IN VITRO HEPATOCYTE-ACTIVITY ENHANCEMENT VIA A LOBULE-MIMETIC ENGINEERED LIVER TISSUE LAB CHIP

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ABSTRACT

A liver labchip integrating the DEP-based cell patterning technique and the microfluidic system for reconstructing the engineered liver tissue with the feature of liver function enhancement is demonstrated and reported in this paper. DEP forces are utilized as the cell-patterning mechanism to manipulate heterogeneous cells for mimicking the inherent hepatic morphology and increasing the cell-cell interaction. An ethoxyresorufin-*O*-dealkylase assay was performed to study the liver function enhancement of the engineered liver tissues. Experiment results show that the P450-1A1 enzyme activity of engineered liver tissues are 68.41% enhanced comparing with non-patterned liver cells.

KEYWORDS: liver labchip, dielectrophoresis, engineered tissue, cell patterning

INTRODUCTION

Over the past two decades, tissue engineering has been of high interest in tissue regeneration, but it is lack of the ability to reconstruct the tissues with complicated architectures. The major barrier is that a functional tissue usually is constructed from multiple cell types organized in the unique structure to perform its specific and complex functions. [1] Thus, the efficient and precise reconstruction of complex tissue structures according to their native morphologies is significant to the *in vitro* developments of functional tissues. [2] In our research, we utilize the dielectrophoresis (DEP) force as the cell-patterning mechanism to reconstruct an *in vitro* engineered liver tissue. Here we report the progress focusing on the enhancement of cytochrome P450-1A1 (CYP450-1A1) enzyme activity by increasing the cell-cell interaction and mimicking the inherent-hepatic morphology.

THEORY

Liver is considered as a very difficult object to be reconstructed in tissue engineering due to its complex cellular architecture. Structurally, the liver is morphologically divided into lobules which take the shape like a hexagonal plate as illustrated in Figure 1 (a). The lobule consists of hepatocytes radiating from the central vein and separating by vascular endothelial cells. From the view of cellular level, the direct cell-cell contact between heterogeneous cells in particularly spatial orientation is essential to maintain the liver tissue function. Thus, the efficient and precise reconstruction of complex liver tissue according to their natural morphology is important toward the developments of functional tissue engineering. [2] Our liver labchip previously demonstrated the proof-of-concept work to *in vitro* reconstruct the heterogeneous liver cells. [3-4] The cell manipulation mechanism used for cell patterning is DEP, which is a phenomenon caused by the induced dipole of the polarizable particles in the solution under non-uniform electric fields. We highlighted the concept that designing the electrodes to form a specific electric-field gradient is equivalent to pattern cells via either negative or positive DEP manipulation. Following up with this concept, the lobule-mimetic-stellate-electrode array shown in Figure 1 (b) is proposed to pattern heterogeneous cells by mimicking liver morphology for engineered liver tissues. Figure 2 illustrates the operation principle of the lobule-mimetic heterogeneous cells patterning. In order to culture the patterned engineered liver tissue in long term, a microfluidic system is developed with our liver labchip as illustrated in Figure 2 (d). According to the hydrodynamics, the flow resistance in the microchannel is roughly proportional to the microchannel length if the cross-sections of microchannels in the microfluidic system are identical. [5] Our microfluidic system is designed to support the triplicate condition of three downstream cell patterning chambers.

