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Designing Electrical Stimulated Bioreactors for Nerve Tissue Engineering

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Abstract. Bioreactor provides a biomimetic ecosystem that is able to culture cells in a physically controlled system. In general, the controlled-parameters are temperature, pH, fluid flow, nutrition flow, etc. In this study, we develop a bioreactor that specifically targeted to culture neural stem cells. This bioreactor could overcome some limitations of conventional culture technology, such as petri dish, by providing specific range of observation area and a uniform treatment. Moreover, the microfluidic bioreactor, which is a small-controlled environment, is able to observe as small number of cells as possible. A perfusion flow is applied to mimic the physiological environment in human body. Additionally, this bioreactor also provides an electrical stimulation which is needed by neural stem cells. In conclusion, we found the correlation between the induced shear stress with geometric parameters of the bioreactor. Ultimately, this system shall be used to observe the interaction between stimulation and cell growth.

Keywords: *bioreactor, biomimetic, cell stimulation, engineered micro ecosystem, neural stem cell.*

INTRODUCTION

Bioreactor, which biological or biochemical processes are developed under a closely monitored and tightly controlled environment, is one of the latest approach that often used to culture NSCs in-vitro [1]. Darling utilizes a bioreactor that uses mechanical means to influence biological processes [2]. In tissue engineering approach, this means that bioreactors are used to stimulate cells and encourage them to produce extra-cellular matrix (ECM).

There were few experiments developed in neural stem cell culturing. In stirred vessels, Gilbertson developed protocols for the extended culture of mouse NSCs by successive passaging the cells over 35 days using 125-250 mL spinner flasks to learn mass transfer, shear stress, and hydrodynamic guidelines [3]. On the other hand, neural stem cell expansion and differentiation has also performed in rotary bioreactors [4,5]. Many experiment employed external stimulations to enhance cell growth, such as mechanical stimulation on bone tissue and cartilage culture [6,7], electrical stimulation on neural and muscular tissue culture [8, 9], pulsatile flow stimulation on cardiac tissue culture [10], and perfusion flow on liver tissue culture [11].

In early development, nervous system construction naturally requires 10-2-1.8 V/mm occurred in frog and chick embryos during neurogenesis [12]. Hence, electrical stimulation affects positively on culturing process of cardiomyocytes [13], spiral ganglion neuron [14], and cochlear neuron [15]. In Thompson experiment, pulse width stimulation rises neurite growth up to two times compared to neurite growth with less neurothropines [14]. Moreover, the stimulation also increases neurite length and enhance neural cell growth significantly on PPy platform [16]. Pavesi conducted an experiment of wave signal stimulation (-2V for 1 ms, 1.2 V for 1 ms, 1Hz) to produce 0.5 V/mm electric field [17]. Small applied DC electric fields, as low as 16 mV/mm, induced significant directional migration toward the cathode [18]

Nowadays, microfluidic bioreactor is developed to create micro cell culture system, that is possible to be used in study of cell viability [19-21], cell migration [22], and toxicity test [23]. The major benefit of using microfluidic bioreactor is the replication and ease to control the environment rather than in common culture media such as micro-well plate. In this paper, we developed a special bioreactor for Neural Stem Cells (NSCs). A dynamic flow of liquid media and electrical stimulation is employed to mimic the in-vivo ambient. We investigate the influence of electric field occurred in fabricating such system that enable us to observe the interaction between cell and flow velocity combined with electrical stimulation.

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MATERIALS AND METHODS

Design Consideration

One of critical consideration of designing this bioreactor is an ability to be used in cell culturing process and measurement of cell viability. By the virtue of their small scale, the microfluidic systems have an extremely higher surface area to volume ratios, than petri dish model. As a consequence, this ratio implies to high wall shear stresses. The shear stress, itself, significantly impacts the cell attachment and acts as mechanical stimulation. Additionally, the bioreactor provides four culturing area. As a consequence, we are allowed to acquire four data of four culturing area in a single experiment.

