LIVER SPECIFIC FUNCTION ENHANCEMENT BY MICROVASCULAR SYSTEM INTEGRATED WITHIN A LAB-ON-CHIP DEVICE

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ABSTRACT

We report a lab-on-chip device to study liver function enhancement by mimicking a liver tissue with its sinusoid on a 3D biomimetic chip. The device procures a thin polydimethylsiloxane membrane with an array of holes etched in it and sandwiched in the center of the microchannel to form upper and lower microchambers. Hepatocytes and fibroblasts were co-cultured on the upper chamber to mimic liver while the endothelial cells were cultured at the bottom micro chamber to provide nutrients to the in vitro reconstructed liver tissue in the upper chamber and as well to mimic vascular system at the cellular scale.

KEYWORDS

Lab-on-chip device, 3D biomimetic chip, polydimethylsiloxane membrane, cell co-culture

INTRODUCTION

The liver is one of the most complicated organs of human body. It has over 500 kinds of important functions such as, secreting albumin, urea producing, bile, carbohydrate storing, supersession and also the first line of defense in human body [1, 2]. We find a lot of obstacles in developing and repairing of the organs. To rebuild an organ is extremely difficult in medical science and therefore we try to reconstruct a liver tissue in vitro in order to carry on the research (drug screening) into liver that function easier.

The liver and blood circulation is closey related. There is a big crack between the cells-cell interjuction and many processes are involved in this micro circulation. Liver is an organ with many microvascular systems present within, in order to synthesize and metabolize various proteins. The blood capillaries present will have minute holes through which the liver in macrocosm receives proteins. It is crucial to synthesize plasma protein and metabolizes the product in combination with blood plasma protein.

The development of MEMS-based biochips that reproduce complex organ-level pathological responses could revolutionize many fields, including toxicology and the developmental process of pharmaceuticals that rely on animal testing and clinical trials. It is important to provide the nutrients, oxygen and growth factors to enhance its function and this is achieved by mimicking the liver sinusoids in this reconstructed tissue. Microfluidic technology offers great advantages over the conventional methods towards mimicking the cellular microenvironment [3] which makes it possible to reconstitute the organ level function on chip [4].

EXPERIMENT

Liver transports the nutrients to the interstitial fluid through the capillary and it uptake the nutrients from the blood through the cell membrane.

Besides, the waste products will be transported through the cell membrane to the interstitial fluid, entering the endothelial cell which is located at the interface between the blood and the vessel wall, next to the plasma to accomplish the metabolism as illustrated in Fig.1.

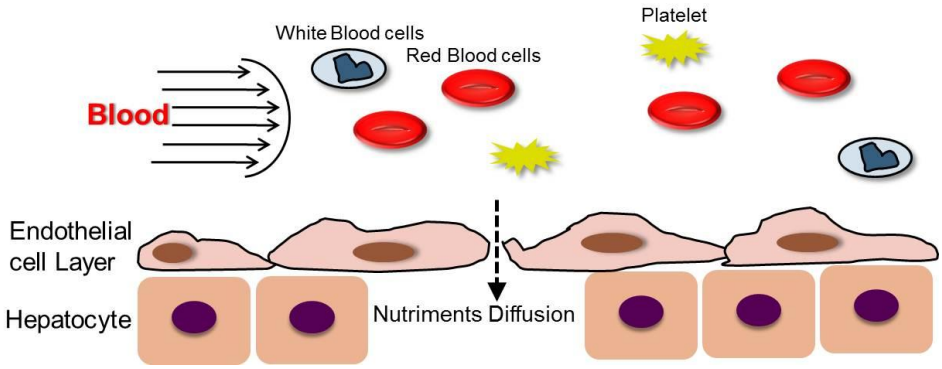

Figure 1:Cross-section of blood capillary. Blood capillary is involved in exchanging nutrients and waste chemicals between the blood and surrounding tissues by diffusion mechanism.

