A MICROFLUIDIC DEVICE FOR CHEMICAL AND MECHANICAL STIMULATION OF MESENCHYMAL STEM CELLS

H. W. Wu[1], C. C. Lin[1], S. M. Hwang[2] and G. B. Lee[1*]

[1]*Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan*

[2] Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan

ABSTRACT

This study presents an integrated microfluidic system capable of chemical and mechanical stimuli on human mesenchymal stem cells (hMSCs) for adipogenic differentiation. It is composed of a dilution module for controlling the concentration of medium and membrane structures for tuning the pneumatic force on cells. Experimental data showed that maximum oil droplets were induced under an insulin concentration of 10 μg/ml. Furthermore, it also revealed that a pressure of 2 psi was optimal for hMSCs growth. The development of the microfluidic system may provide a useful tool for investigating the differentiation of stem cells.

KEYWORDS: microfluidics, stem cell, MSC, differentiation

INTRODUCTION

Recently, hMSCs have shown great potential to differentiate into multiple lineages for cell therapy and therefore have attracted considerable interest [1]. Bio-micro-electro-mechanical-systems (Bio-MEMS) techniques have been demonstrated to be an enabling technology for cell culture and differentiation with many advantages including high surface-area-to-volume ratio, high throughput and automation [2]. Therefore, microfluidic devices have been developed for generating well-controlled mechanical stimuli on hMSCs, which play important roles in the differentiation (chondrogenesis and osteogenesis) studies [3]. However, very few studies on adipogenesis have been conducted, which is also crucial for hMSCs. In this work, an integrated microfluidic system capable of fine-tuning the insulin concentration automatically and applying different normal stress simultaneously is developed to investigate the effects of chemical and mechanical stresses on adipogenic differentiation of hMSCs.

DESIGN AND FABRICATION

A schematic diagram of the microfluidic device is shown in Fig. 1. It is comprised of 5x3 arrayed culture areas, 4x3 arrayed pneumatic chambers, 5x3 arrayed seeding reservoirs, two medium reservoirs, five waste reservoirs, three micropumps, a dilution chamber and fluidic microchannels for connecting these devices. Figure 2(a) shows the working principle of the dilution module. The medium (20 μg/ml insulin) was first pumped into the dilution reservoirs. Then, the dilution medium (0 μg/ml insulin) was loaded into the dilution chamber with three kinds of widths (w_1:w_2:w_3 = 1: 3: 9) to dilute the original medium (20 μg/ml) to the concentration of 15, 10, and 5 μg/ml for inducing hMSCs. For mechanical stimulation, as schematically shown in Fig. 2(b), three different pneumatic forces were produced by deflecting the PDMS membranes with three different thicknesses (200, 350, and 500 μm). Figure 3 shows a photograph of the microfluidic device made of PDMS structures with dimensions of 62 mm × 80 mm.

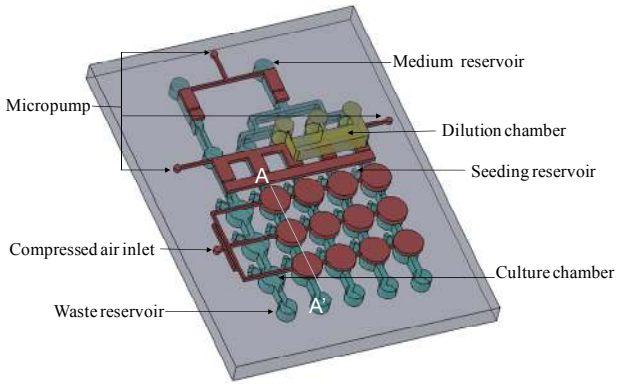

Figure 1. Schematic illustration of the microfluidic device for culture and differentiation of stem cells.