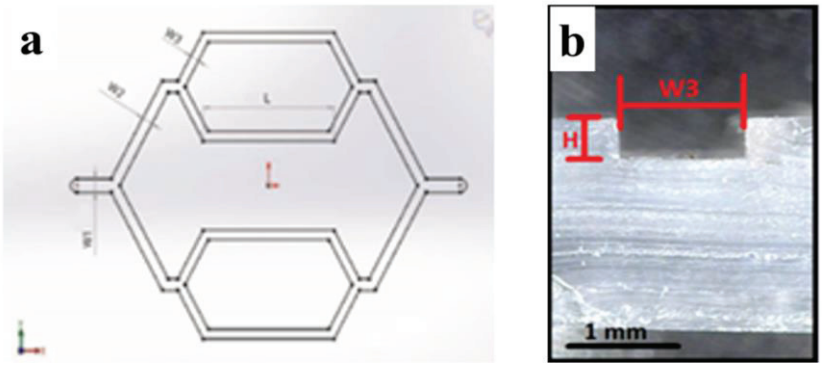

FIGURE 1. a) Design of microfluidic bioreactor; b) Dimension of the channel 1.31mm wide and 0.34mm high.

Bioreactor Fabrication

The fabrication of this bioreactor can be divided into four main steps: mold fabrication; PDMS casting and curing; product assembling; electrical stimulating.

Mold Fabrication: aluminum mold was formed using milling process. The aluminum 7075 was chosen due good economic value rather than copper, good fatigue strength, ease of machining, good thermal conductivity and low corrosion rate. To get high precision manufacturing process, EMCO VMC-200 Milling CNC was demonstrated with accuracy for 1/100mm. At the beginning, bioreactor mold was designed in CAD program and simulated the milling process in CAM program. There are 3 types of flat end mill tools diameter we used: 4 mm, 2 mm, and 1 mm (Seco Tools, Singapore). A machining process parameter was set at 3500 rpm spindle rate, and 50 pps feed rate.

PDMS casting and curing: PDMS (Sylgard 184 Silicone, Dow Corning) was poured on the mold to cast bioreactor. PDMS contained base and agent in a ratio 10 : 1, which mixed on an analytical balance HWH DJ602. Then, the mold was put on a vacuum chamber to reduce air content, which trapped in PDMS while mixing process, that might create a bubble. Vacuum pump VE115N was employed during 45 minutes' vacuum process in an airtight chamber. Afterwards, mold was heated on a hot chamber for 15 minutes at 140o C, then peeled. To enhance bonding process, both upside and downside PDMS were collide and reheated for 15 minutes.

Product assembling: both PDMS were placed between two plates of acrylic. Bolts and nuts were employed in creating compressive force to avoid leakage while fluid was flowing (figure 2a).

Electrical stimulating: An Arduino Uno was employed to create a 16MHz PWM signal with digital output 5V. Duty cycle was regulated using 10 kΩ potentiometer as an analog input. A LCD 16x2 was also applied to indicate duty cycle and theoretical voltage output.

In addition, further investigation will be demonstrated in order to characterize shear effects on each bioreactor. But before going further, all bioreactors need to be characterized based on many manufacture parameters which are very correlated to its function: geometry, shrinkage, and roughness.

Measurement of Flow Velocity

The flow of fluid is captured by Digital microscope AM 4113 ZT camera which is supported by interface program Dino Capture 2.0. Image analysis was then performed using NI Vision Assistant 2015. The velocity of fluid is then measured by dividing the displacement of fluid (Δs) by elapsing time (Δt).

$$\boldsymbol{v} = \frac{\Delta \boldsymbol{s}}{\Delta \boldsymbol{t}} \quad (1)$$

Measurement of Electric Field

Since we assume that electric field between two plates is uniform, they are measured indirectly by measuring potential difference (V) and distance between two plates (d).

$$\boldsymbol{e} = \frac{\boldsymbol{V}}{\boldsymbol{d}} \quad (2)$$

RESULTS AND DISCUSSION

Realization of Bioreactor

Figure 2a presents the assembling results of PDMS bioreactor with the acrylic housing. The process of molding the bioreactor has been thoroughly reported in our previous study. The bioreactor has a rectangular cross section as depicted in figure 1b. Through an image acquisition, the width and depth of cross section are 1.31± 0.01mm and 0.34± 0,00mm, respectively. The bioreactor also consists four channels which the length of one channel is around 15mm.