The micro fabrication process of the chip is represented in Fig. 2. Cylindrical microstructure with dimension 13~15 µm was fabricated on the silicon wafer. A thin layer of PDMS was spun coated on the Si-wafer (Fig. 2d) and another PDMS structure is bonded to the thin layer to form the upper channel of the chip (Fig. 2e). Tetra-n-butylammonium fluoride (TBAF) is injected in the upper channel for etching the PDMS (Fig. 2f) in order to obtain a porous PDMS membrane. The membrane is then peeled off and bonded to PDMS channel to obtain the complete chip (Fig. 2h).

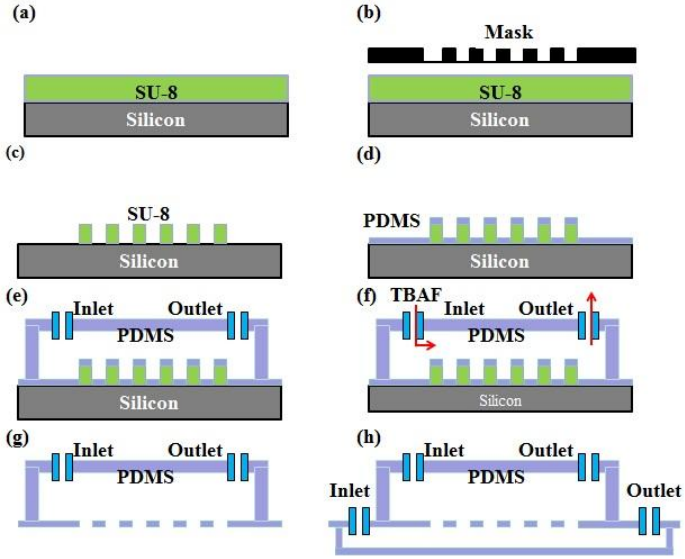

Figure 2: Micro-fabrication process of the biomimetic chip.

HMEC-1 cells are cultured in the lower channel to form a sinusoid like structure as depicted in Fig. 3. The HepG2 and 3T3 cells are co-cultured to mimic liver tissue in the upper channel and the continuous perfusion of the medium from bottom channel to top channel supplies nutrients to the tissue. With the simulation result shown in Fig. 3 we can confirm that fresh nutrient is diffusing to upper structure gradually. As time progress the entire chamber gets filled with the medium. The simulation reveals the concept utilsed in this chip. Thus, the liver tissue can continuously be supplied with fresh nutrient by diffusion from bottom channel.

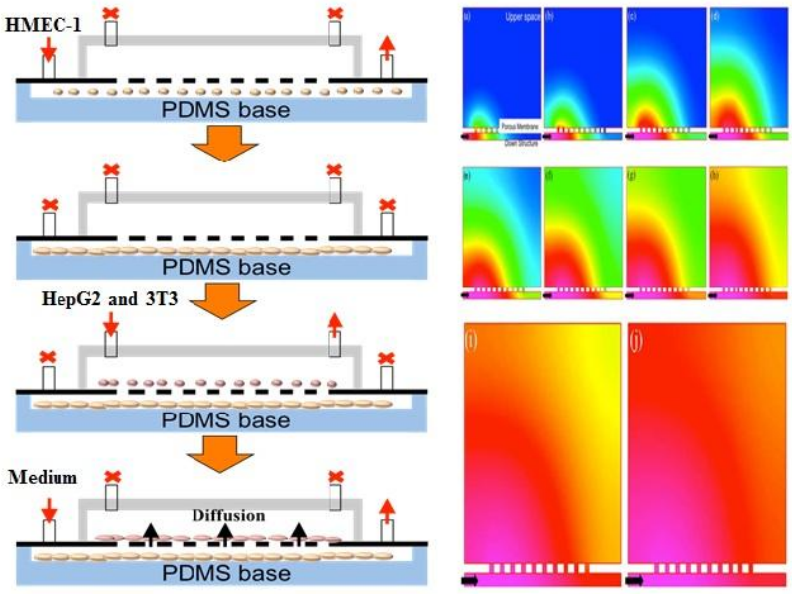

Figure 3: Porous PDMS membrane is sandwiched between two PDMS channels. In the lower channel HMEC cells are cultured to mimic the micro-vascular system. The simulation reveals fresh nutrient (pink color) is diffusing to upper structure gradually. As time progress the entire chamber is filled with medium channel.

