

THREE DIMENSIONAL CO-CULTURE OF NEURON AND ASTROCYTE IN A MICRO-FLUIDIC DEVICE

Young Hun Kim¹, Young Eun Kim¹, Suk Chung², Byungkyu Kim³, Tae Song Kim¹ and Ji Yoon Kang¹

¹Korea Institute of Science and Technology

²Korea University, Republic of Korea

³Korea Aerospace University, Republic of Korea

ABSTRACT

In this paper, we describe a microfluidic platform that enables three dimensional cell culture within defined micro-environments towards a study of axon-glia interaction. The microdevice can offer both 3D axon isolation from cell body and 3D spatial cell separation between neuron and astrocyte. It consists of large fluidic channels for media supply and small channels for collagen filling as a three dimensional ECM (extracellular matrix). Astrocytes were seeded in collagen gel through small fluidic channels and neuron cells were aggregated at an openings of small channel to large channels. This method separated neuronal soma from axon as well as segregated neuron body from astrocyte. The preliminary study on the effect of astrocyte to the axon growth showed that axon growth was faster and richer in co-culture condition without growth factor. This 3D co-culture chip can provide a new method to observe the effect of astrocyte on the axon growth in three dimensional culture system.

KEYWORDS: microfluidic chip, 3D cell culture, neuron, astrocyte, co-culture

INTRODUCTION

Nerve system contains neuron and glial cells that transmit reciprocal signals between organs and nerve system. Most of animals have two parts of nerve system: central nerve system (CNS) and peripheral nerve system (PNS). Especially, CNS plays a critical role in our body and get involved in serious diseases such as spinal cord injury, Parkinson's and Alzheimer's disease. The causes of these chronic neuronal diseases are apparently relevant not to one kind of cell but to malfunctioned interaction between cells. Therefore, researchers need *in-vivo-like* experimental models to elucidate the mechanism of chronic diseases through genetic manipulation of neuronal cells. Especially, we are focused on spinal cord injury model because there is no therapies to recover the neuromuscular function and affects a significant deterioration on quality of life [1].

The major reasons of spinal cord injury are damage of axons and the effect of reactive astrocyte to axons. Some molecules in inhibiting axon growth are also known. Nevertheless, however, there are no effective therapeutic methods to cure the spinal cord injury yet. The difficulty lies in the variety of patients' damage status and the interactions in healing process among the several cells such as astrocyte, oligodendrocyte, microglia, fibroblast and other immune cells are very complex and time-varying. Since the observation of axonal injury and axon-glia interactions is difficult in *in-vivo* environment, many researchers are pursuing *in-vitro* experiment model. The Campenot chamber, which enables manipulation of axons independently from neuronal soma, was developed to compartmentalize axons from neuronal cell bodies. However, embodiment of *in-vivo-like* environment to observe interaction between axon-glia has been precluded within Campenot chamber [2]. Also, it is difficult to study the interaction in monolayer culture plate because it induces astrocyte proliferation and activation by itself. To mimic *in-vivo* environment, new experimental devices are required to realize three dimensional soft ECM and to cultivate more than two kinds of cells. Therefore, as a beginning to build up *in-vitro* neuronal model, we co-cultured both astrocyte and neuron on 3D soft collagen in micro-fluidic chip to compare axon growth according to the existence of astrocyte [3].

EXPERIMENTAL

We used a microfluidic chip to three dimensionally locate neurons and astrocytes. SU-8 negative photoresist was used as a master mold for main channel and collagen filling channel on silicon substrate. For easy detachment of polydimethylsiloxane (PDMS) mold from SU-8 pattern, the master was coated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane. After mixing with curing agent, PDMS was poured up to 4 mm thickness on the master and was cured at 80 °C for 1 hour. The cured PDMS slab was bonded to a slide glass by plasma cleaner. The dimension of observation zone is 800 μm x 200 μm and height of all channels is about 165 μm (Fig.1).