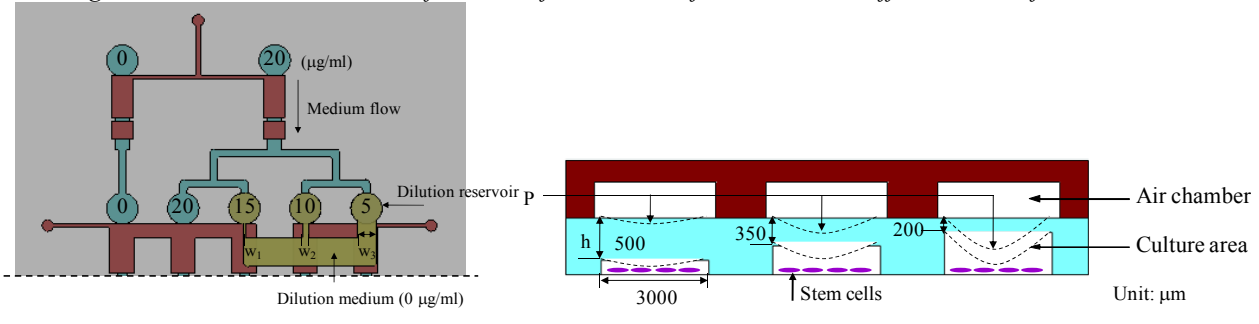

Figure 2. Working principle of the developed device.(a) Medium diluted by the dilution chamber with three kinds of widths (w_1,w_2,w_3);(b)The cross-section view of AA', indicating that three different thickness of membranes are used to produce different force on cells.

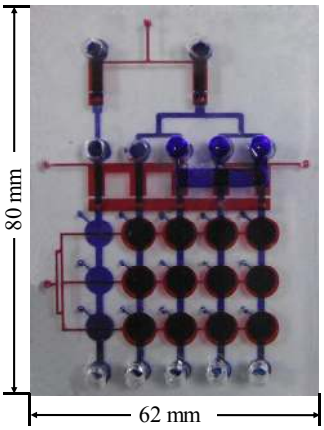

Figure 3. A photograph of an assembled microfluidic chip. The dimensions of the chip are measured to be 62 mm and 80 mm in width and length, respectively.

RESULTS AND DISCUSSION

The relationship between the fluorescent intensity and the concentration of fluorescent dye (Rhodamine B) obtained by the traditional method and the proposed dilution module, respectively, is shown in Fig. 4. It is confirmed that the dilution module has a comparable performance. Figure 5(a) shows a series of images regarding the adipogenic differentiation of hMSCs induced for one and two weeks with five different medium concentrations of insulin (0 μg/ml, 5 μg/ml, 10 μg/ml, 15 μg/ml and 20 μg/ml). Adipogenic differentiation was verified by Oil Red O staining. At a concentration of 10 μg/ml, an optimum growth of adipocytes was observed. The oil droplet number and OD value under different insulin concentrations are shown in Fig. 5(b).

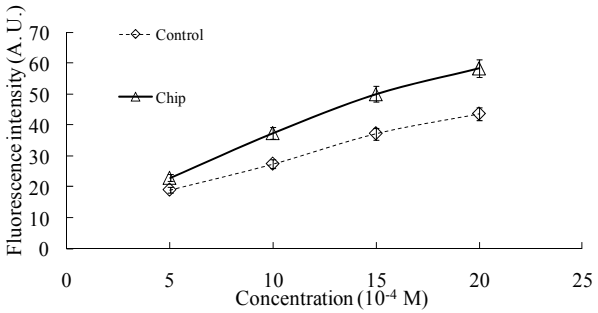

Figure 4. The relationship between the fluorescence intensity and concentration of dye (Rhodamine B) using the traditional system and the dilution device.