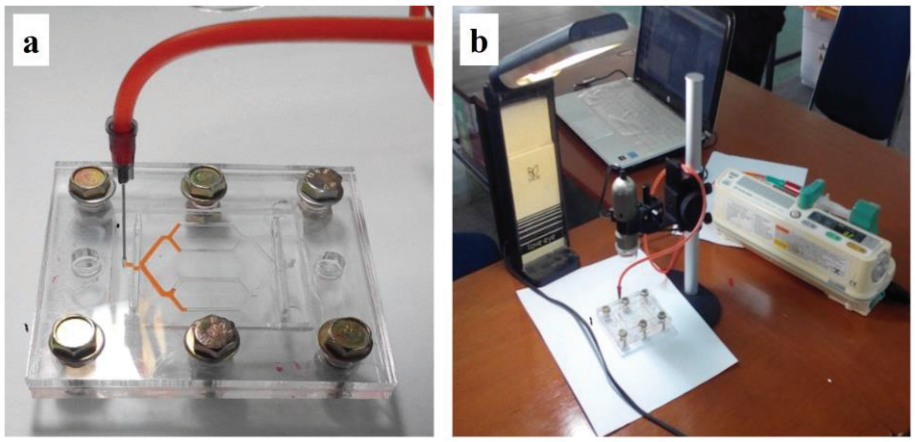

FIGURE 2. Experimental Step : a) Flowing the fluid into bioreactor; b) Observation of flow

The roughness measurement shows that the channel is around 0.014±0.002µm which is smother than the mold (0.043±0.010 µm). The channel roughness is relatively small to compare with the flow area of fluid. Therefore, it can be indicated that fluid flows smoothly in the channel.

The flow in the channel was also observed using Dinolite microscope. The velocity of fluid is determined through image processing method. However, we are more interested in the shear rate magnitude rather than the velocity flow. Therefore, we need to transform the velocity into shear rate by using below equation:

$$\gamma = \frac{32.Q}{\pi.D_h^3} \quad (3)$$

where:
D : debit
Dh : diameter.

Figure 3 shows the measurement of shear stress in the reactor. It is obtained that the shear stress is around 0.14 ± 0.01 dyne/cm^2 during the observation at pumping rate of 200 µL/hour (figure 3a). It shows that the value of shear stress becomes stable after a certain time. Shear force is calculated by multiplying shear rate and its dynamic viscocity. This value also consider as below 100 dyne/cm^3, which is allowable for cell culturing.

Figure 3b also shows the measurement of shear stress at pumping rate of 400 µL/hour (figure 3a). It shows that the value of shear stress more stable at the initial time compare to previous trial pumping rate. The Shear stress is achieved at around 0.16 ± 0.01 dyne/cm^2. This value also consider as safe environment according to Sbrana report on interstitial blood on cell membrane which is upto 0.01 dyne/cm^2 [24]. This is also far below the report of Serbest which is 50 dyne/cm^2 to cause 5% mortality [25].

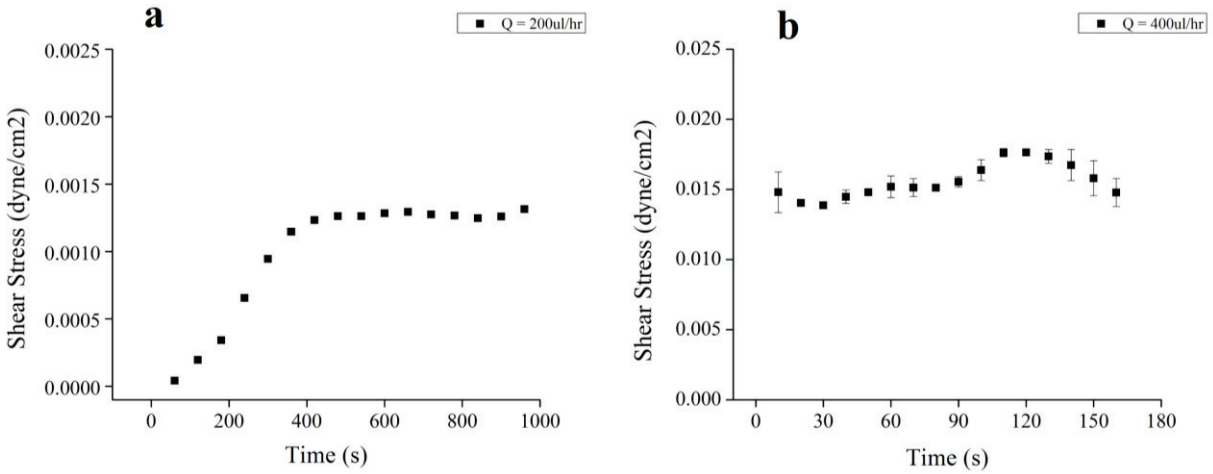

FIGURE 3. Measurement result of wall shear force measurement on the wall at (a) 200 µL/hour and (b) 400 µL/hour

Electric Field Simulation & Measurement

The second part of this experiments is conducting varying duty cycle to investigate resulted electric field in the bioreactor. In doing so, an electric stimulator is realized to perform the stimulation by connecting to electrode plates in the bioreactor (figure 4a). The stimulation is designed to give potential up to 5 DC Volt with a certain pulsatile flow (figure 4b).

