10 cm between the paper foil (cathode) and the needle tip (anode). The syringe pumping speed was 1 ml/h and PLGA nanofibers were formed on the paper foil. PLGA nanofibers also accumulated on the slide glass to observe the migration of nHDFs. Prior to performing the experiment, the electrospun scaffolds were dried for three days in a fume hood to remove the solvents. PLGA nanofibers accumulated on the slide glass and electrospun scaffolds were sterilized under UV light overnight. The thickness of electrospun PLGA nanofibers were measured by scanning electron microscope (SEM, Hitachi S-4700, Tokyo, Japan).

### Attachment assay of nHDF on nanofibers

The weights of both 8.5% (w/v) and 20% (w/v) electrospun PLGA scaffolds were prepared with 80 mg and each scaffold was placed in a separate well of a 24 well tissue-culture plate for cell seeding and attachment test. nHDFs were seeded on the scaffolds at a density of  $1 \times 10^5$  cells of each well and inside of silicon O-ring (inner diameter = 15.6 mm) on the slide glass. After 4hr incubation in the 5% CO<sub>2</sub> incubator at 37°C, the unattached cells and culture medium were simultaneously removed by pipette, followed by washing the specimens with phosphate-buffered saline (PBS). Then, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reagent (5 mg/ml MTT in PBS) was added to each well and inside of silicon O-ring on the slide glass. After removal of the media and MTT reagent, 80 mg of electrospun PLGA scaffold (DMSO soluble) was placed in the silicon O-ring on the slide glass then dimethylsulfoxide (DMSO) and glycine buffer were added onto the



**Figure 1.** Schematic diagram of the real time observation system for the evaluation of cell migration.

specimens to dissolve the blue crystals. The optical density (OD) of the dissolved solute was then measured by a microplate reader (Molecular Devices, California, USA) under a light source of 570 nm wavelength.

### Real time observation system

We used the real time observation system (Figure 1) to observe the migration of the cells on the PLGA nanofibers. The real time observation system consisted of incubator system installed with the microscope to observe live cells migration. The incubator was regulated by temperature and gas composition controlling program (CCP ver. 3.8, Live Cell Instrument, Korea) under proper environment for cell (CO<sub>2</sub> 5%, 37°C).

### Image acquisition

The cells were cultured in the incubator placed on the microscope stage, and cell images were recorded every 5 minutes for 24 hours by the change-coupled device (CCD) camera (Electric Biomedical Co. Ltd., Osaka, Japan). Images were conveyed directly from a frame grabber to computer storage using Tomoro image capture program and memorized them as JPEG image files.

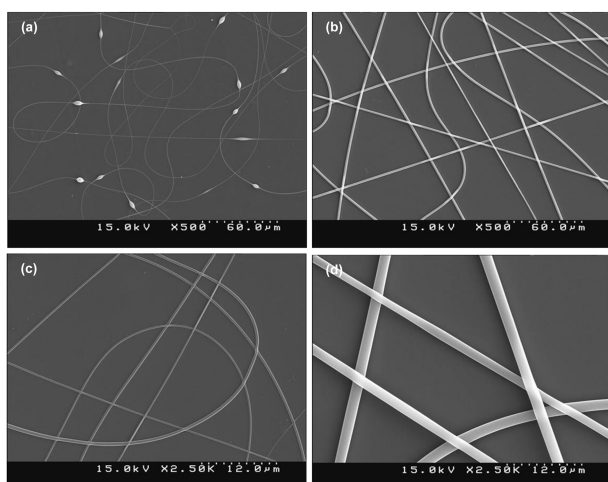
### Cell tracking and evaluation of cell migration

PLGA nanofibers accumulated on slide glasses were placed on the mini incubator (Live Cell Instrument) and then nHDFs were seeded on the 8.5% (w/v) and 20% (w/v) at a density of  $4 \times 10^3$  cells/sample. After a 2 hr attachment period, fresh medium was added. PLGA nanofibers accumulated slide glass seeding nHDFs was mounted on the bottom plate of real time observation system. For data analysis, captured images were imported into Adobe Photoshop 7.0. Image analysis was carried out by manual tracking that put a dot on the position of cell nuclei in picture and plotted cell migration pathway by connecting the dots. Cells undergoing division, death, or migration outside the field of view were excluded from the analysis.

