DESIGN AND FABRICATION OF STIMULATED BIOREACTORS FOR NEURAL STEM CELL ENGINEERING (SIMBION PROJECT)

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Abstract- Microfluidic bioreactor gives a unique environment for culturing cells with its perfusion flow as a growth stimulant. This microenvironment was made of polydimethylsiloxane (PDMS) that offers a high biocompatibility and low toxicity. This bioreactor was made of polydimethylsiloxane (PDMS). It offers an opportunity to overcome previous limitations and recreate critical elements of in vivo microenvironment in order to investigate cellular responses. A dynamic flow of liquid media is employed to mimic the in vivo ambient. Here, we developed bioreactors that equipped with electrical stimulant for neural cells. This paper investigates the fluid dynamic study specifically the shear rate that generated during the flow. Ultimately, this system enables us to observe the interaction between cell and the stimulant.

Keywords- microfluidic bioreactor, growth stimulant, microenvironment, neural cell

I. INTRODUCTION

Stem cell engineering strategies can contribute for studying the mechanism controlling cellular events such as proliferation and differentiation (Vazin T., et al., 2010). There are many experiments in developing stem cells which have been potentially developed. In this paper, we are going to discuss further about Neural Stem Cells (NSCs). Neural stem cells (NSCs) itself have the potential to differentiate into all cell phenotypes present in the central nervous system: neurons, astrocytes, and oligodendrocytes. Primary fetal murine NSCs are typically grown in vitro as suspended spherical aggregates, known as neurospheres (Reynolds and Weiss, 1992).

Bioreactor is one of the latest approach that often used. Bioreactors are devices in which biological or biochemical processes are developed under a closely monitored and tightly controlled environment (Niamh A. Plunkett, et al., 2010). A tissue engineering bioreactor can be defined as a device that uses mechanical means to influence biological processes (Darling, et al., 2003). In tissue engineering, this generally means that bioreactors are used to stimulate cells and encourage them to produce extra-cellular matrix (ECM). Moreover, the need to develop fully controlled large-scale bioreactors arises not only from the limited number of cells that can be obtained from available donors, but also from the need to comply with strict regulatory guidelines (FDA, EMEA) (Cabral JMS, 2001).

The petri dish and microwell plate has become a common media in cell culture (Chen A., et al., 2009). But, there are many constraints using this method, such as the number of microcolonies appeared on petri dishes (6 ± 4% of the number of cells inoculated) (Kaeberlein T., et al., 2002). Besides, the microcolonies that grew after passage to petri dishes appeared to represent mixed cultures and only those that produced rapidly growing macrocolonies, visible to an unaided eye, seemed capable of sustained growth on petri dish (Kaeberlein T., et al., 2002). The complexity of the physiological environment cannot be replicated in petri dishes or microplates.

In most cases, the bioreactors are usually customized based on specific requirements and necessitate the use of particular seeding methods (D. Mazzei, et al., 2010). Furthermore, most microfluidic bioreactors are fabricated using polydimethylsiloxane (PDMS) or other elastomeric polymers, which are known to adsorb small hydrophobic molecules (Toepke, et al., 2006). This bioreactor will adapt the material of microfluidic bioreactor which has proven in cell viability study.

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 (Gilbertson J. A., et al., 2006). On the other hand, neural stem cell expansion and differentiation has also performed in rotary bioreactors (Lin H.J., et al., 2004; Low H. P., et al., 2001). Baghbaderani engineered 500mL computer-controlled suspension bioreactors presented protocols for serum-free generation of clinical quantities of human telecephalon-derived neural precursor cells (NPCs). It is shown that human NSCs have already been successfully expanded in bioreactors (Baghbaderani B.A., et al., 2008).

Therefore, all cells growth in cell culture are very sensitive to their microenviroment, which is

interfered from other cells and from mechanical stimuli. So, there must be main design criteria for bioreactors that maximizing mass transport between cells and culture medium and on the application of mechanical, electrical, chemical, or other stimuli. Mechanical stimulation, in terms of flow, can be used to encourage stem cells down a particular path and hence provide the cell phenotype required (Niamh A. Plunkett, et al., 2010).

In terms of flow, a shear force has to overcome the frictional resistance for cell viability, which allowed 10-100 dyne/cm2 leads to a death rate of 20-80% after 10 mins (Sittinger, et al., 1994). The shear forces will dominate at the boundary layers where the velocity and they are affected by the viscosity that measure transfer impulses. Successful in vitro models will therefore enable the study of the mechanism and dynamics of stem cell differentiation and organ development (Abranches E., et al., 2009).

