A MICROFLUIDIC CULTURE SYSTEM FOR ANALYSIS OF NEUROTOXICITY OF OLIGOMERIC ASSEMBLIES OF AMYLOID BETA PROTEINS

Yoon Jung Choi[1], Sukyung Chae[1], Jeong Hun Kim[1], Joong Yull Park[2], and Sang-Hoon. Lee[1]

[1]*Department of Biomedical Engineering, College of Health Science, Korea University, Seoul, Republic of Korea*
[2]*School of Mechanical Engineering, College of Engineering, Chung-Ang University, Seoul, Republic of Korea.*

ABSTRACT

In this paper, we introduce a microfluidic system to analyze the effect of oligomeric assemblies of amyloid beta on neurons quantitatively with well-organized *in vivo*-like microenvironment. By generating extremely slow flow rate and gradient of oligomeric assemblies of amyloid beta, our microfluidic platform simulates the regulated intramembrane proteolysis (RIP), a convergence physiological mechanism.

KEYWORDS

Oligomeric assemblies of amyloid beta, Microfluidic gradient chip

INTRODUCTION

The generation of pathogenic amyloid beta protein is one of the examples of RIP. In the endoplasmic reticulum, pathogenic amyloid beta proteins are carved from the amyloid precursor protein (APP) and then secreted into biological fluid. As amyloid beta has a unique propensity of self-aggregation, cleaved amyloid beta proteins aggregate and form oligomeric assemblies which have neurotoxicity (Figure 1A). Because of the small size, oligomeric assemblies of amyloid beta diffuse through the biological fluid and a gradient of assembled oligomeric proteins is generated at the interface of the interstitial flow. Based on the ideas of process of formation and diffusion of pathogenic amyloid beta, we designed a microfluidic system consisting of a microchannel-patterned polydimethylsiloxane (PDMS) piece bonded against a glass coverslip, osmotic pump, and flexible tube for connecting the osmotic pump and the PDMS piece (Figure 1B). In this system, both the Reynolds number (inertia vs. viscous effect) and the Peclet number (convective vs. diffusive effect) are small, therefore, the flow is laminar with diffusion-dominant flow characteristics in which diffusion mixing at the interface of laminar streams occurs and lasts for a prolonged time (Figure 1C) [1]. We use an osmosis-driven pump generating slow flow rates comparable to those of interstitial flow and a gradient profile which mimics the microenvironment (Figure 1D).

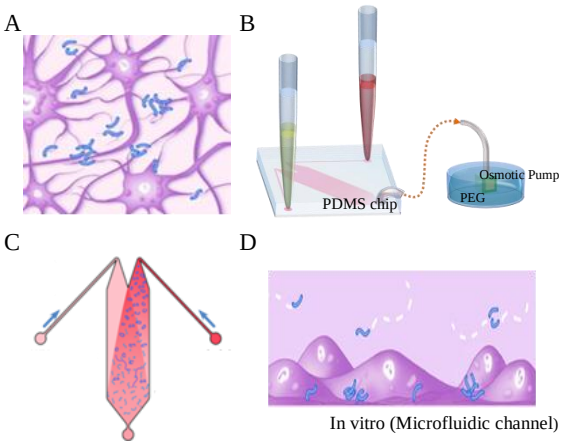

Figure 1. Concept of microfluidic system; (A) Neurotoxicity of oligomeric assemblies of amyloid beta; (B) Experimental setup; (C) Gradient of oligomeric assemblies generated in the main channel of microfluidic chip; (D) Biomimetic condition in the main channel of microfluidic gradient chip.

EXPERIMENTAL

Neuronal cells were allowed to adhere in the main channel of the chip and were maintained for 7 days after plating by culturing with a continuous flow of pure media. For the last 3 days, pure media and 5 μM amyloid beta dissolved in the pure media were introduced via two respective inlet ports, creating a gradient of oligomeric assemblies. Figure 2 shows optical microscopy images of neural networks formed in the main channel of a microfluidic gradient chip. Compared to neural networks without amyloid beta exposure (Figure 2A and Figure

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2B), neural networks exposed to a gradient of oligomeric assemblies of amyloid beta for 3 days (Figure 2C and Figure 2D) show neuronal loss and destruction of neurites. Figure 2E shows the ratio of cytoplasm to nucleus of neural networks of Figure 2A and Figure 2D. Compared to the ratios of neural networks without treatment of amyloid beta, those of neural networks with amyloid beta is small, which indicates the atrophy derived from neurotoxicity of amyloid beta. Figure 3A shows optical microscopy images of neural networks formed in the main channel after exposure to oligomeric assemblies of amyloid beta gradient. Neuron cells were treated with live/dead assay reagent and live cells (green fluorescence) were stained by Calcein and dead cells (red fluorescence) were stained by Ethidium homodimer. The slope of the linear fit of the plot shows a decreasing trend which indicates the neurotoxicity of the oligomeric assembly gradient (Figure 3B).

