31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Fabrication of microfluidic system for the assessment of cell migration on 3D micropatterned substrates

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Abstract— Cell migration and proliferation are major process in wound healing, cancer metastasis and organogenesis during development. Many cells are related to recovery process of wound. Especially, fibroblasts act an important role in wound healing. Various cytokines such as platelet derived growth factor (PDGF) can induce fibroblast migration and widely studied to investigate the cell response under controlled cytokine microenvironments during wound healing. In real tissue healing process, cell microenvironments change with tissue types and anatomical characteristics of organs. With microfluidic system, we tried to mimic the natural microenvironment of wound healing, with gradient of PDGF, a fibroblast migration inducing cytokine, and patterned substrate with different orientation to PDGF gradient. Fibroblasts cultured in PDGF gradient micro fluidic chip showed cell migration under various micro environmental gradient conditions. Cells were cultured under PDGF gradient condition and different substrate pattern. Mouse fibroblast L929 cells were cultured in the microfluidic gradient. The results showed that most cells migrated along the substrate topological patterns under high concentration of PDGF. We developed long range sustaining micro fluidic channel and could analyze cell migration along the gradient of PDGF. Also, the cell migration on patterned extracellular environment shows that cells migrate along the extracellular 3D pattern rather than directly along the cytokine gradient when the pattern height is less than 1 μm. In this study, we could demonstrate that the extracellular pattern is more dominant to cell migration in combination with cytokine gradient in the wounded tissue when the environmental cues are 20 μm.

I. Introduction

Cell migration is a fundamental behavior of embryonic stem cell in gastrulation and organogenesis during developmental process. The harmonized movement of cells toward a particular direction and to a specific location occurs during embryonic development, immune responses and wound healing process. Cell migration is also a major mechanism in cancer development and propagation. Cells in animal tissues often migrate in response to, and toward specific external signals, a process called chemotaxis.

Fibroblast is one of dominantly migrating cell type in wound healing process according to biochemical signals secreted from defected tissue regions [1]-[3]. Fibroblasts are principal origin of ECM (extracellular matrix) and produce collagen and fibronectin that form granulation. When cells migrate to tissue defect and form a new tissue, the microenvironments such as tissue texture of cell alignment, ECM pattern can affect cell migration direction and speed to wound sites. For better understanding of the basic mechanism of wound healing and tissue response, it is important to control biochemical and physical microenvironment of cells which initiates cell migration.

The purpose of this study is to suggest a micro fluidic system providing controlled cell migration microenvironments. Cytokine gradient and 3D textured extracellular matrix-like structures were generated in the cell culturing micro fluidic chip [4]. For this purpose, we fabricated a microfluidic system to observe fibroblast migration under PDGF gradient and different substrate conditions. To investigate tissue texture effect versus chemical effect, we tried to mimic extracellular matrices by assembly of 3D groove patterns with cytokine gradients. For this purpose, we made microfluidic gradient chip system on the substrates with different orientation with PDGF gradient [2]-[3]. The effect of pattern topology on cell migration was also observed when the pattern height changes from 1 to 20 μm. Fibroblasts showed active movement under PDGF gradients moving along the patterns [5]-[6]. We could fabricate microfluidic chip system which can give reproducible results by controlling biophysical microenvironments in designed conditions. We could observe migration, proliferation and elongation of fibroblasts in microenvironment which mimic the wound healing process in real tissues.

II. MATERIALS AND METHODS

Micro fluidic chip design and fabrication

Micro fluidic chip used in this study was composed of two-layer PDMS channel structure, as illustration in Figure 1.

Manuscript received April 7, 2009. This research was supported by a grant (M103KV010026-08K2201-02610) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, the Republic of Korea.

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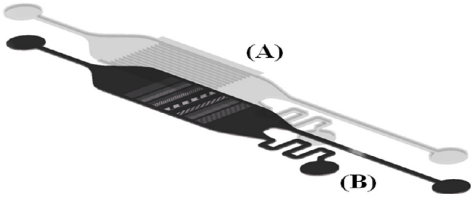

Figure 1. Schematic diagram of micro fluidics chip, and device consist of a two-layer PDMS channel structure. (A) layer height is 250μm, (B) bottom pattern height is 0, 1, and 20μm.

The main function of the upper layer channel was reducing lateral diffusion in the cell culture region and bottom layer pattern had biomimetic design of extracellular matrix.

The pattern orientation was designed to have specific angle to chemical gradient of growth factors, with 45, 90 and 145 degrees, and pattern spacing was set to 20μm irrespective of pattern angle.