In this paper, Neural Stem Cells (NSCs) culturing will be discussed further. A dynamic flow of liquid media is employed to mimic the in vivo ambient. We investigate the influence of shear stress in fabricating such system that enable us to observe the interaction between cell and flow velocity combined with perfusion stimulation.

II. EXPERIMENT

2.1. Fabrication

Fabrication process of this bioreactor can be divided into three main steps: mold fabrication and preparation, PDMS casting and curing, and channel assembling. The mold can be reused to make an unlimited number of castings.

Mold Fabrication and Preparation: aluminum mold was formed using milling process. The aluminum 7075 was chosen because of 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. To begin with, bioreactor model was created in CAD program and imported to be processed in the next steps: compose tools path and to simulate milling process. There are 3 types of flat end mill tools diameter that we used: 4 mm, 2mm, and 1 mm (Seco Tools, Singapore). Machining process specification was also convenient with tools itself which were 3500 rpm spindle rate, and 50 pps feed rate.

PDMS casting and curing: PDMS (Sylgard 184 Silicone, Dow Corning) was poured on molding to cast the bioreactor. PDMS contained base and agent with ratio 10 : 1, which mixed on an analytic balanced Shimadzu DJ602. Hence, mold and fluid PDMS was put on vacuum chamber in order to reduce air content, which trapped in PDMS, created a bubble and hinder range of interest. Pump VE115N was employed during 45 minutes' vacuum process inside airtight chamber. Afterwards, mold was emplaced in hot chamber and heated in 15 minutes with power at 200 Watt, then peeled. To enhance bonding process, both upside and downside PDMS were collide and reheated in the same parameters

Bottom and top channel assembling: both PDMS were pressed between two plates of acrylic. Bolts and nuts were used to create compressive force and to align each layer. Furthermore, those plates and bolts were clamped to avoid leakage while fluid was flowing.

2.2. Design Consideration

One of critical consideration of designing this bioreactor is an ability to be used in cell culturing process and be measured of cell viability. Three models were proposed, ie the conventional petri dish and microfluidic channel were compared. Those three models will be characterized by its shear stress. The shear stress significantly impacts to cell attachment and acts as mechanical stimulation which enhance cell growth.

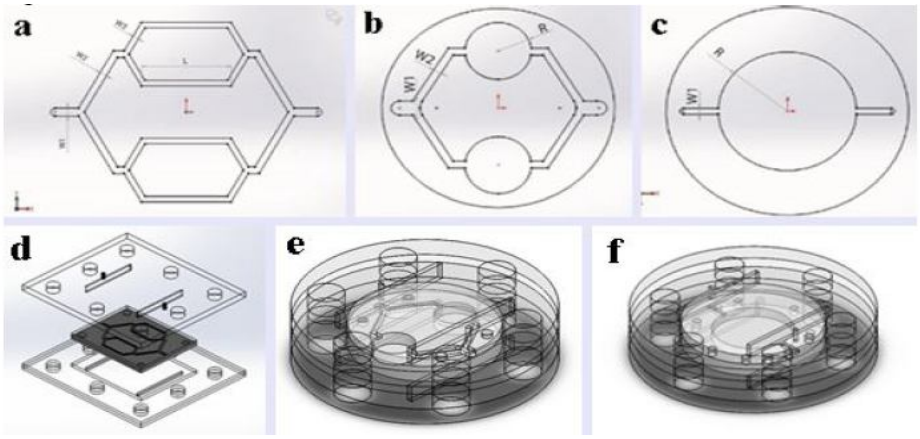

Figure 1. Design of Bioreactor Simbion A, B, and C from top view (a,b,c) and the perspective view (d,e,f)

Table 1. Comparison of Simbion-A, Simbion-B, and Simbion-C

General Feature	Simbion-A	Simbion-B	Simbion-C
Number of Culturing Area	4	2	1
Width (mm)	1.31 (channel width)	4.02 (radius of well)	10.15 (radius of well)
Culturing Area	19.83 mm^2	50.74 mm^2	323.75 mm^2
Culturing Volume	5.13 mm^3	151.69 mm^3	1206.47 mm^3
Ratio of Length: Height in culturing area	44.72	2.68	5.45

In addition, further investigation will be demonstrated on this paper in order to characterize shear effects on each bioreactor. Before going further, all bioreactors need to be assessed based on some manufacture parameter which is very correlated with the purpose to culture cells: dimension and roughness. At least five measurements were taken on each surface, and the average and standard deviation were calculated.

2.3. Measurement of Dimension and Flow Velocity

Dimension of bioreactors are measured using AccretechSurfcom 2900 D3. The dimension measured are width, length, and depth of the bioreactors. Additionally, Surfcom also used to measure roughness of the surface. To ensure repeatability aspect, each measurement was done in five times.