## Results and Discussion

### Surface morphology of PLGA nanofibers

Figure 2 shows the morphologies of 8.5% (w/v) and 20% (w/

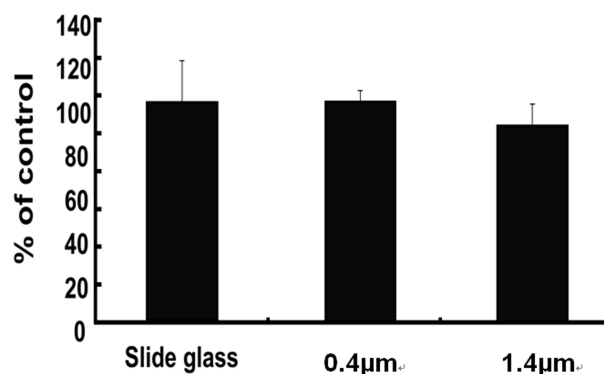


**Figure 2.** SEM micrographs of the 8.5% w/v (a,c) and 20% w/v (b,d) PLGA nanofibrous scaffold.

v) PLGA nanofibrous scaffolds. The electrospun nanofiber deposits randomly to form a fused fiber mesh, and fiber diameter can be controlled by varying electrical potential, throw distance, needle diameter, and solution concentration.<sup>11-14</sup> The fiber diameter was regulated by changing the concentration of PLGA solution or voltage. High viscosity of PLGA solution induces thicker fibers than that with low viscosity, and the thinner electrospun fibers are produced as the voltage of power supply is increasing. To manufacture thick electrospun fibers, however, we set the low voltage as possible and use high viscosity of PLGA solution. Two electrospun fibers of different thickness were needed to compare the migration and cell adhesion on thin fibrous scaffold with those on thick fibrous scaffold. Small beads were connected with thin 8.5% (w/v) PLGA nanofibers (Figure 2a) and these beads are related to the viscosity of polymer solution, net charge density, instability of the jet of polymer solution, fiber diameters.<sup>15-18</sup> The 8.5% (w/v) PLGA nanofibers with few beads were distributed evenly at 18 kV and the fiber diameter was 0.4  $\mu\text{m}$  as estimated from SEM images (Figure 2a, b). Figure 2(c) and (d) represent the 20% (w/v) PLGA fibers were spread uniformly and connected with no beads at 20 kV. The diameter of 20% (w/v) PLGA fiber was 1.4  $\mu\text{m}$  (Figure 2d).

#### Cell attachment of slide glass and PLGA nanofibers

PLGA is better than non-hydrophilic polymers in respect to cell adhesion and proliferation due to its hydrophilic property.<sup>19</sup> Addition of PLGA to poor hydrophilic PCL mats enhances attachment and proliferation of L-929 cell and NIH 3T3 cell.<sup>11</sup> Nevertheless, we need to find out the effect of the thickness of PLGA fibers on cell attachment because the differences between cell adhesions in thin and thick PLGA fibrous scaffolds may affect cell migration. Figure 3 shows the results of nHDF attachment tests for 4 hr on the slide glass, 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$



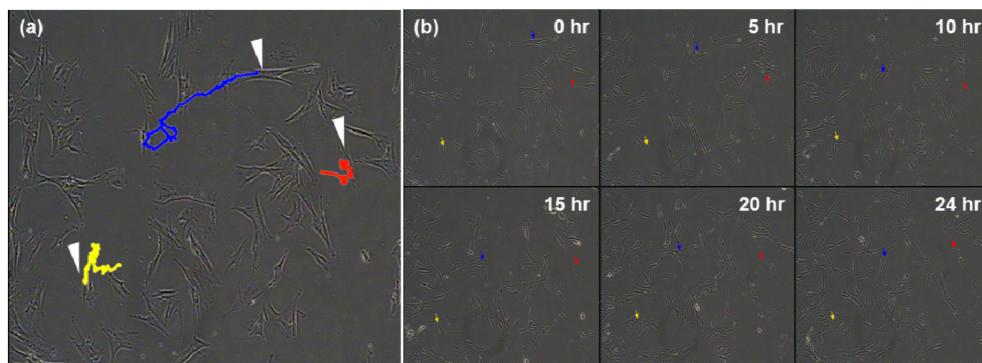
**Figure 3.** Attachment of nHDF on the slide glass, 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$  PLGA fibers.

PLGA meshes. There were no significant differences in nHDFs attachment between the slide glass and PLGA meshes. This indicates that the adhesion of nHDFs is less influenced by the thickness of PLGA nanofibers. In cell affinity as slide glass required situation, however PLGA fibrous scaffolds would not need specific treatments to increase cell attachment.

