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NEURONAL CULTURE AT SINGLE CELL LEVEL FOR CREATION OF BIOLOGICAL NEURONAL NETWORK
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ABSTRACT
To understand the electrophysiological dynamics of neurons, the use of micro-electrode array (MEA) is common because it is non-invasive and it allows long-term monitoring. However, as the culture are randomly arranged on fixed electrodes, it is not capable of recording electrophysiology at single cell level. Here, we present a fully transparent neuronal culture device capable of monitoring cells growth or activity at single scale level. To trap cells singularly, dielectrophoresis is used on a ITO glass coated with fluoropolymer component called CYTOP® to manipulate cells.

KEYWORDS: ITO electrode, Brain on a Chip, Cell control, Neuronal network.

INTRODUCTION
The use of indium tin oxide (ITO) has been employed for recording neuronal cell activity to investigate the biological phenomena such as dynamic neuronal function in a network. Microelectrodes array (MEA) is one of commercially available devices with ITO electrodes on glass that does not influence the healthy environment and it is usually applied in the investigation of neuronal activity experiment. Cell arrangement on such devices are usually randomized and cannot record each neurons. To understand the electrophysiological network pattern triggered by each neuron, there’s a need to study single cell recording [1]. Existing devices only records one by one or in small network of three. Here, a device capable of recording larger network of neurons with organized neuronal arrangement is concepted to understand cell dynamics in a patterned network, heterogeneity and single cell physiology. A fluoropolymer called CYTOP® which can make thin film coating of less than 1µm is employed for patterning and guiding the neuronal growth. Among the techniques of cell manipulation, dielectrophoresis (DEP) which uses electrokinetic phenomena is used for leading cell in a designated well. However, the use of DEP is very challenging for realizing neuronal network of in vitro culture due to high vulnerability to unfamiliar physiochemical environment such as material properties, humidity and pressure.

EXPERIMENTAL
Firstly, primary hippocampal neurons from mice embryo of D-18 were plated on a device with single channel made with CYTOP on ITO glass. After 10 days of maturation, neurons forming into an assembly were arranged in a single line with guided axonal projection (Fig1). Absence of neurons was also observed on CYTOP coated area. Secondly, a trap and drop system [2] which was presented at MicroTas 2018 by Dr. Soo Hyeon Kim on microwell-array was reproduced for trapping the neurons singularly with DEP manipulation and dropping the cells to polydimethylsiloxane (PDMS) structured wells (Fig 2, Fig. 3). Larger wells made with PDMS material that rarely influence with neurophysiological environment were fabricated to enable spatial growth. As result, the cells were successfully trapped in the designated CYTOP wells of 10µm (Fig 4) and were dropped in the PDMS wells when the generator turned off.

![Figure 1: Guided axon extension on patterned CYTOP](image1.png)

![Figure 2: Overview of the trap and drop system](image2.png)
Figure 3: Concept of trap and drop device using dielectrophoresis technique. (A) primary neurons are attracted to the ITO glass coated with CYTOP patterned layer when electric field is applied. (B) primary neurons are dropped into a bigger well made with PDMS allowing (C) cells to grow.

Figure 4. Primary neurons inside trap and drop device. Yellow arrows show single neural cells trapped in one well. Red arrow shows two different neuron cell sizes trapped in a well.

Figure 5. (A) Transversal view of CYTOP wells on ITO glass. (B) Designed channels for 4 possible guided axonal extensions. (C) bi-directional axonal projection with larger well at the bottom of smaller cell trapping well for spatial growth.

RESULTS AND DISCUSSION
The use of thin coating of 1µm CYTOP successfully arranged the assembly of neurons in a single channel and guided the axonal projection on ITO glass electrode. Additionally, the completely transparent trap and drop device allowed single neuron trapping while having a full visibility though a microscope. However, only several cells were trapped and grew in the device and lacked axonal extension. To enhance the neuronal health, the device will be cultured with glial feeder layer in bilaminar model to support the neurons homeostasis and axonal projection [3] The device was also innovated into a simpler design (without additional PDMS wells) with concepted channels between the wells to allow guided axonal projection (Fig 5).

CONCLUSION
Primary hippocampal neurons were manipulated successfully on a designated pattern on ITO electrode glass with 1µm patterned CYTOP. This single neuronal culture allowed cell monitoring for long term. An innovated neuronal network device with axonal projection channel is created and we hope to present the results on MicroTas 2019.

REFERENCES

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