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 by elapsing time.

$$v = \frac{\Delta s}{\Delta t} \qquad 1$$

III. RESULTS AND DISCUSSION

3.1. Metrology of Fabrication

Figure 2a depicts the cross section view of microfluidic bioreactor which indicated a rectangular area. On the other hand, figure 2b shows the cross section of bioreactor Simbion B and C which has a petri dish form. The measurement of each dimension was employed using Surfcom in contour mode measurement. The result is tabulated in table 1.

The surface roughness of mold and product are also measured using Surfcom. This roughness is a critical parameter since it might affect affects the fluid flow inside the bioreactor. Figure 3 shows that roughness between mold and product. The statistical analysis shows that roughness of Simbionmolds are significantly different. Whereas, the product has a similar surface roughness.

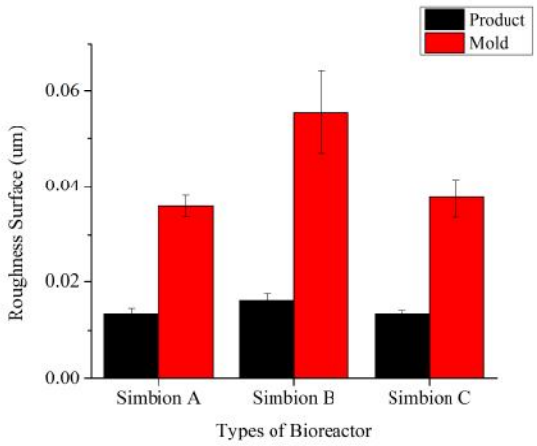

Figure 2. Roughness Measurement of Simbion Bioreactors

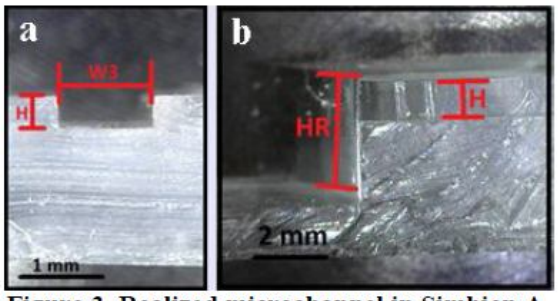

Figure 3. Realized microchannel in Simbion A and dish-like in Simbion B/C

Table 2. Comparison of Width/Diameter and Height

	Width/Diameter	Height
Simbion A	1.379	0.354
Simbion B	3.04	2.93
Simbion C	10.66	2.93

3.2. Flow Simulation

Culture Area become range of interest of each bioreactors, where cell possibly grow and being observed. Therefore, a range of volumetric rate (100 to 1000 µL/hour) was determined to investigate the resulted shear rate using Comsol Multiphysics 4.4. There are several parameter we set before simulation : physics, material, geometry setting, and laminar flow. First, we defined physics in laminar flow and study's model in stationary. Second, we set a block

on the wall of each bioreactor to gather mass flow and shear rate data on the expected location. Third, we defined the area of flow occurred and we set many parameters, such as ambient temperature. Hence, we also converted that mass flow from µL/hour to kg/s by multiplied density since these unit is not available. Through this simulation, we could investigate correlation between mass flow rate and shear rate

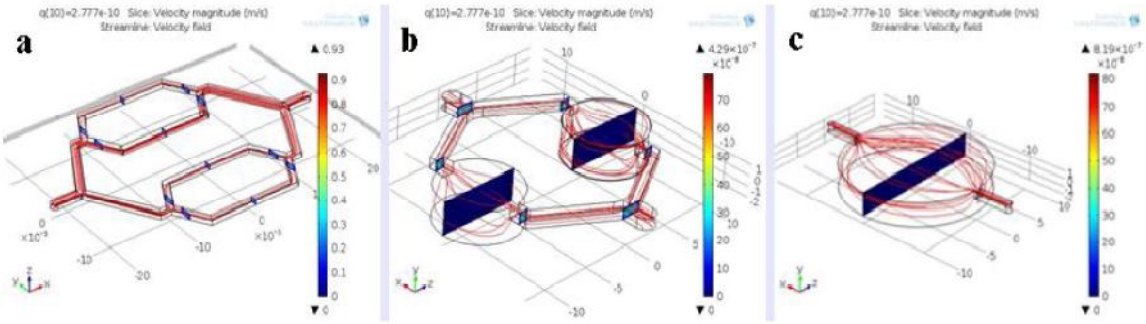

Figure 4. The Fluid Simulation on Simbion Bioreactors

At the end of the simulation, a relation between volumetric rate and wall shear rate of bioreactor is plotted. Thus, through shear rate data, shear stress on each bioreacto can be determined. Based on our simulation, Simbion-B and Simbion-C have a similar characteristic rather than Simbion-A.Figure 4 shows that Simbion-A generatesan average shear rate of 764,854.55 1/s.On contrary, Simbion-B and Simbion-C generatesan average shear rate of $1.86x10^{-5}$ 1/s and $3.15x10^{-5}$ 1/s.

