MICROFLUIDIC CELL STIMULATOR USING BEAD IMPACT

Young-Hun Kim, Tae-Jin Kim, Hyung-Joon Kim, Min-Suk Park and Hyo-Il Jung
School of mechanical engineering, Yonsei University, Seoul, Republic of Korea

Keywords: Mechanical stimulation, Micro device, Rolling bead, CPAE, PDMS.

Abstract: Recently many researchers are focused on cell stimulation regarding observation of cells to specific stimulation factors. We introduce new mechanical stimulation method using micro beads without any chemical reagents. CPAE (calf pulmonary artery endothelial cell) were cultured in PDMS (polydimethylsiloxane) microfluidic device. After starvation process, sterilized 10μm glass beads were rolled by only gravitational force for 10 minute. To find optimal stimulation time, 16 devices were made by PDMS and each device was slanted every hour. Results show that cell exposed under micro bead stimulation perform at a higher growth rate than normal conditions and 1 hour stimulation time represents more effective than other stimulation times. This new cell stimulation method will be able to help make artificial organ such as blood vessels in the future.

1 INTRODUCTION

Studies about observation of cell behaviour are performed to explore factors which are related with inhibition and promotion of cell growth. It is true that cells in human and animal are exposed under various stimulation such as mechanical (Matteucci, 2007), chemical (Nakashima, 2005), and electrical stimulation (Mattei, 2004). These studies were tried to find alternative stimulation of cell growth, thus could contribute tissue regeneration. However the effect of mechanical stimulation is not to be accomplished and possibility of stimulation is still opened.

Miniaturized bioreactor for cell stimulation was developed by soft lithography and PDMS (polydimethylsiloxane) molding technique. It is considered inexpensive and time saving method and makes disposable device and requires small culture media and other reagents. Moreover PDMS has bio-compatible and gas permeability. By using PDMS device, we could fabricate micro cell stimulation system with a small amount of culture media and other reagents (Kim et al., 2008). Various methods for physical cell stimulation such as fluidic shear stress (Brown., 2008), pneumatic pressure (Sim, 2006), microfluidic motion (Pioletti, 2003), and etc. have developed. In light of this perspective; we expect that micro-beads can be one of effective sources for physical stimulation to improve cell growth.

Now, we demonstrate a novle stimulation method using micro-bead impact and microfluidic device. The mechano-stimulation by bead impact will be one of the efficient stimulation methods.

2 MATERIAL AND METHOD

2.1 Fabrication of Stimulation Device

Various experiments were conducted in order to find an optimal condition for cell culture in microfludic culture chambers, and the first step is to determine the appropriate method to fabricate devices. Our choice was to use soft lithography and PDMS to easily acquire the required microfluidic devices.

A set of single straight channels were created on a Silicon wafer using negative photoresist Su-8 mold. After treating a bare silicon wafer with acetone, methanol and DI (deionized) water, respectively, Su-8 2050 (MicroChem, MA, USA) negative photoresist was spin-coated at 1,300 rpm for 30 seconds to acquire a channel height of 100 μm. After the soft baking process, the wafer was exposed under UV light followed by additional heat treatment for post exposure baking process. The wafer was etched using Su-8 developer and was

Kim Y., Kim T., Kim H., Park M. and Jung H. (2009).
MICROFLUIDIC CELL STIMULATOR USING BEAD IMPACT.
In *Proceedings of the International Conference on Biomedical Electronics and Devices*, pages 426-429
DOI: 10.5220/0001780204260429

rinsed using iso-propyl alcohol (Figure 1).