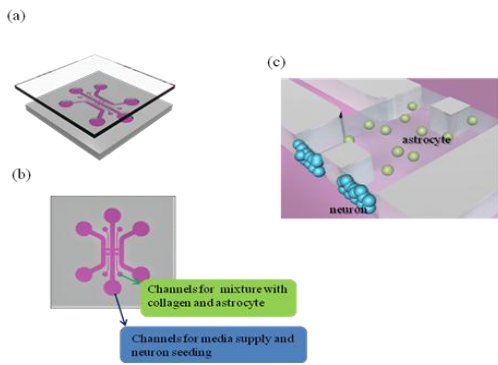


Figure 1: Schematic diagram of the microfluidic chip for 3D astrocyte cell culture: It had three main channels for supplying cell culture media and four small channels for filling mixture of collagen and cells (a) overall view of the device (b) descriptions about each channel (c) schematic diagram of observation zone: After solidification of mixture with astrocyte and collagen, neuron cells are attached in a side of collagen wells

RESULTS AND DISCUSSION

Because astrocyte cells could be activated after treatment of trypsin, we observed astrocyte morphology every day to verify resting state of astrocyte. To make stable condition, we seeded primary neurons after 3 days of astrocyte seeding in the microfluidic chip. After 7 days, axons under co-culture with astrocyte were thicker, longer and healthier than control (without astrocyte) (Fig. 2 and Fig. 3). There is no deterioration in cell viability of both culture conditions until up to 7 days.

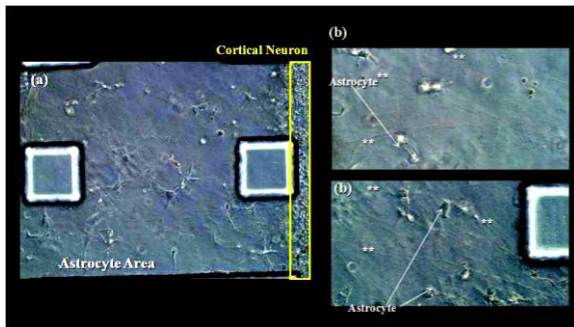


Figure 2: Microscope image of co-culture of neuron and astrocyte (a) a photograph of observation zone in the microfluidic chip (20X, after 7 days) (b) a photograph about axon growth with astrocyte: asterisks (**) appear axons. Most of axons showed preference of astrocyte (40X, after 7 days)

The device was consisted of two channels: large channels were for offering culture medium and primary cortical neuron seeding and small channels were for filling collagen mixed with primary astrocyte cells. We obtained cortical rat neurons from E18 fetal rat cortex and cortical astrocytes from P1 rat cortex. These cells were cultured in neural basal media containing 2% B27 (vol/vol), 0.25% glutamax (vol/vol) and 2M L-glutamine.

The devices were stored at 80 °C overnight to recover hydrophobicity before filling collagen gel scaffold. The sterilized PDMS chips were filled with 2 mg/mL collagen Type (pH 7.4). Collagen solution was made with a mixture of 10x PBS, 1 M NaOH and sterilized deionized water. For 3D primary astrocyte culture in the device, cells were seeded at a density of 5 million cells/ml in collagen gel solution and stored in a cell culture incubator for 30 min for gelation. After 3 days, primary cortical neurons (cell density was between 2.5 million cells/ml and 8 million cells/ml) were loaded in large channels.

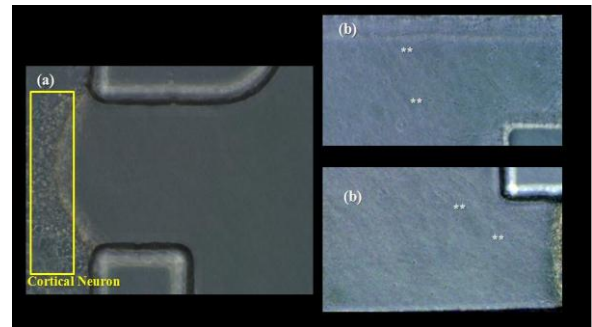


Figure 3: Microscope image of neuron culture without astrocyte (a) a photograph of observation zone in the microfluidic chip (20X, after 7 days) (b) a photograph about axon growth : asterisks (**) appear axons. In contrast to co-culture condition, axons were short and even axon degradation was appeared.