In Fig. 4 the green fluorescence exhibited by HMEC-1, shows the sinusoid like structure that mimics the micro-vascular system whereas HepG2 and 3T3 cells cultured in the upper channel exhibit red fluorescence.

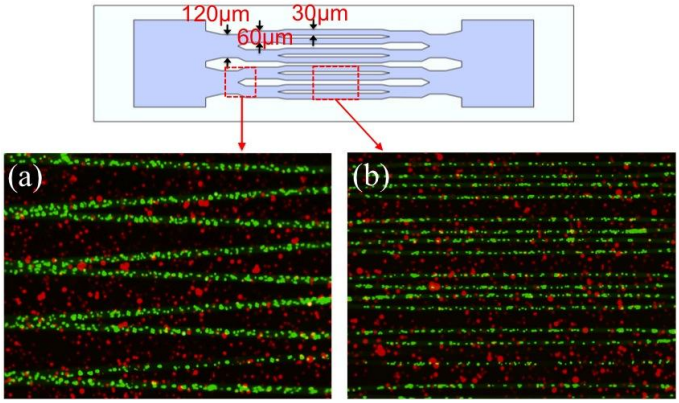

Figure 4: Green fluorescence shows that HMEC-1 cells are cultured in the lower channel to exhibit a sinusoid like structure for mimicking the micro-vascular system. HepG2 and 3T3 cells exhibit red fluorescence cultured, in the upper chamber.

In Fig.5 illustrate urea secretion for the microvascular system integrated chip and co-cultured tissue assayed and the results suggested enhancement in liver function for biomimietic chip. The urea secretion affirmed that nutrients were transported by the micro-vascular system integrated in the bio-chip.

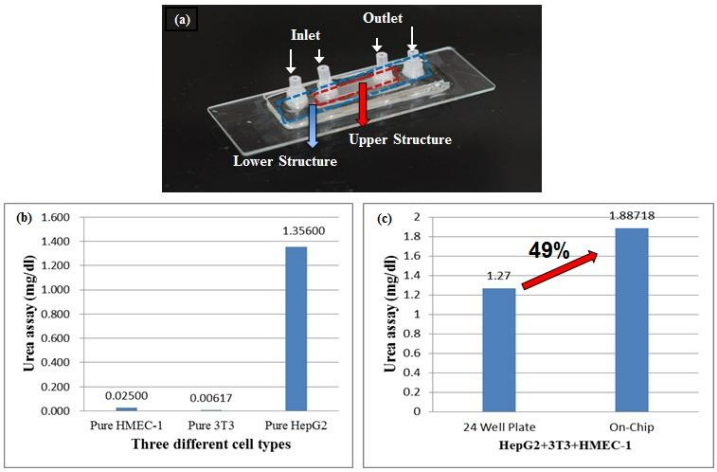

Figure 5: (a) The image of the chip used throughout the experiment. (b) Urea synthesis for individual trait of cell is elucidated. Here we compare three different cell types. (c) Urea Assay results emphasize that using this system the liver function would be enhanced up to 49%.

CONCLUSION

We were successful in mimicking the nutrient transport function of microvascular system in the liver from Urea assay results. We believe that this cell co-culture device with continuous perfusion system will serve as a platform for studying hepatocyte derived functions and analyze the effect of hepatotoxic chemicals and drugs.

ACKNOWLEDGMENT

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