3D CIRCULATORY PERFUSION-CULTURE SYSTEM BY USING HIGH EFFICIENCY PROPORTIONAL CELL CONTACT

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ABSTRACT

This paper reports a three dimensional circulatory cell perfusion-culture system with difference medium by using proportional cell contact. The system was consisted of two dimensional cell contact chambers and three dimensional cell loading chambers. For cell contact chambers, we used dielectrophoresis pattern method to make three difference contact area for difference proportional communication of HMEC-1 cells and 3T3 cells. For cell loading chambers, we used the PDMS-based micropillars to mimic three dimension lobule-mimetic structures in vitro. Finally, we showed liver functional albumin testing. The albumin secretion of liver pattern chambers with HMEC-1 and 3T3 co-culture communication was 64.8% higher than the albumin secretion of liver chip without cells communication.

KEYWORDS

3D cell culture, perfusion-culture, cell contact, HepG2, cell-cell interaction.

INTRODUCTION

In recent research, the bulk of the lobule consists of hepatocytes which are arranged into hepatic cords that are separated by sinusoid spaces [1]. On the other hand, photopolymerizable PEG-DA hydrogels integrated with dielectrophoresis (DEP) has been utilized to trap cells to form 3D structures [2]. By the way, a 3D cell perfusion-culture in microfluidic system microenvironment is used in 3D cell-matrix interaction via a microfluidic channel based system [3]. An artificial liver sinusoid with a silicon-based endothelial-like barrier capable of transporting mass is reported [4].Therefore, the re-establishment of 3D cell pattern is important for the 3D cell culture in vitro. However, complex cell co-culture was not easy in vitro. In this paper, we have developed the cell communication base on cell contact by DEP and artificial lobule pattern by the PDMS-based micropillars. HepG2 cells were loading with three dimensional PDMS-based microstructures for artificial lobule pattern.

METHODS

We had designed a liver cell-to-cell communication circulatory perfusion-culture system chip for three dimension hepatocyte culture as showed in figure 1 (a). It consisted of cell contact chamber and cell loading chamber. For cell contact, we used positive DEP method to pattern HMEC-1(green cells) and the 3T3 (red cells) were seeded into the chambers in figure 1 (b). It is to make difference contact area. For cell loading, we used the soft lithography process to form the PDMS-based micropillars to mimic three dimension lobule-mimetic structures in vitro. The HepG2 was loaded by the micropillars from the center and the direction of the medium was pushed by the red arrowhead in figure 1 (c).

In figure 2 (a), we showed difference proportional electrode pattern to make difference cell-to-cell contact ration for cell communication. For example, condition 1 is 300um×300um area, condition 2 is 200um×300um area, and condition 3 is 100um × 300um area. The black area in figure 2 is electrode pattern. The white area is the HMEC-1 cells concentrated area. Schematic diagram in figure 2 (b) is operation principles of cell contact patterning. Uniformly distributed HMEC-1 cells were loaded into the chamber. The HMEC-1 cells were attracted and positioned via the DEP patterning electrodes. The 3T3 cells were seeded.

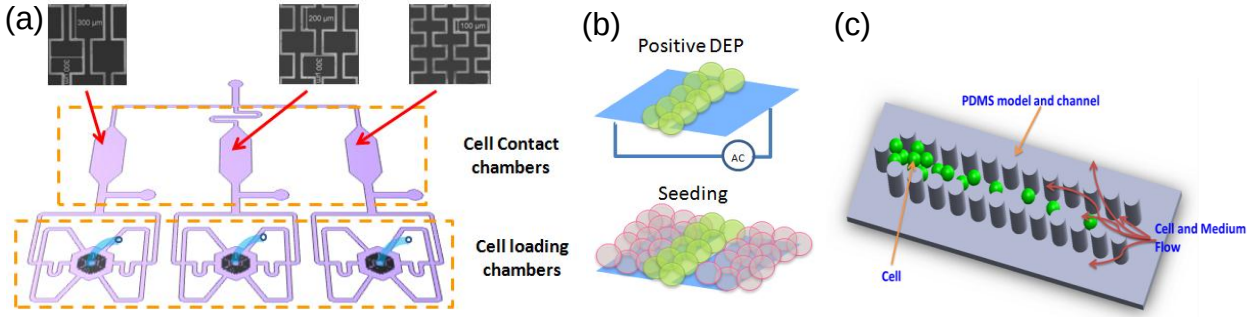

Figure 1.(a) A liver cell-to-cell communication circulatory perfusion-culture system for three dimension hepatocyte culture. It consisted of cell contact chamber and cell loading chamber. (b)For cell contact, we used positive DEP method to pattern HMEC-1(green cell) and the 3T3 (red cell) was seeded. It is to make difference contact area. (c)For cell loading, we used the soft lithography process to form the PDMS-based micropillars to mimic three dimension lobule-mimetic structures in vitro. The HepG2 was loaded from the center and the direction of the medium was pushed.