Since, fluid in bioreactor was not been mixing, viscocity (µ) assumed to be homogeneous and water is Newtonian Fluid, represented by $0.91x10^{-3}$ constant. The shear stress is calculated indirectly by multiplying shear rate and viscocity constant. Among three models of bioreactor, similarity of shear stress in culturing area is determined and plotted in Figure 5 and 6 below.

Based on calculation, Simbion-A has an average shear stress of 6,965.53 (dyne/cm^2), whereas Simbion-B and C have an average shear stress of $1.69x10^{-7}$and $2.87x10^{-7}$respectively. This results shows that the dimension of bioreactor plays a significant role. It also is indicated that Simbion-A needs less number of mass flow to create significant shear stress on bioreactor.

3.3. Flow Measurement

In order to validate the result of our simulations, a simple measurement of mass flow rate in Simbion-A is employed. The flow is captured using an AM 4113 ZT camera. Image analysis was then performed using NI Vision Assistant 2015 to measure water volume in bioreactor. Moreover, mass flow rate was calculated by dividing volume and elapsing time using equation 1.

Hence, it can be measured that the average mass flow rate is $3.46.10^{-12}$ m^3/s. Then, average shear rate is then calculated using formula:

$$\gamma_{wall} = \frac{32\,Q}{\pi\,D^3} \qquad (2)$$

It obtained a shear rate of (γ) 0.25 ± 0.09 dyne/cm3 as standard deviation. Shear force is calculated by multiplying shear rate and dynamic viscocity. This value also consider as below 100 dyne/cm^3, which is allowable for cell culturing. Figure 6 shows the measurement of shear force in the Simbion A reactor. It shows that the value of shear force becomes stable after a certain time.

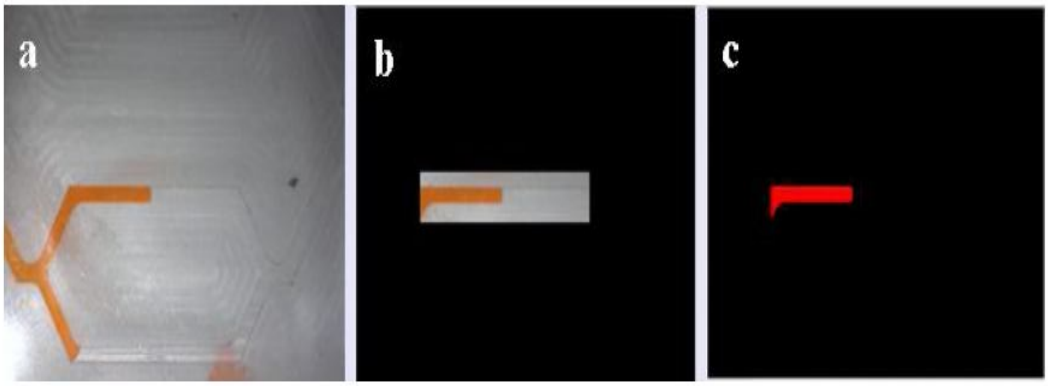

Figure 5. Image Processing Step : a) Original Image; b) Masking; c) Color Extraction

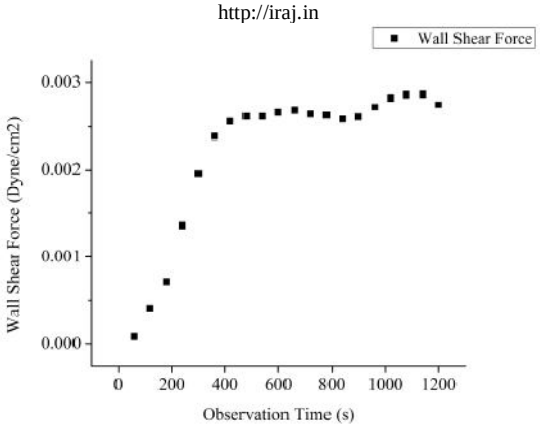

Figure 6..Measurement result of wall shear force measurement on the wall of Simbion A

CONCLUSIONS

We successfully investigated the shear rate resulted by a mini to micro bioreactors. Furthermore, shear force would be the main concern in realizing such bioreactor that enables to stimulate the hosted cells. Based on fluid dynamic study, an optimum design of bioreactors is formulated that shall become a suitable dynamic cell culture device.

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