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# Lobule-mimetic Reconstruction on a Liver Lab Chip

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## 1. Introduction

Biomedical studies are carried out either in vitro or in vivo. In vivo studies utilizing laboratory animals can provide high resemblance to the physiological conditions in humans. However, data variation is relatively high. It is also difficult to observe the molecular processes in whole animals. In vitro studies, on the other hand, could well control environmental factors in experiments but are unable to reflect the complex physiological responses found in vivo. The objective of this study is therefore to construct the multi-cell type liver tissue mimicking the hepatic tissue in the animal body that may bridge the current gap between in vivo and in vitro studies. The engineered organoid can find applications in drug screening and disease research models, as well as in organ transplantation in patients. One of the most challenging areas in liver tissue engineering is how to construct a highly vascularized tissue to achieve normal cell-cell and cell-ECM interactions, and mass transfer of nutrients and metabolites. Despite several world-leading research groups are competing to reconstruct complex liver organoids in vitro, there has not been any successful report.

## 2. Approach and Results

The formation, homeostasis, and regeneration of tissues, i.e., tissue dynamics, is the result of intricate temporal and spatial coordination of numerous individual cell fate processes, each of which is regulated not only by cell-autonomous processes but also by extracellular microenvironmental stimuli. Hence, the ability to approach tissue-mimetic reconstruction and manipulate the cellular microenvironment to facilitate cell-cell interactions, cell-extracellular-matrix interactions, and soluble stimuli is essential to maintain cell/tissue physiological functions and to engineer tissues for applications such as drug screening and regenerative medicine.

Up to now, there is no efficient technology to massively pattern hepatic-endothelial cells by mimicking the lobule (the hepatic primary functional unit) genetic morphology which has been optimally developed in nature to support the huge oxygen, nutrient and mass transport to preserve the viability and activity of liver [1]. Conventional approaches for tissue engineering require a porous scaffold seeded with suitable cells in a culture environment, which is designed to provide nutrients, physical stimuli and growth factors to cells for cellular differentiation and assembly into tissue. Although advanced biodegradable scaffolds, which morphologically mimic the human tissue, are developed as the cultured matrix for the cell attachment, it is still insufficient to precisely guide, place and distribute the heterogeneous cells to reconstruct complicated architectures of tissue especially like kidney and liver. Recent reports on a variety of other cell patterning techniques also demonstrated the great promising and interests on engineering tissues. Recent advances in passive cell patterning techniques, which focus on the modification of cell-adhesive substrate via photolithography[2], microcontact printing[3] and microfluidic patterning[4], usually require complicated pretreatment processes and are limited by the intrinsically slow and irregular cellular adhesion. Active cell-patterning techniques, such as ink-jet patterning[5] and laser-guided writing[6], can handle different cell types, but the course patterning resolution and the laser energy loading on cells, respectively, are still the major problems. Moreover, most of these cell-patterning techniques deal only with relatively simple tissues but fail to reconstruct complex organs and suffer from poor cell viability after cell patterning. The appropriate position control/manipulation of multiple cell types to reconstruct a complex tissue pattern according to its genetic architecture with good cell viability after cell patterning is one of the major challenges for engineered tissues. In this talk, we report a liver lab chip which engineers liver tissue by rapidly organizing millions of hepatic and endothelial cells into the complex lobule-mimetic tissue structure.

The liver chip consists of a designed PDMS-based microstructure and a metal electrode patterned on a glass surface. The function of the microchannel is to provide fresh cell culture medium containing nutrients to the cells patterning on the electrode surface. A microchannel is fabricated to guide cells and a metal electrode is patterned to generate an electric field (Fig. 1). The electrode is defined on a glass substrate using the photolithography process, with E-gun evaporation of a 20 nm titanium layer, and using AZ4620 positive photoresist. After exposure to mercury and development by the etching solution ($NH_4OH:H_2O_2:H_2O$ = 1:1:2), the electrode is patterned on the glass substrate. In Fig. 1 (d) to (f), the piranha clean process prepares a 4" silicon substrate. The chamber and microchannel are defined by using SU-8 negative photoresist. After mercury lamp exposure and development, the silicon substrate is 80 μm in height. Following mixing of the elastomer base and the curing agent of PDMS (Sylgard