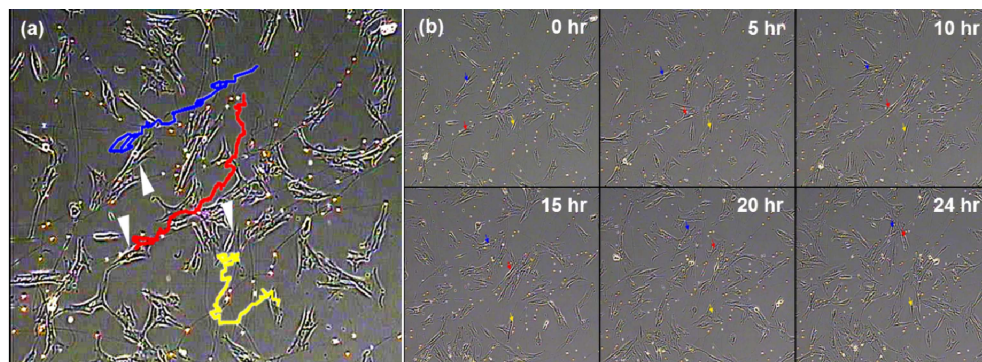
#### Migration of nHDFs on slide glass and PLGA nanofibers

The migration of nHDFs seeded on the slide glass, 0.4  $\mu\text{m}$  and 1.4  $\mu\text{m}$  thick PLGA nanofibers deposited on the slide glasses was observed for 24 hr to figure out the relation of diameter of nanofiber to migration of cells. Implanted scaffolds should have an internal structure for vascular invasion. Subsequently, the cells are able to migrate on the surface of the implanted scaffolds and also the infiltration of the cells in to the scaffolds.<sup>20</sup> Therefore we evaluate the migration of the cells on PLGA fibrous scaffolds. To characterize the migration pattern of nHDF, the position of cell nuclei was indicated with a dot and represents the cell pathway by connecting the dots. nHDFs on the normal slide glass migrate without space constraints (Figure 4a), and did not show directional migration (Figure 4b).

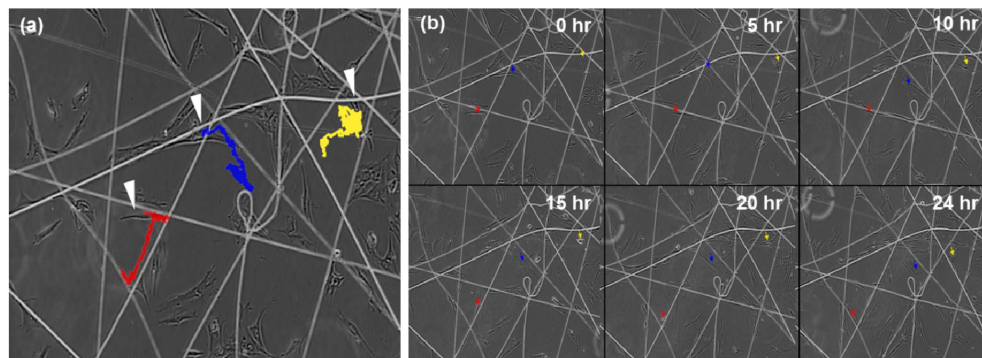
We observed nHDFs on 0.4  $\mu\text{m}$  thick PLGA nanofibers deposited on the slide glass migrate along the PLGA nanofibers and pass the nanofibers easily (Figure 5). This aspect may relate with the cell migration on micro patterned surface. Transmembrane proteins interacting with the micro patterned surface triggers the intra-cellular signaling pathways, and this pathway controls the cell responses.<sup>21,22</sup> Cell micropatterning and bio-microelectromechanical (Bio-MEMS) systems guide cell migration because the length scale of the features is similar to the length scale of mammalian cells. NIH 3T3 fibroblast on the “zigzag” patterns of microislands showed “zigzag” migration pathway.<sup>23</sup> It is considered that PLGA nanofibers played the role of microarray in this experiment. Figure 6 indicates that migration of nHDF is restricted by the 1.4  $\mu\text{m}$  thick PLGA nanofibers. Cells did not pass the 1.4  $\mu\text{m}$  thick nanofibers and were locked in small space surrounded by PLGA fibers. Thick PLGA fibers block the natural migration of cells, and conse-



**Figure 4.** nHDF migration pathway on the slide glass for 24 h (a), location of nHDF at different time (b).



**Figure 5.** nHDF migration pathway on the 0.4 μm PLGA fibers for 24 h (a), location of nHDF at different time (b).



**Figure 6.** nHDF migration pathway on 1.4 μm PLGA fibers for 24 h (a), location of nHDF at different time (b).

quently cell proliferation would be decreased. Cell infiltration into scaffolds also might be inhibited by blocking the migration of cells. Therefore, proper thickness of nanofibers should be considered when fibrous scaffolds are fabricated.

### Conclusion

In conclusion, cell adhesion was less dependent on the thickness change of PLGA nanofibers from 0.4 μm to 1.4 μm because of no significant differences of cell attachment between the normal slide glass and electrospun PLGA scaffolds. Migration of nHDFs was affected by the thickness of PLGA nanofibers. The cells passed the 0.4 μm thick fibers and migrate

along the PLGA fibers but did not cross the 1.4 μm thick fibers. Therefore, diameter of electrospun nanofibers affect cell migration and should be regulated properly for enhancing cell migration, infiltration on the electrospun scaffolds. In this research, it has been evaluated the effects on the cell migration in different diameter of fiber, and it could be applied for the development of the fibrous scaffold in tissue engineering.