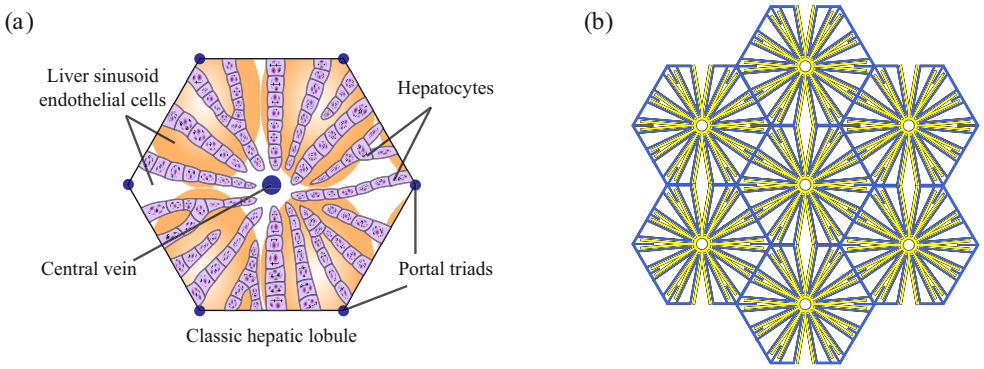

Figure 1: (a) The illustrated configuration for the classic hepatic lobule, a basic functional unit of liver. (b) The lobule-mimetic-stellate-electrodes array.