The micro fluidic chip was designed using AUTOCAD 2007 (AUTODESK, USA). Fabrication process of micro fluidic chip is illustrated in Figure 2. The design was exposed onto an emulsion mask (Nepco, Ansan, KOREA). A SU-8 master mold for making a micro fluidic chip was fabricated on a 3inch-diameter Si wafer. The 50 μm thickness of first layer was prepared by spin coating SU-8(50) (MicroChem, USA) at 2000 rpm for 25 s. After soft-baking at 65 °C for 10 min, the temperature was raised to 95 °C and left for 30 min. The pattern on a first layer mask was exposed to SU-8 coated silicon wafer at 365 mJ/cm^2 using contact aligner (Shin-Wo, KOREA). After exposure of the first layer, exposed layer of 200 μm thickness was made by spin coating SU-8(50) at 700 rpm for 20 s. After soft-baking at 65 °C for 10 min, the temperature was raised to 95 °C and left for 50 min.

The pattern on a first layer mask was exposed again to SU-8(50) coated silicon wafer at 365 mJ/cm^2 using Shin-Wo A200 contact aligner. The second layer of SU-8(25) was spin coated at 2000 RPM for 30 s to create a 20 μm thickness layer. The second layer was exposed to 365 mJ/cm^2. Then, post-exposure baked at 65°C for 1 min and 95°C for 10 min, and the master mold was developed in a SU-8 developer solution for 10 min with agitation. PDMS mixture was prepared with 10:1 weight ratio of prepolymer and curing agent. After degassing to remove air bubbles, the mixture was gently poured onto a SU-8 master mold. The PDMS was cured at 80°C for 120 min and the PDMS structure was cut and peeled off from the master mold. Manufactured micro fluidic chip device was treated with O_2 plasma bonder for 2 min and immediately bonded together reversely (Figure 2). Inlet and outlet holes were punched with 24 gauge stainless steel needle [7]-[10].

Osmosis pump was used for keep constant flow rate of cell culture medium. To test osmosis pump, diluted yellow and blue dye were supplied into different inlet (Figure 3). Flow rate of osmosis pump was determined by concentration of PEG (0.082 mol) [11]. Cell culture fluid (500$\mu\ell$) with 10.0 nM/$m\ell$ of PDGF was introduced into one side inlet, and only

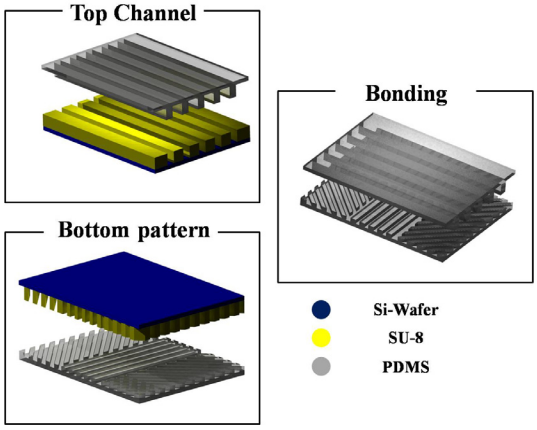

Figure 2. Fabrication process of micro fluidics chip.

cell culture media (500$\mu\ell$) only introduced to the other side of inlet. These were experimented in manufactured micro fluidic chip for real time observation. Also, osmosis pump was connected to outlet of micro fluidic device. Cell is used L929 mouse fibroblasts (ATCC, NCTC clone 929, USA) in this study. Cells were seeded by density of 5x10^{5}cell/$m\ell$. Cells were kept in incubator (5% CO_2, 37°C) during 4 hours after seeding in micro fluidics chip. Cells in device were observed at interval 5 minutes through microscope (Olympus BX50).

III. Result and Discussion

Micro fluidic chip for cell migration

The cross sectional view of the microfluidic chip is also illustrated in Figure 2. The bottom substrate of micro fluidic channel was grooved with different height. The purpose of this substrate height modulation was to investigate the guidance effect during cell migration. This substrate pattern was to resemble the microenvironment of cells with 3D grooved patterns.

The resulting microstructure demonstrated ECM of cells in *in vivo* tissues which may affect the directional cell migration. This PDMS based substrate was made by SU-8 photo resistor spin coated on Si wafer and replicated by soft lithography. The size of pattern used in this study was 20 μm in width and pacing to form grooves.

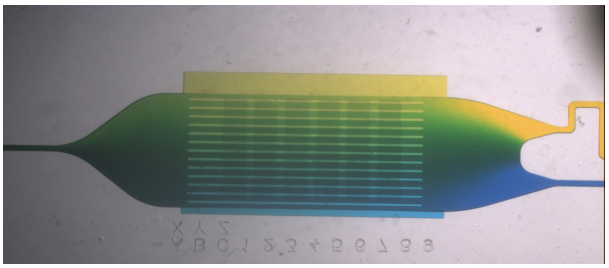

Figure 3. Image of generated constant gradient in the microfluidic chip. Horizontal lines are grooves for minimization of lateral diffusion across the gradient and vertical bands are 3D micropatterned substrate. Osmosis driven pump was used for pumping yellow and blue dye, colored for visualization of diffusion field in the microfluidic chamber.

