ENCAPSULATION OF CELLS TO FEIGN VASCULAR NETWORK FOR LIVER TISSUE ENGINEERING

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ABSTRACT

3D bio-artificial liver systems and transplantable devices are gaining significance due to the increase in number of patients with liver diseases. We demonstrate an ex-vivo system that mimics liver tissue and enhance the liver specific function with better viability. The proposed in vitro microchip takes advantage of micro contact printing and encapsulating process to achieve the goal. A multiple layer microfluidic channels were conjugated with this system to enable encapsulation of cell within sodium alginate to feign vascular network for the liver tissue. Urea secretion confirmed the cell functionality and the cell viability were affirmed by MTT assay throughout the culture period for different culture pattern. The aforementioned method will further be applied to mimic 3D liver tissue so that the microenvironment can be much closer to clinical trials in bio-artificial liver support systems.

KEYWORDS

Vascular network, Encapsulation, Liver, Microcontact printing.

INTRODUCTION

Tissue engineering is finding importance to develop new therapeutic agents. To evaluate drug efficacy and safety, preclinical models are desired as it furnishes with minute details into human physiology. Advances in biomedical engineering have proposed umpteen in vitro systems to provide an effective 3D tissue via DEP patterning [1], hydrogel encapsulation [2], microcontact printing [3], microscale co-culture [4], patterning encapsulated hydrogels [5] and so on. According to a survey by British Liver Trust, many patients with liver diseases like cancer cirrhosis, hepatitis, acute or chronic liver failure are increasing year by year. The mortality statistics paint a picture of the increasing number of liver disease deaths. Though liver transplantation is the remedy to cure these diseases, the shortage of donors and expensive post-operation treatments have led to the developments of in vitro/ bio-artificial liver devices. Although many attempts have been made by researchers, to reconstruct liver tissue, it is still far away from clinical trials due to the intricate design of liver tissue. Liver is the largest gland with conglomerate design in the body that performs large number of tasks and has an impact on all body systems. The functional unit of liver is the hepatic lobule. Structurally the liver is morphologically divided into many lobules that take the shape of irregular polygonal (hexagonal) prisms. Blood enters the lobules through branches of the portal vein and hepatic artery, and then flows through small channels called sinusoids that are lined with primary liver cells (i.e., hepatocytes). The hepatocytes remove toxic substances, including alcohol, from the blood, which then exits the lobule through the central vein (i.e., the hepatic venule). Under normal physiological conditions the flow within vascular capillaries and tissues is known to have Reynolds numbers <100, resulting in predominantly diffusion based characteristics, over spatial distances of approximately 100 μm,[6, 7] within which cellular metabolite uptake, gaseous exchange and waste removal occurs [8].

Therefore it is importunate to build some ex vivo systems that can delivery nutrients to and remove toxins from the reconstructed liver tissue at distance of approximately 100 μm. The proposed system is one such approach that encapsulates endothelial cells within alginate gels. Endothelial cells when co-cultured with 3T3 fibroblast cell and HepG2, the reconstructed tissue maintained better viability when compared to the one without inculcation of endothelial cells. Precise placement of cells on a device and controlling of the cell culture environment are important for mimicking a liver tissue which is essential for tissue engineering, drug screening and fundamental studies of cell behavior. In this paper we have achieved this goal via micro contact printing and encapsulating process. A multiple layer microfluidic channels were conjugated with this system to enable encapsulation of cells within sodium alginate and thus feign vascular network for the liver tissue. Urea secretion confirmed the cell functionality and the cell viability were affirmed by MTT assay throughout the culture period for different culture pattern. On the whole our results emphasized that this device could be promising for tissue engineering applications. It allows favorable attachment of cells on glass substrate, encapsulation of cells and maintenance of the tissue function

EXPERIMENT

Two major steps were involved in the process as depicted in Figure 1A. At first, patterning of HepG2 and 3T3 fibroblasts is achieved via polydimethylsiloxane (PDMS) microcontact printing. Secondly, endothelial cells (HMEC-1) were encapsulated by alginate and calcium chloride amalgamation. Once the cells were encapsulated the entire system was continuously perfused with fresh culture medium at a flowrate of 0.2 μl/min. the experimental result is shown in Figure.1B.

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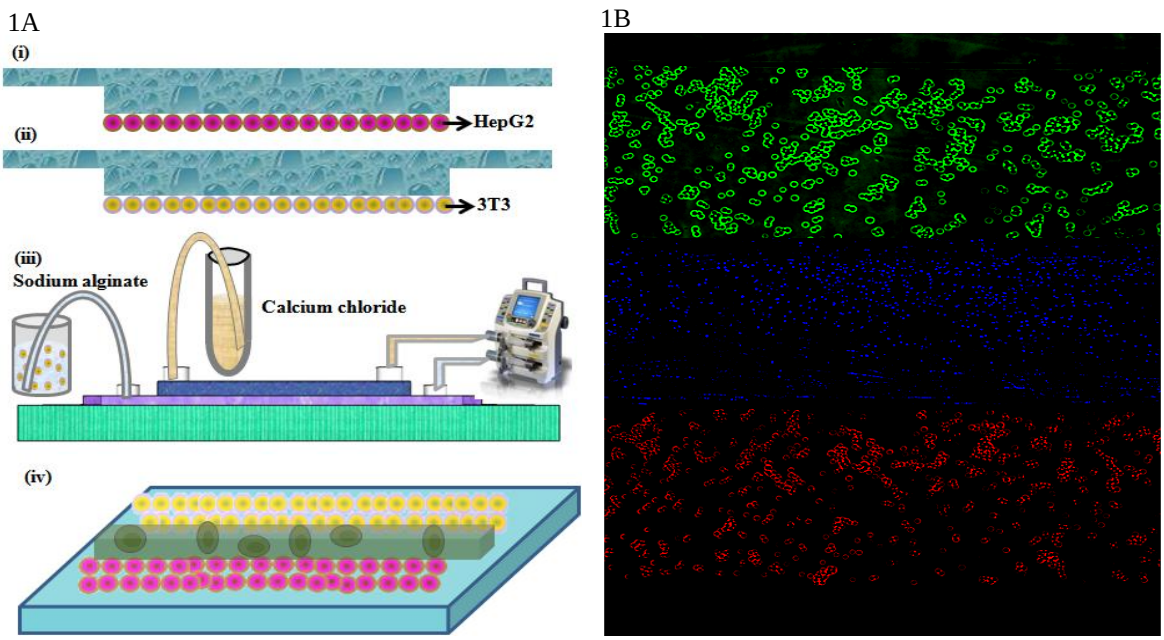