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## References

1. S. Li, J. L. Guan, S. Chien, "Biochemistry and biomechanics of cell mortality," *Annu. Rev. Biomed. Eng.*, **7**, 105-150 (2005).
2. D. A. Lauffenburger and A. F. Horwitz, "Cell migration: a physically integrated molecular process," *Cell*, **84**, 359-369 (1996).
3. R. Vasita and D. S. Katti, "Nanofibers and their applications in tissue engineering," *Int. J. Nanomedicine*, **1**, 15-30 (2006).
4. J. R. Venugopal, Y. Zhang, and S. Ramakrishna, "In vitro culture of human dermal fibroblast on electrospun polycaprolactone collagen nanofibrous membrane," *Artif. Organs.*, **6**, 440-446 (2006).
5. E. K. Yim and K. W. Leong, "Significance of synthetic nanostructures in dictating cellular response," *Nanomedicine*, **1**, 10-21 (2005).
6. N. T. Hiep and B.T. Lee, "Electro-spinning of PLGA/PCL blends for tissue engineering and their biocompatibility," *J. Mater. Sci. Mater. Med.*, **21**, 1969-1978 (2010).
7. J. D. and D. H. Reneker, "Electrospinning process and applications of electrospun fibers," *J. Electrostat.*, **35**, 151-160 (1995).
8. B. Duan, X. Yuan, Y. Zhu, Y. Zhang, X. Li and Y. Zhang, "A nanofibrous composite membrane of PLGA-chitosan/PVA prepared by electrospinning," *J. Eur. Polym.*, **42**, 2013-2022 (2006).
9. C. M. Vaz, S. V. Tuijil, C. V. C. Bouten and F. P. T. Baaijens, "Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique," *Acta. Biomater.*, **1**, 575-582 (2005).
10. Y. Zhang, J. R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su and C. Lim, "Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering," *Biomaterials*, **29**, 4314-4322 (2008).
11. C. A. Bashur, L. A. Dahlgren and A. S. Goldstein, "Effect of fiber diameter and orientation on fibroblast morphology and proliferation on electrospun poly(D,L-lactic-co-glycolic acid) meshes," *Biomaterials*, **27**, 5681-5688 (2006).
12. H. Fong, I. Chun and D. H. Reneker, "Beaded nanofibers formed during electrospinning," *Polymer*, **40**, 4585-4592 (1999).
13. P. Gupta, C. Elkins, T. E. Long and G. L. Wilkes, "Electrospinning of linear homopolymers of poly(methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent," *Polymer*, **46**, 4799-4810 (2005).
14. L. S. Nair, S. Bhattacharyya, J. D. Bender, Y. E. Greish, P. W. Brown and H. R. Allcock, "Fabrication and optimization of methylphenoxy substituted polyphosphazene nanofibers for biomedical applications," *Biomacromolecules*, **5**, 2212-2220 (2004).
15. H. Fong, I. Chun and D.H. Reneker, "Beaded nanofibers formed during electrospinning," *Polymer*, **40**, 4585-4592(1999).
16. V. M. Entov and L. E. Shmaryan, "Numerical modeling of the capillary breakup of jets of polymer liquids," *Fluid Dynamics*, **32**, 696-703 (1997).
17. R. Jaeger, H. Schonherr and J. Vancso, "Chain packing in electrospun poly(ethylene oxide) visualized by atomic force microscopy," *Macromolecules*, **29**, 7634-7636 (1996).
18. R. Jaeger, M. Bergshoef, C. Battle, H. Schonherr and J. Vancso, "Electrospinning of ultra-thin polymer fibers," *Macromol. Symp.*, **127**, 141-150 (1998).
19. J. Y. Kim and D. W. Cho, "Blended PCL/PLGA scaffold fabrication using multi-head deposition system," *Microelectron. Eng.*, **86**, 1447-1450 (2009).
20. M. Mastrogiacomo, A. Muraglia, V. Komlev, F. Peyrin, F. Rustichelli, A. Crovace and R. Cancedda, "Tissue engineering of bone: search for a better scaffold," *Orthod. Craniofac. Res.*, **8**, 277-84 (2005).
21. D. Falconnetta, G. Csucs, H. M. Grandina and M. Textor, "Surface engineering approaches to micropattern surfaces for cell-based assays," *Biomaterials*, **27**, 3044-3063(2006).
22. H. RO, "Integrins: bidirectional, allosteric signaling machines," *Cell*, **110**, 673-87 (2002).
23. G. Kumar, C. C. Ho and C. C. Co, "Guiding cell migration using one-way micropattern arrays," *Adv. Mater.*, **19**, 1084-1090 (2007).