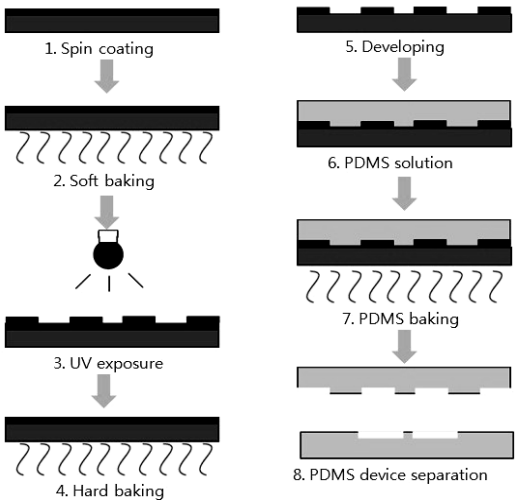

Figure 1: Soft lithography fabrication process using Su-8 photoresist.

2.2 Cell Culture

CPAE (calf pulmonary artery endothelial cell) was selected to investigate the change in growth rate when exposed to micro bead impact. These cells are thought to be exposed to blood cells impact in a blood vessel. It can be thus, mimicry of blood cells impact that micro beads collide against the surface of CPAE. Sterilization was performed under UV for 24 hour 70% ethanol. The cells were seeded into the microfluidic chamber with a concentration of $1.65x10^6$/ml and were incubated overnight. Before experiments, starvation process was conducted with 0.5% FBS RPMI media for 24 hours to fix cell phase in G1.

2.3 Experimental Setup

In previous work, two types of cell lines, HeLa cell and MC3T3 cell, were selected to investigate the changes in growth rate when exposed to micro-bead impact. The cells were seeded into the microfluidic chamber with a concentration of 5 x 10^5 cells/ml and were incubated overnight. Fresh DMEM was supplied in HeLa cell cultured chambers and aMEM in MC3T3 cell cultured chambers every 12 h. Since the concentration of 11µm micro-beads in culture medium is 10^6/ml, and the flow rate is 3µl/min, the number of 11µm micro-beads passing through a single straight cell culture chamber per minute is calculated as 3 x 10^3beads/min (Figure. 2)

However, this simulation system was designed except important aspects. It had two mechanical stimulus factors, flow and bead impact and it is not considered about cell cycle which the series of events that take place in a cell leading to its replication. Therefore we developed idea to minimize other stimulus sours and maximize bead impact to cells. Also we performed experiment with considering cell cycle to find optimal stimulation time and to get effect of the number of micro-bead rolling in cell growth.

Sixteen micro devices have been fabricated because cell cycle is about 16 hour and each device has 10 cell culture chamber which dimensions are height = 100µm, width = 40µm, length = 80µm (Figure 3). There were two inlets, one for cell seeding and media and other for beads. The concentration of 10 µm glass beads is $1.9x10^5$/ml. Before tilting device, micro beads were gathered on inlet for bead. Slating process was performed for 10 minutes in incubator.

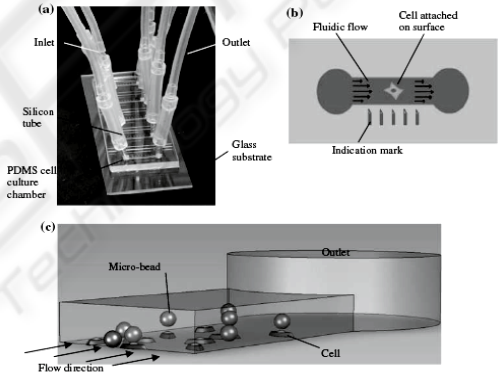

Figure 2: Photograph and schematic diagram of previous work about cell stimulation.

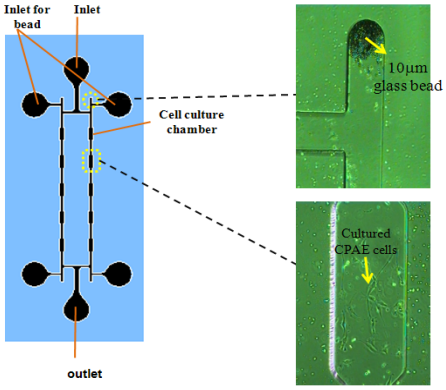

Figure 3: Schematic diagram and photograph of device.