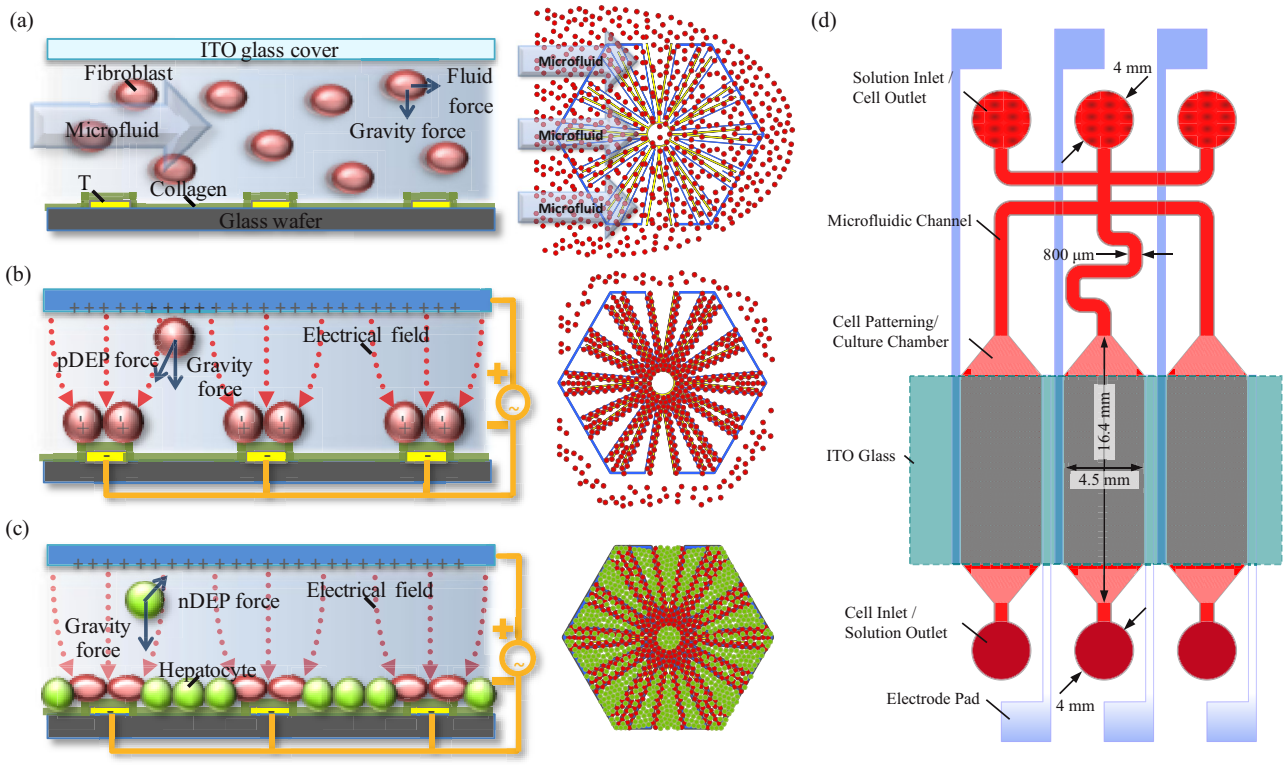

Figure 2: (a-c) The configuration and operation principles of heterogeneous lobule-mimetic cell patterning. (a) Randomly distributed fibroblasts are loaded into the microfluidic channel. (b) The fibroblasts are captured and patterned onto the DEP patterning electrodes to form the radial cell-string pattern after the vertical positive DEP voltage is applied. (c) The hepatic cells are, then, loaded, repelled and positioned in-between the patterned hepatic cells via applying the vertical negative DEP. The final pattern forms the heterogeneous integration of interlaced hepatic and endothelial cells, which mimics the hexagonal lobule of human liver tissue. (d) A schematic view of the microfluidic system consisting of three branching microchannels.

EXPERIMENTAL SETUP

Human hepatocellular carcinoma cell line, HepG2, and mouse fibroblasts, 3T3, were adopted for cell patterning and CYP450-1A1 enzyme activity assay. Isotonic sucrose medium was used as the DEP-manipulation buffer [3]. A syringe pump was used to infuse and withdraw the flow streams of the fluidic sample. A function generator was used to produce the ac signals for DEP manipulations. CYP450-1A1 enzyme activity of engineered liver tissue was measured by using an ethoxyresorufin-*O*-deethylase (EROD) assay [5]. The engineered liver tissues were cultured with the fresh medium containing the CYP1A1 inducer β-naphthoflavone (20 μM) and incubated for 48 h. After 48 h, the engineered tissues washed with the PBS and serum-free medium containing 10 μM ethoxyresorufin, along with 25 μM dicumarol, was introduced to each chamber under a flow rate of 15 μl/min. After a further 60 min of incubation, the culture supernatants were collected and pipetted into black 96-well plates. The fluorescence intensities of resorufin were determined at em/ex 530/595 nm by using a fluorescence plate reader and calibrated by the total cell number of HepG2.

RESULTS AND DISCUSSION

Figure 3 (a) shows the fabricated liver labchip. Figure 3 (b) shows the patterned 3T3 cells which mimic the pattern of the sinusoid-like vascular endothelial lining cells in the classic hepatic lobule. Figure 3 (c) shows the heterogeneous lobule-mimetic-stellate patterns after patterning the HepG2 cells. The heterogeneous integration via DEP manipulation in our labchip exhibits the lobule-mimetic pattern to provide the intimate contact for cell-cell interaction during cellular development. CYP450-1A1 enzyme activity is a liver-specific function which can be used to quantitatively assess the utility for drug metabolism of liver tissues. The results of CYP450-1A1 enzyme activity on our engineered liver tissue compared to non-patterned HepG2, patterned HepG2, non-patterned coculture, and patterned coculture are shown in Figure 4. The enhanced P450-1A1 enzyme activity of patterned HepG2 was observed compared to the non-patterned HepG2. The P450-1A1 enzyme activity on patterned HepG2 is 32.72% higher than that on the non-patterned HepG2. P450-1A1 enzyme activity was significantly ($P<0.01$) enhanced in the patterned HepG2 as compared with the non-patterned HepG2, suggesting that the intimate cell-cell interaction of HepG2 cells facilitates the enzyme activity. By coculturing with 3T3 under patterning condition, the much higher P450-1A1 enzyme activity is observed compared to the non-patterned coculture. The highest P450-1A1 enzyme activity on patterned coculture is 68.41% and 18.39% higher than the non-patterned HepG2 and non-patterned coculture, respectively. P450-1A1 enzyme activity was significantly ($P<0.01$) enhanced in the patterned coculture as compared with the non-patterned coculture, suggesting that the patterning of heterogeneous cells and mimicking of inherent-hepatic morphology facilitates the enzyme activity.