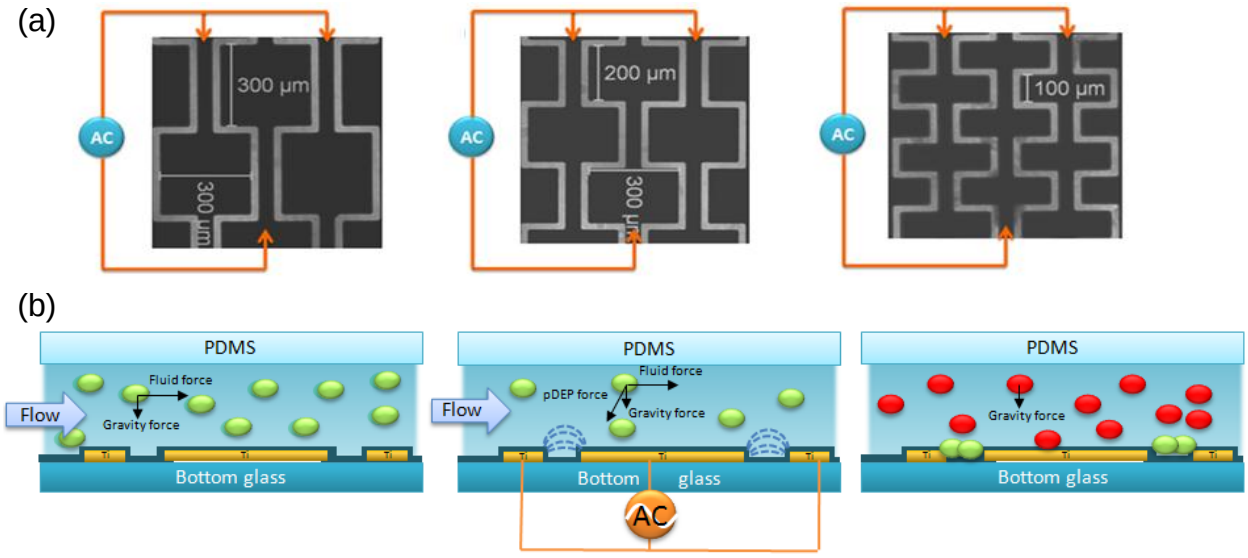

Figure 2. (a)We have designed difference pattern of the electrode to make difference cell to cell contact ration for cell communication. The black area in this figure is electrode. The white area is the HMEC-1 cells concentrated area. (b)Uniformly distributed HMEC-1 cells were loaded into the chamber. The HMEC-1 cells were attracted and positioned via the DEP patterning electrodes. The 3T3 cells were seeded.

RESULTS

In our experiment, the contact area is depending on the long size of the pattern of the HMEC-1 cells. The chamber in the most contact area is 100um×300um which enhance the best liver albumin testing. HMEC-1 cell were attracted on the white area as shown in this figure3 (a) by the 1MHz, 10V. The scale bar is 100um. In this condition, we also used the flow rate by 0.5 microliter per one minute. In figure 3(b), these photos are showed the on-chip cell-patterning result of HMEC-1 cells and 3T3 cells in 300um×300um area case. Figure 4 (a) is showed the schematic diagram of the operation principle of cell loading chamber. These outlets of cell contact chambers were sealed. Additionally, we show the photo is the SEM photo of the SU-8 mold in Figure 4 (b). This photo was showed the result of the HepG2 loading by the micropillars as showed in figure 4(c).

Finally, we show the result in liver albumin experiment result on the third day in figure 5. The condition setup is the albumin secretion of liver pattern chamber with HMEC-1 and 3T3 co-culture communication and the control setup is the albumin secretion of liver chip without cells communication. The condition setup was 64.8% higher than the control setup.