3 RESULT

The micro-bead and cell culture medium mixture was introduced into the microfluidic chamber using syringe pump infusion mode, and deflections in the micro-bead pathway were observed as they flowed over or by the adhered cells. This motion indicates that micro-beads flow along the adhered cell's boundary layer and the outer membrane of the cells are stimulated through this manner. The cell population of HeLa and MC3T3 cells under different micro-bead stimulation was counted through microscopic observation at a 12-h interval, and the proliferation rate was then achieved by dividing the cell number in each time interval by the initial population. The cell increase rate of HeLa cells in the experimental chamber with 11-lm micro-bead stimulation performed the highest growth rate, whereas 2μm micro-bead stimulation performed the lowest growth rate. For the case of MC3T3 cells, the cell increase rate of the 2μm micro-bead stimulation device was significantly higher than that of the 11-lm micro-bead stimulation device, while the control group performed the lowest stimulation rate (Fig. 4).

Cell growth was measured in 16 type device: control device and other related with each stimulation time. The cell population of HeLa and MC3T3 cells in the control and experimental chambers was observed through an inverted optical microscope (Olympus, Japan) and compared after 16 hours. All fluids were supplied by capillary force to remove effect of flow. Figure 5 shows increase of CPAE cell according to the stimulation time. The proliferation rate (Figure 6) was then achieved by dividing the cell number in each time interval by the initial population.

As mentioned above, cells are divided by cell cycle which consists of G1, S, G2, and M phase. First, G1 phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Next, S phase is related with DNA synthesis. Third, G2 phase significant protein synthesis occurs which are required during the process of mitosis. Finally in M phase the cell is divided by two cells.

According to figure 6, we could conclude that micro bead stimulation was more effective at 1 hour data than other stimulation time. We analyzed that 1 hour stimulation time was involved in G1 phase because cells were fixed by starvation process. G1 phase takes part in synthesis of diverse proteins about DNA synthesis. Therefore mechanical stimulation by rolling micro bead plays an important role to proliferation of cells. Following this data, we performed other experiment to verify effects of the number of rolling micro-beads. Figure 7 shows that more frequent micro-beads stimulation could be better effect in cell growth.

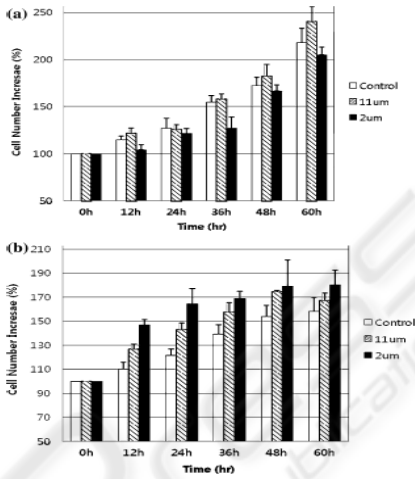

Figure 4: Cell number increase comparison of control group. : (a) HeLa cells and (b) MC3T3 cells.

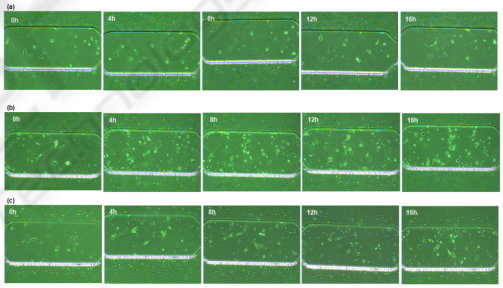

Figure 5: Photograph of CPAE cell in micro chamber : (a) control device, (b) device at 1 hour stimulation time,(c) device at 15 hour stimulation time.