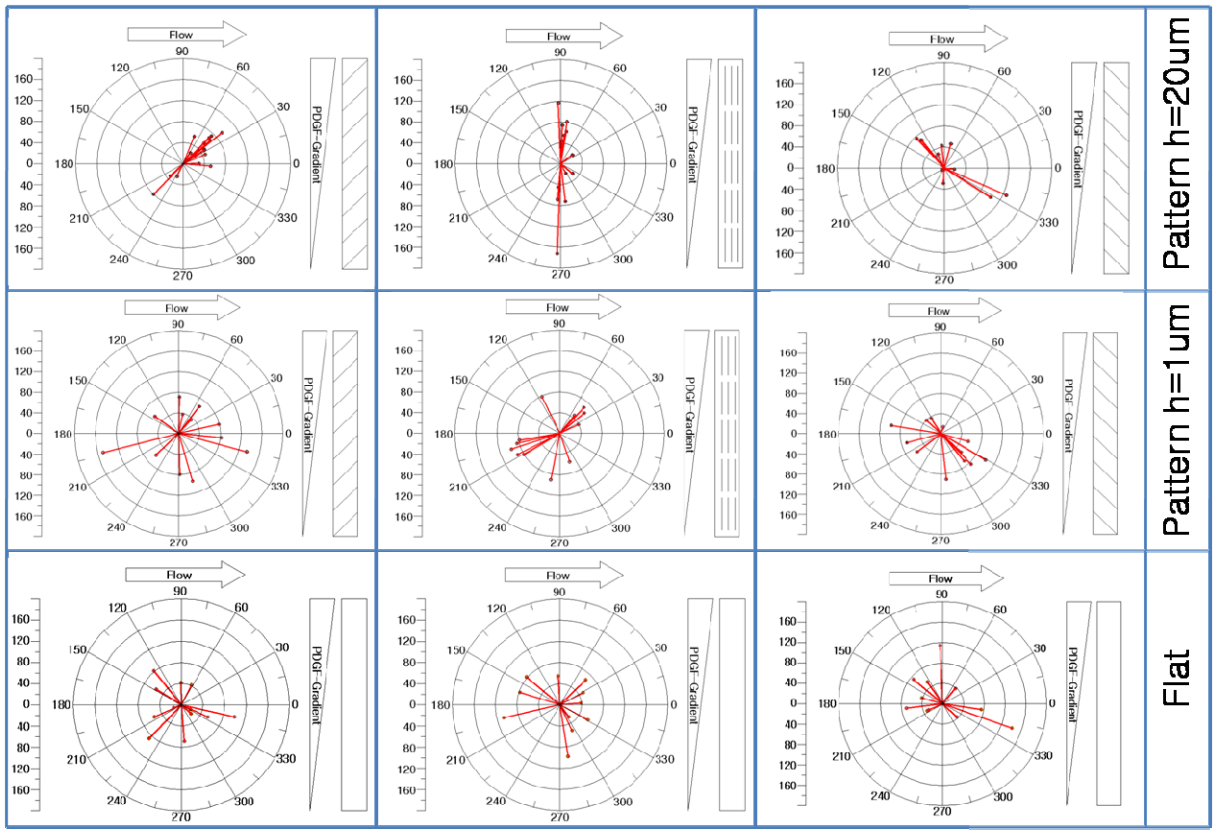

Figure 4. Graph of distance and direction of cell migration by pattern height

To generate chemical gradient, we used osmotic pump driven passive flow during cell culture. This osmotic pump was connected to the flow out port of chip and induced passive pumping. In the inlet port, the 1000 μL micropipette tip was filled with medium with and without PDGF. As shown in Figure 3, chemical gradients demonstrated with edible dyes with osmotic pump. Due to stability of osmotic pump in low speed, adequate flow rate was achieved. In Figure 3, diffusion based mixing was shown for gradient generation without complex mixing structures within micro fluidic channels. To maintain chemical gradient generated by diffusion, there were guidance grooves from the top surface of the PDMS in microfluidic channel. Because of the low flow rate, the initial chemical gradient could change by diffusion mixing in distal part of chip. As shown in Figure 3, the grooves successfully maintain the initial gradient with minimized mixing along the gradient.

