THE EFFECT OF EXTRACELLULAR MATRIX ON ACTIVATION OF ATROCYTE IN 3D CO-CULTURE CHIP FOR NERVE INJURY MODEL

Young Hun Kim[1], Young Eun Kim[1],Suk Chung[2], Tae Song Kim[1]andJi Yoon Kang[1*]

[1]Korea Institute of Science and Technology

[2]Korea University,Republic of Korea

** tel: +82-2-958-6747; jykang@kist.re.kr*

ABSTRACT

This paper investigated the effect of extracellular matrix (ECM) on the morphology and activation status of astrocyte in microfluidic cell culture chip. The device provides a 3D co-culture system mimicking *in vivo* environment of central nervous system as well as axon isolation from neuron soma. Astrocyteswere seeded in collagen gel through small fluidic channels and neuron cells were aggregated at openings of small channel to large channels. This method not only separated neuronal soma from axon but also segregatedneuronalbody from astrocyte. The interaction of axons and astrocyte in the chip showed that the properties of extracellular matrix determines the activation status of astrocytes.The morphology of astrocytes was compared in two extracellular matrix such as collagen, a mixture of collagen andlaminin. The shape of astrocytes in laminin mixture was more star-like that was almost same as *in-vivo* astrocyte than that in collagen only.Also the effect of TGF-β1 was higher in laminin mixture, which imply that the astrocytes in laminin mixture were deactivated and those in collagen appeared quiescent. Theexperimental results showed thatthe activation status of astrocytes can be controlled in 3D cell culture chip by the control of extracellular matrix material and the treatment of TGF-β1. Hence the suggested co-culture chip is expected to provide a model of axon growth after the damageof nervous system like spinal cord injury.

KEYWORDS: Extracellular matrix, 3D cell culture, neuron, astrocyte, co-culture

INTRODUCTION

Nervous 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 playsa 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 areapparently relevantto inappropriate interactions 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 interested in spinal cord injury model because no therapies are available to recover neuromuscular function andit affects a significant deterioration on quality of life[1].

The Campenot chamber has been widelyused to segregate axons from neuronal soma to study axon growth. However, this system is not enough to embody *in vivo*-like environment to observe interactions between axon and glia. Although 2D culture models have been used tofind out important information regarding the reactivity of astrocytes as well as their effects on axon growth, there are limitations inrecapitulating*in vivo* environment since astrocytes in 2D culture are highly reactive and proliferative. We usedmicrofluidic deviceswith three dimensional cell culturefor facile observation of interactions between neuron and astrocyte. The activation status of astrocytesdepends on microenvironment of ECM. They are activated in 2D culture and they turn into 'resting state'when they are seeded in 3D culture [2]. Hence co-culture of neuron and glia in 3D culture provides the opportunity to observe the mechanism of activation of astrocyte, while it was hard to test in 2D culture. ECM component alsoplays important role in controlling cell proliferation, migration and differentiation [3]. We tested collagen type I and investigated the effect of addition oflaminin to modulate physical and structural properties of the ECM scaffold. Transforming growth factor–β1 (TGF-β1) was used to activate astrocyte sinceincrease of TGF-β1 levels in astrocytes is associated with astrocytic scar formation [4].

EXPERIMENTAL

We used a microfluidic chip to locate neurons and astrocytes in 3dimension. SU-8 negative photoresist was used as a master mold on silicon substrate. For easy detachment ofpolydimethylsiloxane(PDMS) mold from SU-8 pattern, the master was coated with (tridecafluoro-1,1,2,2-tetrahy-drooctyl)trichlorosilane. After mixing with curing agent, PDMS was poured up to 4 mm thickness on the master and was cured at 80℃ for 1hour. 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).

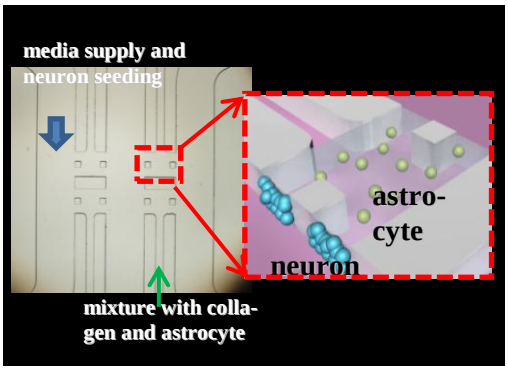

Figure 1: Schematic diagram of the microfluidic chip for 3D astrocyte cell culture: It had three main channels for supplying cell culture media and four small channels for filling mixture of collagen and cells (a) overall view of the device (b) descriptions about each channel (b) schematic diagram of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

The device consists 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 E18fetal rat cortex and cortical astoryctes from P1rat 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 ℃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 solutionand 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. To realize in vivo-like spinal cord injury environments, we treated 10ng/ml TGF-beta1 for 7 days in each device.

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 3days of astorycte seeding in the microfluidic chip. Weobserved that astrocyte cells represented different morphology according to the ECM components. In our study, we used two kinds of ECM for three dimensional astrocyte culture; collagen type1 and a mixture of collagen type I and 100μg/ml laminin. Astrocyte cells have more star-like shape in laminin mixture condition than only in collagen condition. The experimental result means that the mixture of collagen and laminin is closer to*in vivo* microenvironment because the astrocytesappeared almost same morphology *in vivo* (Fig.2).Spinal cord injury model in the microdevice was demonstrated by the treatment of TGF- β1 and its effect on axon growth was investigated.