The differences of axons between control and co-culture were verified by immunostaining of MAP 2 (Microtubule-associated protein 2, a marker for neuron cells) and GFAP (Glial fibrillary acidic protein, a marker for astrocyte). The axons with astrocyte are denser and healthier than the control because axonal degradation and axon end-bulbs were appeared in only control (Fig. 4). The images showed that soma of neurons were isolated by collagen and only axons were grown in three dimensional collagen scaffold. Also, only astrocytes in the scaffold and no cell body was infiltrated into collagen. To quantify these immuno-fluorescent data, we used RGB (red, green, and blue) mean value of confocal microscope. Each values (red, green, blue) in the image were obtained and plotted in graph (Fig.5). The values in red and blue mean background noise and green means intensity of immuno-fluorescence because neuronal soma and axons were stained by only green. These results demonstrated that axon elongation was more clear in co-culture condition. The quantified data were acquired by Image J program.

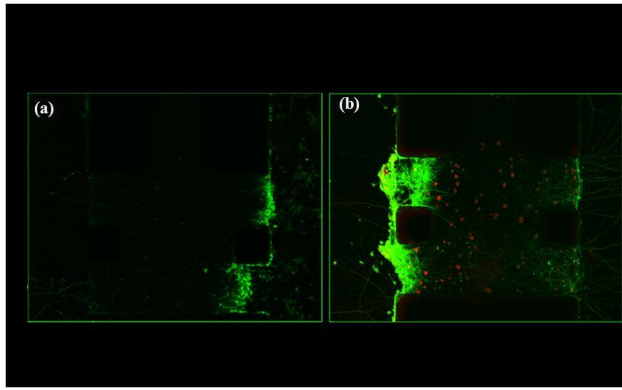


Figure 4: Confocal microscope image about immunocytochemistry (MAP2- green, GFAP- red)(a) a chip for only neuron culture (b) a chip for neuron-astrocyte co culture In contrast to neuron culture, a lot of axons are shown in co-culture condition.

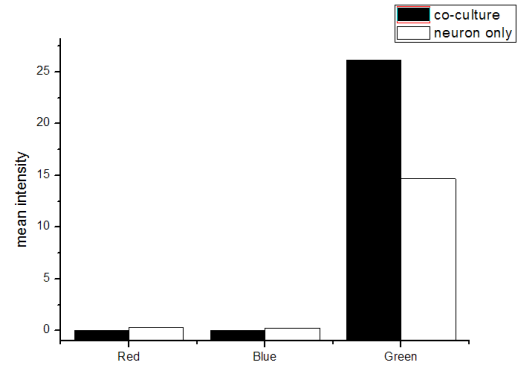


Figure 5: Quantification of MAP2 (green) staining: It shows mean intensity of staining significantly increased in co-culture condition after 7 days in culture compared to only neuron cultures at the same time point (n=3, data are acquired by ImageJ and graph are plotted by Origin)

CONCLUSION

We developed a new method for co-culture of neurons with astrocyte. The experimental results confirmed that neuronal soma could be isolated by collagen scaffold and axon could grow into three dimensional collagen scaffold. Also, the device can be used as a co-culture platform of neuron and astrocyte and the co-culture enhanced the axon elongation. In conclusion, the 3 dimensional microfluidic device was effective in culturing primary neuronal cells such as neuron and astrocyte and it is expected that the microfluidic chip can be utilized as a new platform to study neuron-glia interaction by offering *in vivo*-like environment.

REFERENCES

- [1] "Therapeutic interventions after spinal cord injury", Sandrine Thuret, Lawrence D.F. Moon, Fred H. Gage, Nature Reviews-neuroscience , **7**, 628- 642 (2006)
- [2] "Circular compartmentalized microfluidic platform: Study of axon-glia interaction,"Suneil Hosmane, In Hong Yang, April Ruffin, Nitish Thakor, Arun Venkatesan, Lab On a Chip , **10**, 741- 747 (2010)
- [3] "A versatile 3D culture model facilitates monitoring of astrocytes undergoing reactive gliosis," Emma East, Jonathan P. Golding, and James B. Phillips, Journal of Tissue Engineering and Regenerative medicine, **3**, 634-646 (2009).

CONTACT

*Ji Yoon Kang, tel: +82-2-958-6747; jykang67@gmail.com