Cell migration under PDGF gradient

Cell migration experiment was produced with L929 mouse fibroblasts. Before cell seeding, fibronectin coating was used for cell adhesion. Pumping started after cell adhesion and generated PDGF gradient across the micro fluidic region. Cells attached on the bottom angled with the gradient direction along the substrate micro pattern. This is due to the cell guidance grooves, which were angled with the flow direction. We could observe cells migrating across the channel after forming PDGF chemical gradient with osmosis pump. We could observe the cell migration from series of time lapse image for 20 h captured by optical microscope (Zeiss Axiovert). The analyzed cell migration results were summarized in Figure 4. Vector plot (ignoring the intermittent position during migration) shows the cell migration characteristics. The polar coordinate expression clearly shows the cell migration direction and distance in Figure 4. Analyzed cell migration data shows that the cells has directionality along the substrate patterns. Especially in 20 μm height pattern, result shows almost same direction along the substrate pattern. As the pattern height decrease, cell migration result shows less preference to substrate patterns. Also, cell migration was not fully consistent to PDGF gradient.

Figure 5 shows the immunostaining of cells with actin(red), vinculin(green) and nucleus (DAPI, blue). Cells show alignment along the groove direction. We observed actin filament, a marker of cell movement, was also aligned following pattern direction. However, cells on flat substrate did not showed preferred direction. Also, most cells showed their migration to higher PDGF region. The result of 1 μm-height pattern shows less guiding cells along the patterns. This micro fluidic chip based cell migration research will be useful for study of cell migration during organ generation and wound healing. Also, the gradient can also be applied to screening of various kinds of drugs with different tissue origin cells for targeted drug development.

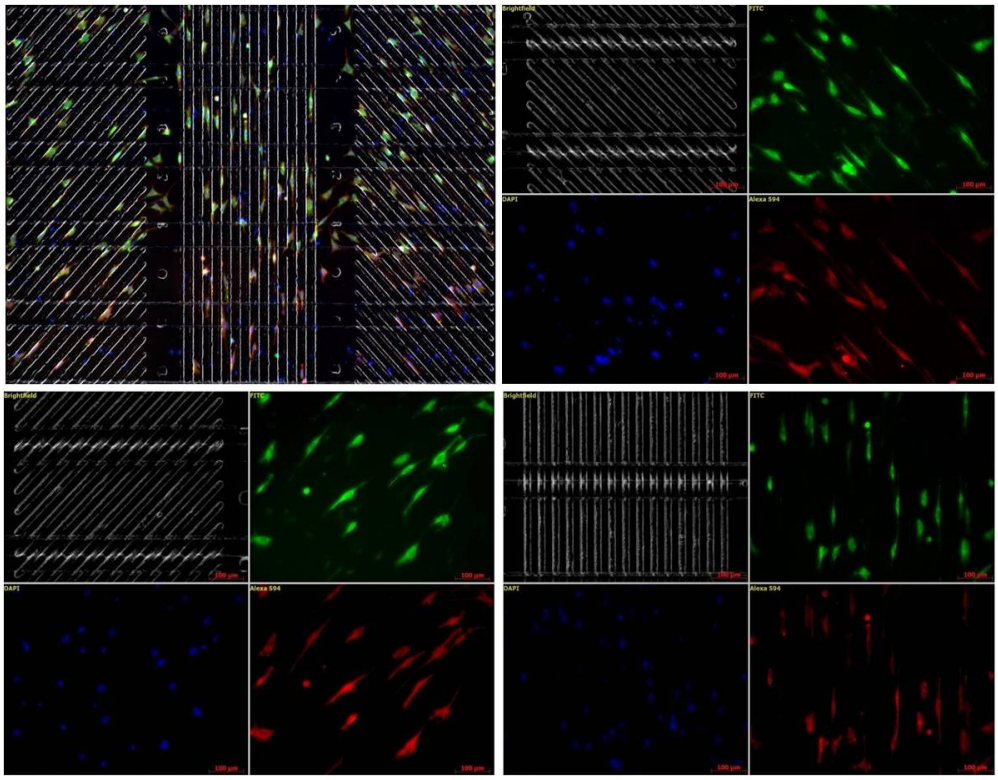

Figure 5. Fluorescence microscopy images of L929 after seeding onto Microfluidic channel for 20hr; staining with Actin(red), Vinculin(green), DAPI(blue).

IV. Conclusion

In this study, we demonstrated biomimetic system with chemical gradient and topological cues in the micro fluidic chip. This system generates stable gradient in the channel and has anisotropic topography on the substrate to mimic the ECM microenvironments with 3D substrate patterns. Cells on the surface migrated to the chemical gradient of PDGF. During migration, cells moved along the substrate topographic cues in 20 μm height. When the pattern height was 1 um cell migration direction was less oriented to growth factor gradients. This cell migration controlling microfluidic system has many applications for biological and medical fields. We can also investigate other cell types for further understanding of cells, such as embryonic stem cells during differentiation and organ generation process.

Acknowledgment

This research was supported by a grant (M103KV010026-08K2201-02610) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, the Republic of Korea.

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