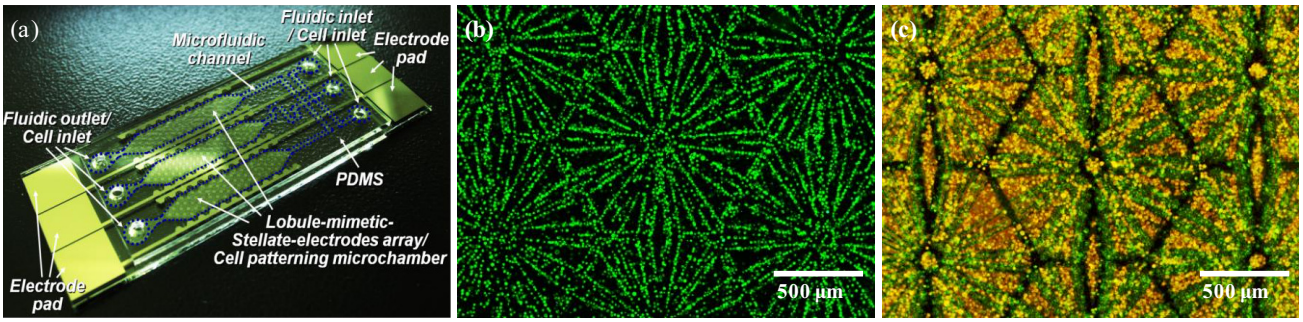

Figure 3: (a) A full view of the fabricated liver labchip consisting of lobule-mimetic-stellate-electrode array and a microfluidic system. (b) On-chip cell-patterning demonstration of 3T3 cells (pre-labeled with biocompatible fluorescent dye, DiO, green). (c) A heterogeneous lobule-mimetic-stellate patterns constructed of HepG2 (pre-labeled with biocompatible fluorescent dye, DiI, yellow) and 3T3 (green) cells to mimic the native morphology of the classic hepatic lobule.

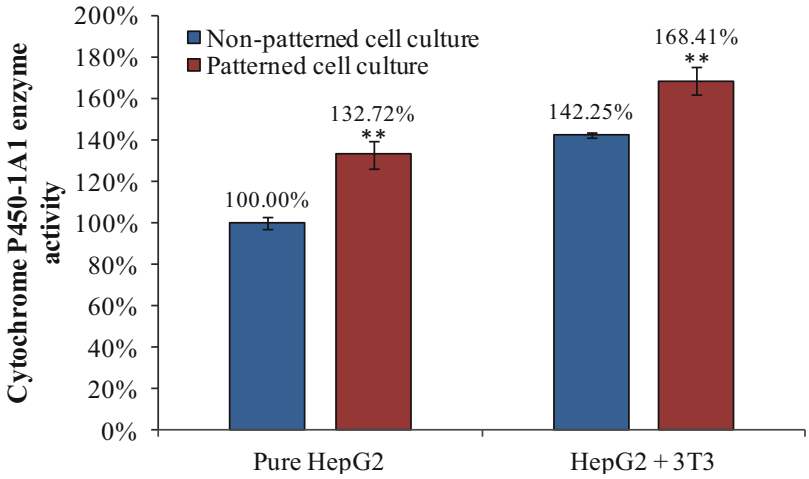

*Figure 4: The comparison of CYP450-1A1 enzyme activity between non-patterned tissue culture and engineered/patterned tissue culture. The results reveal that we enhance the liver-specific function via our engineering arrangement. All data represent mean ± s.d. (n = 3). *p<0.05, ** P < 0.01 versus control (control: Non-patterned tissue culture).*

CONCLUSION

In this research, we demonstrate the *in vitro* lobule-mimetic tissue reconstruction. We also demonstrate the promotion of hepatocyte activity by lobule-morphology-mimetic arrangement. These are significant progress for the *in vitro* reconstruction of complex artificial organ.

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