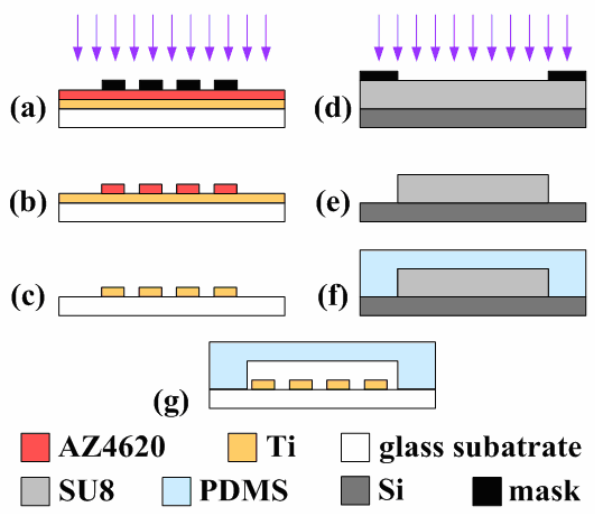


Fig. 1 Fabrication processes of the liver lab chip. (a) to (c) metal electrode pattern process. (d) to (f) PDMS-based microchannel fabrication process.

184, Dow Corning, USA) (10:1), the mixture is poured on the silicon-SU8 mold, with degassing, and cured at 90°C for 30 min. Holes of 1 mm diameter are then mechanically punched through the cured PDMS structure with the purpose of creating a cell injection inlet and a fluidic connection outlet to outside tubing. Finally, after oxygen plasma treatment on both the glass and PDMS top surface, these two elements are aligned under a microscope and bonded together.

Providing external AC voltage to the metal electrode pattern fabricated on the glass substrate generates a non-uniform electric field near the electrode edge. The cells, thus, experience DEP force. Applying positive/negative DEP locates the cells to specific regions: the surface of the metal pattern or the space between electrodes. These are the processes by which cell patterning using electrokinetic phenomena occur. Fig. 2 (a) shows the random distribution of cells suspended in DEP-manipulating buffer on the electrode pattern injected by the syringe pump. Following application of the external AC voltage to provide negative DEP operational conditions (5 Vpp and 100 kHz) the patterned electrodes generated a local minimum electric field gradient in the space between each electrode. The cells, therefore, experienced negative DEP force and repelled toward these spaces. Fig. 2 (b) shows the negative cell patterning processes. The aggregation of cells by positive/negative DEP manipulation on the liver-lab-chip provided the lobule-mimetic pattern and intimate contact for HepG2/3T3 interaction during 2D cellular tissue engineering.

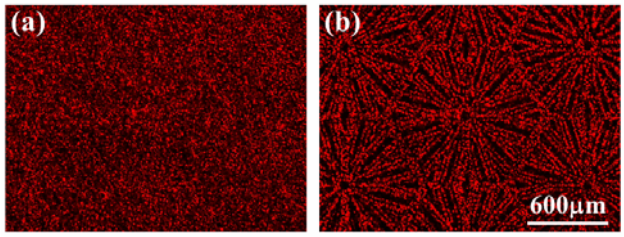


Fig. 2 (a) Cells spread on the surface. (b) Positive dielectrophoresis patterns cells on the electrode.

## 3. Conclusions

One of significances in this liver-labchip research is the lobule-mimetic reconstruction of heterogeneous cells. The liver contains over a million classical liver lobules. Each lobule is morphologically shaped like a hexagon plate which is filled with cords of liver parenchyma cells, hepatocytes, radiating from the central vein and separated by sinusoid-like vascular endothelial lining cells. This unique radiate structure ensures the high metabolism reactions exchanging from blood to hepatocytes.

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