To embody glial scar model in the device, TGF-β1 was treated every two days during a week. As a result, in lamininmixture ECM condition, the effect of TGF- β1 on astrocyte activity was stronger than in collagen condition since axon growth was reduced by TGF- β1 more effectively (Fig.3 &Fig.4). We believe that ECM component influences cell shape and the function in 3D culture condition, which means that *in vivo*-like shape astrocyte can be activatedmore effectively by growth factor than round shape astrocyte in collagen by TGF- β1.

CONCLUSION

We developed a new method for co-culture of neuron 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 was used as a co-culture platform of neuron and astrocyte and the co-culture enhanced the axon elongation. In conclusion, our three dimensional microdevice was fairly effective in mimicking*in vivo* condition of spinal cord injury. We hope that the chip will be a useful tool for the experiment of spinal cord injury to substitute animal model.

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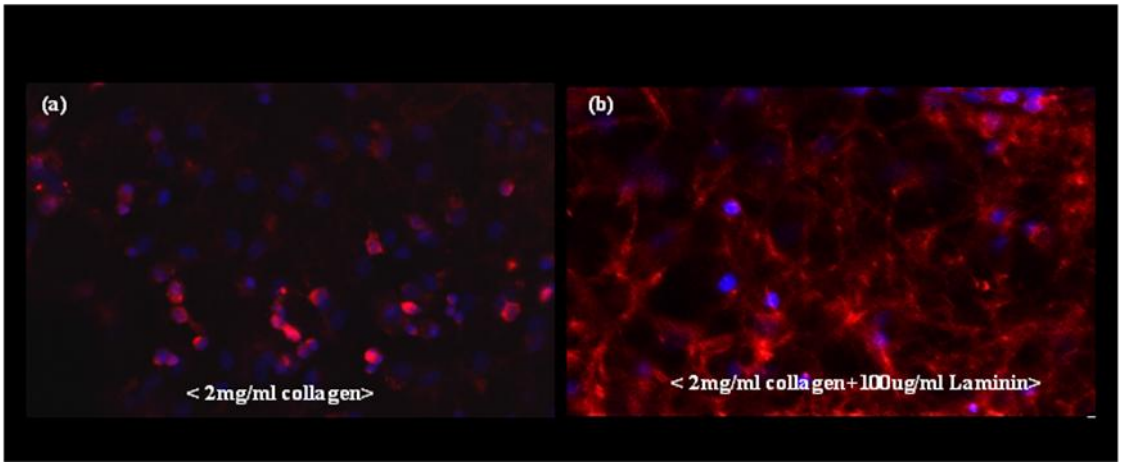

Figure 2: Immunostaining image of astrocyte in 3D culture condition (red-GFAP blue-DAPI): astrocyte cell shows different cell morphology according to components of ECM (a) in collagen type 1, astrocyte shape is round and (b) in laminin added condition, astrocyte shape is star. It is very similar to in vivo environment.

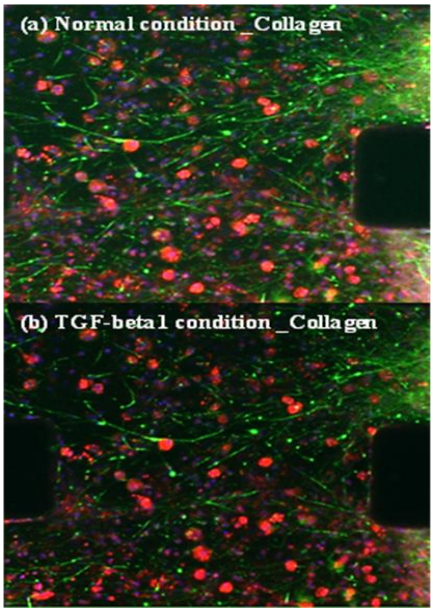

Figure 3: Microscope image of neuron culture without astrocyte (a) a photograph of observation zone in the microfluidic chip (20X, after 7 days) (b) a photograph about axon growth : asterisks (**) appear axons. In contrast to co-culture condition, axons were short and even axon degradation was appeared.

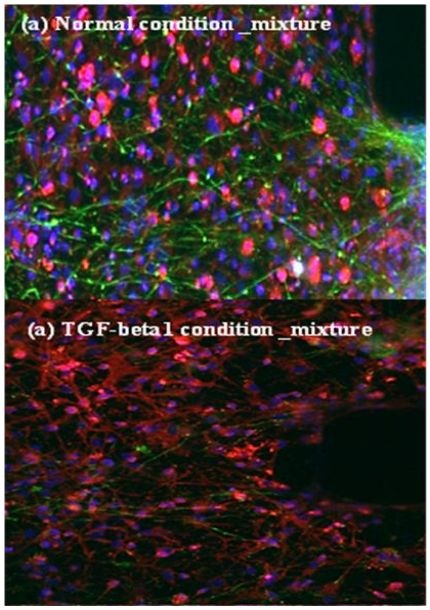

Figure 4: Confocal microscope image about immunochemistry (MAP2- green, GFAP- red)(a) a chip for only neuron culture (b) a chip for neuron-astrocyte co culture In contrast to neuron culture, a lot of axons are shown in co-culture condition.