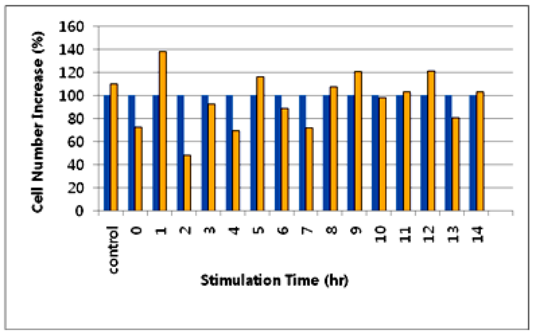

Figure 6: Comparison of growth rate of CPAE cells under different stimulation time: blue bar for data at 0 hour and yellow bar for data at 16 hour.

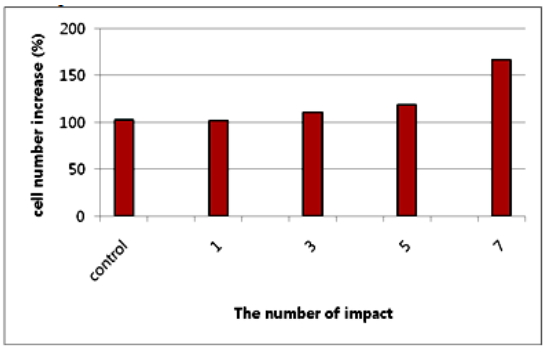

Figure 7: Comparison of growth rates of CPAE cells under different the number of beads rolling.

4 CONCLUSIONS

A new method for stimulating cells has been introduced. Previous our work was conducted with two types of polystyrene micro-beads: 2 and 11μm micro-beads. The cell population increase rate was the highest when stimulated by 11 μm beads in the case of HeLa cells and 2 μm beads in the case of MC3T3 cells. From the comparison between control device and experimental device, it can be seen that cells in the experimental group performs at a higher growth rate. This suggests that the growth rate is accelerated when stimulated by micro-beads. We designed new device to maximize micro bead stimulation and minimize other factors: beads were rolled by only gravitational force. Thus, we get data about more effective simulation time, 1 hour which is involved in G1 phase and cells exposed under micro bead impact represent better proliferation. Moreover, the number of bead impact is one of the important factors in cell stimulation and more frequent impacts show better effects to the cells. When our new devices which were designed to maximize beads impact was compared with previous our device, cell proliferation data by only beads impact represent similar increase rate (about 10%~40%). Therefore we could conclude that micro-beads stimulation can be one of the effective physical stimulation to enhance cell proliferation.

Now, we are conducting additional experiments to convincing our experiments including cell viability test and we will perform other analyzing test to compare with other research data such as protein analysis.

ACKNOWLEDGEMENTS

This work was supported by National Core Research Center (NCRC) for Nanomedical Technology of the Korea Science & Engineering Foundation (Grant no. R15-2004-024-01001-0), Seoul Research & Business Development (R&BD Program, 11128) and Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2007-313-D00073).

REFERENCES

Matteucci M, 2007, *Preliminary Study of Micromechanical Stress delivery for Cell Biology studies.* Microelectron, Eng 84:1729–173

Nakashima Y, 2005, *Microfluidic device for axonal elongation control*, Transducers, Seoul, Korea, 5–9 June

Mattei MD, 2004, *Effects of Physical Stimulation with Electromagnetic Field and Insulin growth factor-I treatment on proteoglycan synthesis of bovine articular cartilage*. Osteoarthritis Cartilage 12:793–800

Tae-Jin Kim, Su-Jin Kim, Hyo-Il Jung, 2008, *Physical stimulation of mammalian cells using micro-bead impact within a microfluidic environment to enhance growth rate*, Microfluid Nanofluid on-line published

Brown TD, 2000, *Techniques for mechanical stimulation of cell in vitro: a review*, J Biomech, 33: 3-14

Sim WY, 2006, *A pneumatic micro cell simulator for the differentiation of human mesenchymal stem cell (hMSCs)*, MicroTAS, Tokyo, Japan,5-9

Pioletti DP, 2003, *Effect of micromechanical stimulations on osteoblast: development of a device stimulating the mechanical situation at the bone-implant interface*, J Biomech 36: 131-135