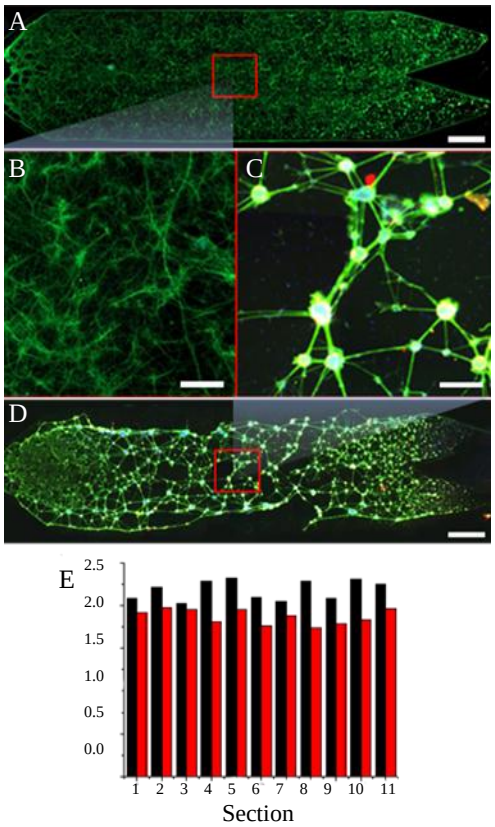

Figure 2. Optical microscopy images of neural networks formed in micro-fluidic gradient chip. Cells were immunostained using antibodies against tubulin and nucleus. (A) and (B) represent neural networks without treatment of amyloid beta and (C) and (D) represent neural networks with exposure of amyloid beta. (E) The ratio of cytoplasm to nucleus of neural networks formed in main channel. Black bars represent cells without amyloid beta treatment whereas red bars represent cells with amyloid beta exposure. Scale bars are 1 mm in A & D, and 200 μm in B & C.

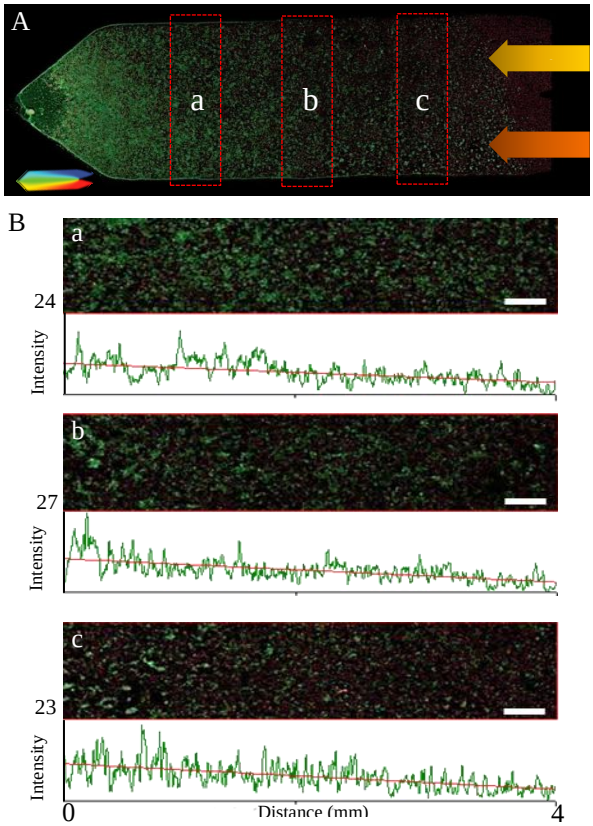

Figure 3. Gradient of oligomeirc assemblies generated in microfluidic chip. (A) Viability of neuronal cells was assessed by using live/dead assay. (B) Graphs of normalized green fluorescence intensity. Scale bars are 1 mm in B

CONCLUSION

We proposed a microfluidic system which provides a useful tool for analyzing neurotoxicity of oligomeric assemblies of amyloid beta. The microfluidic gradient chip is capable of generating extremely low-speed flow (0.25 μL/min) comparable to interstitial flow rates (0.1-0.3 μL/ min·g) [2]. After proteolysis process, known as regulated intramembrane proteolysis (RIP), pathogenic amyloid beta forms and it associates to make oligomeric assemblies. Oligomeric assemblies, known as toxic moieties of amyloid beta are small enough to diffuse and therefore the gradient is generated. Creating a gradient of oligomeric assemblies mimicking *in vivo* condition is

important for accurate understanding of pathophysiologic mechanism derived from oligomeric assemblies. The proposed method can be used to study other examples of regulated intramembrane proteolysis (RIP), and furthermore, efficiency of drug delivery in the interstitial space can be assessed more accurately. Our microfluidic platform will give plenty of benefits to the biomedical fields.

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CONTACT

Sang-Hoon Lee +82-2-940-2881 or dbiomed@korea.ac.kr