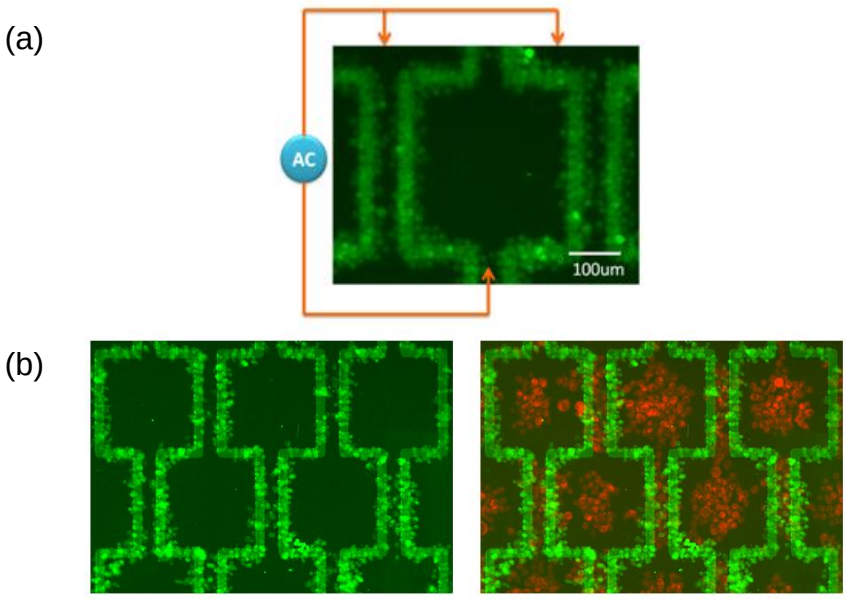

Figure 3. HMEC-1 cell were attracted on the white area as shown in this fluorescent photon. The scale bar is 100um. These photos are showed the on-chip cell-patterning result of HMEC-1 cells (green) and 3T3 cells (red) in 300um× 300um area case.

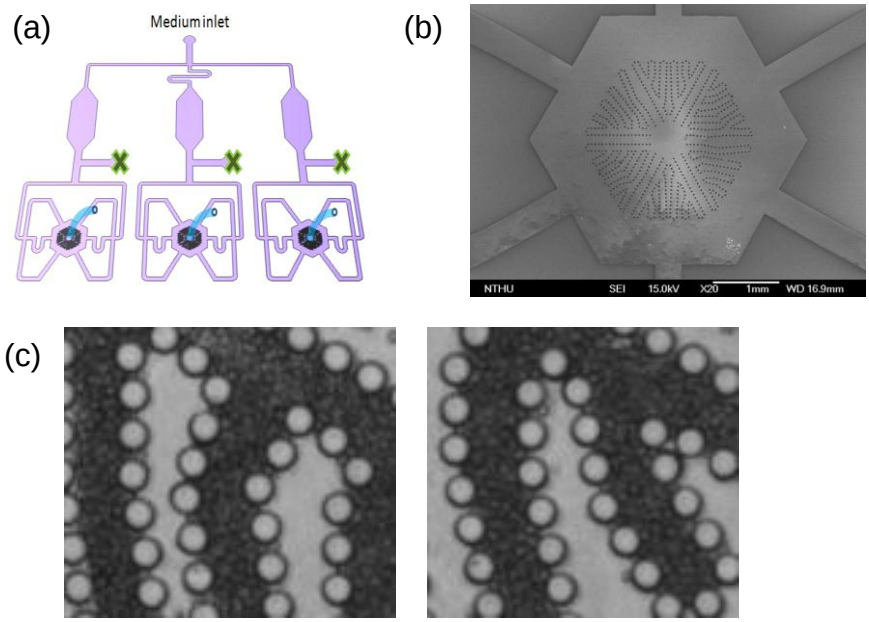

Figure 5. (a) Schematic diagram is the operation principle of cell loading chamber. (b)We showed the photo is the SEM photo of the SU-8 mold. (c) This photo was showed the result of the HepG2 loading.

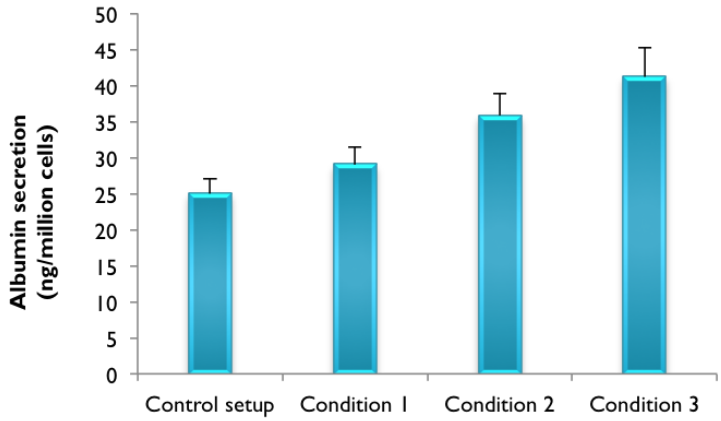

Figure 6. In liver albumin experiment result on the third day, the albumin secretion of liver pattern chamber with HMEC-1 and 3T3 co-culture communication (condition setup) was 64.8% higher than the albumin secretion of liver chip without cells communication (control setup).

CONCLUSION

We have demonstrated a complex three dimensional cell culture chip. In this chip, we have discussed the effect of the medium on the HepG2 cells by the difference proportional HMEC-1 and 3T3 cells contact for the study of liver cells culture.

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