The electrode plate is placed top and bottom of the bioreactor. Electric field is calculated using equation (2) by measuring potential difference and distance between two plates, as shown in Figure 5a. In a constant distance of two plates which are 3.98 ± 0.09 mm, the average electric fields distributed as in Figure 5b. The average electric fields generated is from 10 V/mm- 40mV/mm with variation of duty cycle from 20%-100%, respectively.

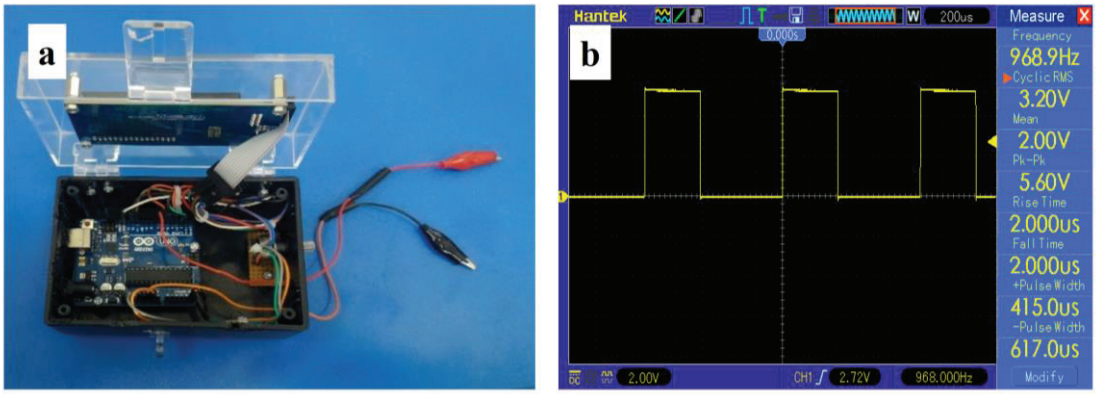

FIGURE 4. (a) Homemade electric stimulator tools as designed; (b) Pulsatile electric field that generated during the testing of the stimulator.

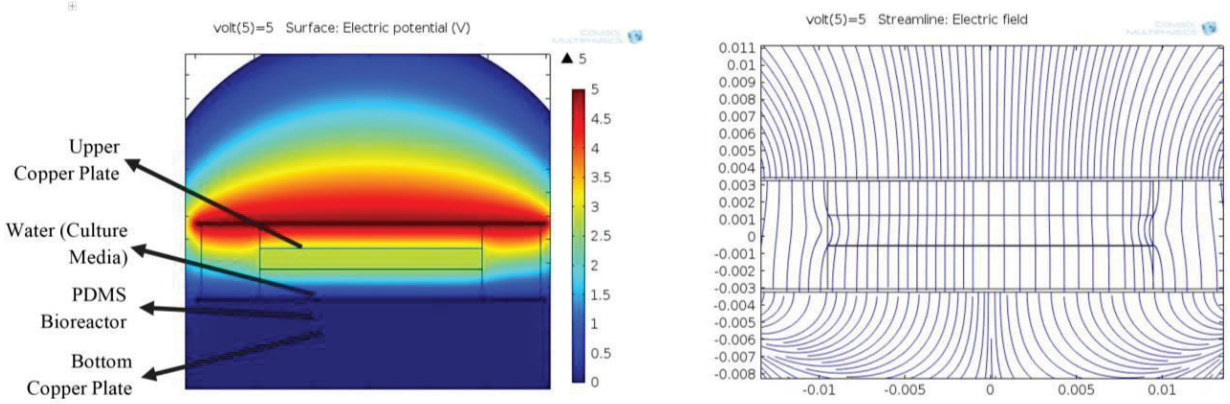

FIGURE 5. Electric Field simulation of bioreactor using Comsol Multiphysics: (a) Electric potential distribution; (b) Electric field streamlines

CONCLUSION

We successfully investigated a potential of bioreactor that enables to stimulate the hosted cells in two modes: perfusion flow and electrical stimulation. The geometry of microfluidic bioreactor design so that the flow will not harm the hosted cells. We also realized a electrical stimulator that easy to use during the culturing process. Additionally, the bioreactor also provides advantages in observing the cells in bioreactor due to specific observation areas.

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