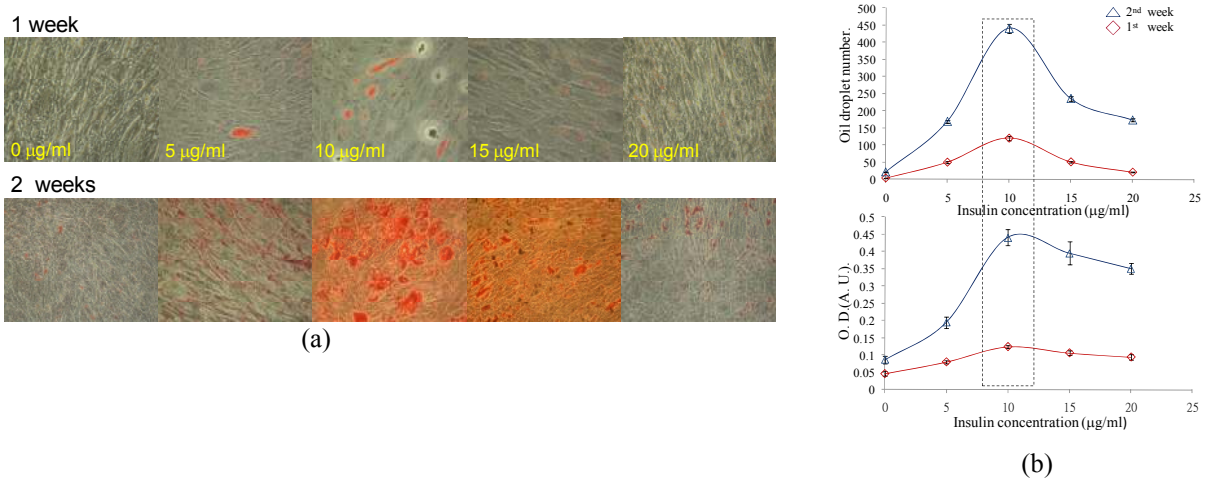

Figure 5. (a) Photographs for the adipogenic differentiation of hMSCs verified by Oil Red O staining. (b) The measured oil droplet number and OD values under different insulin concentrations, respectively.

The effect of the insulin concentration was further investigated. For detecting the specific mRNA expression, total RNA in each sample was isolated using Trizol reagent (Invitrogen, Carlsbad, CA) for reverse transcription. Two primer sequences of polymerase chain reaction (PCR), β-actin and PPARγ2, were used to explore the effect of the insulin concentration on adipocytes differentiation. Note that PPARγ2 is a specific marker of adipogenic cells. The relative expression level of β-actin was used as an internal control to normalize PPARγ2 gene expression in each sample. The results shown in Fig. 6 indicate an optimum PPARγ2 gene expression at a concentration of 10 μg/ml for one week differentiation, which is consistent with the results from oil droplet and OD value measurements.

The effect of the applied force on the hMSCs was also investigated. Figure 7 shows that hMSCs can survive under a pressure of lower than 2 psi for at least 10 min in this chip.

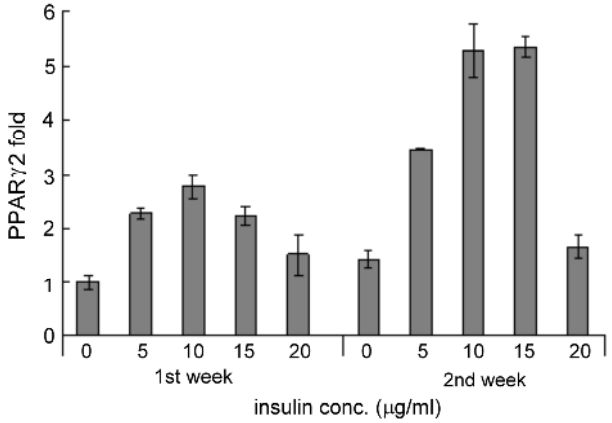

Figure 6. The measurement of PPARγ2 gene expression with five different concentrations using real-time PCR.

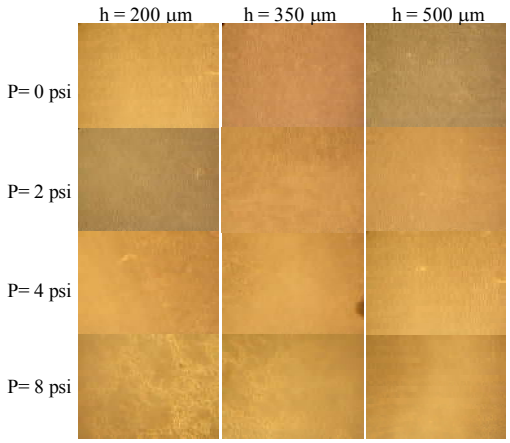

Figure 7. Images of hMSCs stimulated by four static pressures (P= 0, 2, 4, 8 psi) for 10 min, respectively.

CONCLUSION

The current study has successfully demonstrated an integrated microfluidic system capable of chemical and mechanical stimuli on human mesenchymal stem cells (hMSCs) for adipogenic differentiation. The insulin concentration can be fine-tuned automatically and different normal stress can be applied simultaneously. The experimental results showed that an optimum insulin concentration of 10 μg/ml for adipocytes growth can be determined. The survival of hMSCs under a pressure of lower than 2 psi was observed. In summary, the developed microfluidic system may provide a promising tool for hMSCs study.

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CONTACT INFROMATION

*Dr. G. B. Lee, Tel: +886-6-2757575 Ext. 63347; gwobin@mail.ncku.edu.tw