Figure 1A: Working principle. (i) pattern HepG2 cells via PDMS microcontact printing.(ii) pattern 3T3 cells via PDMS microcontact printing.(iii) a two layered step micro channel is bonded with the patterned substrate for encapsulating endothelial cells. Sodium alginate is mixed with the endothelial cells and injected in the bottom channel and calcium chloride solution is injected in the top channel to form a gel. (iv) the overview of the phenomena is illustrated. 1B: The patterning of HepG2 and 3T3 via PDMS microcontact printing and the encapsulation of endothelial cells using biocompatible gel is demonstrated.

UREA SYNTHESIS

In order to evaluate the liver specific function, in this in vitro liver tissue, we have assayed for urea synthesis. Urea is an organic chemical compound from the human body metabolism. It is produced when the liver cells break down amino acids, protein, and ammonia and is transferred from the blood to the urine via kidneys. Therefore, to quantify the urea synthesis concentration would provide an index for the metabolism of mimetic liver labchip.

To compare the urea synthesis between an in vitro liver tissue without patterning and patterning (via microcontact printing & encapsulation) we assayed for urea synthesis from both the liver constructs. Figure 2 plotted the results of the urea assay on our liver lab chip and culture plate.

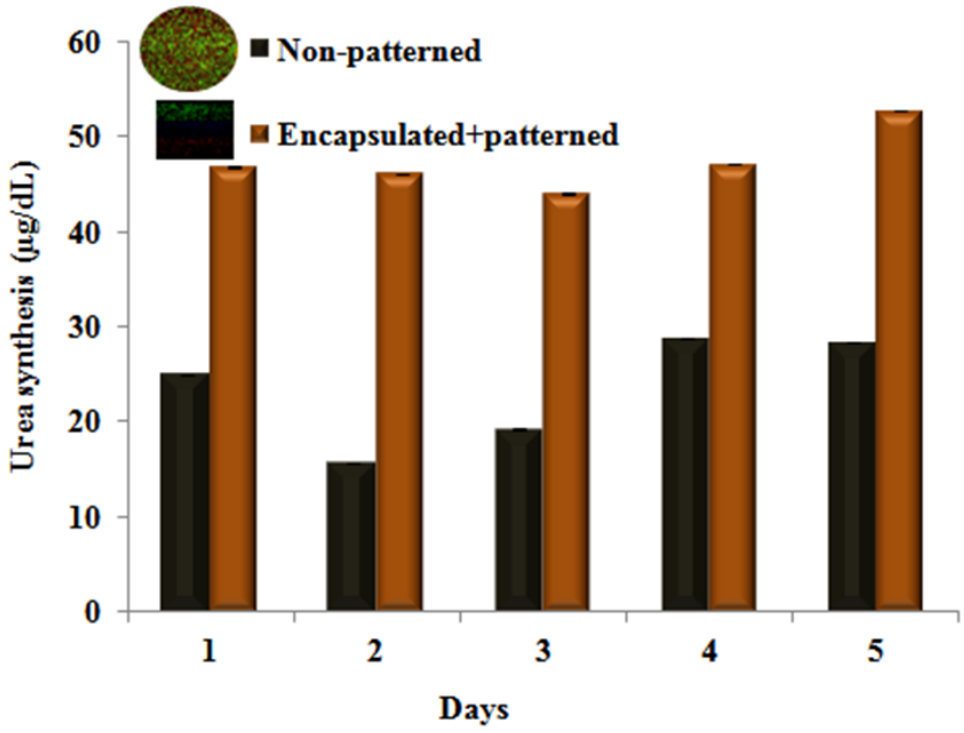

Figure 2. Urea synthesis of the non-patterned and the constructed tissue for 5 days

MTT ASSAY

The MTT assay is based on the ability of mitochondrial dehydrogenase enzyme to cleave tetrazolium rings of the pale yellow MTT forming dark blue formazan crystals. Formazan is impermeable to cell membranes, resulting in its accumulation within healthy cells. Cell viability is directly proportional to the amount of formazan produced monitored by the absorbance at 570 nm. Dead cells do not show any enzymatic activity, hence will not form formazon crystals, therefore the absorbance is only due to live cells containing the active enzyme. The absorbance at 570 nm thus gives an idea of the number of viable cells when compared with controls. The assay was performed for three different culture types: a) pattern only HepG2 and 3T3 via microcontact printing, b) pattern HepG2 and 3T3 via microcontact printing and encapsulate endothelial cells in alginate gels, c) non pattern i.e. just seed cells in 6-well plates. Figure 3 plots the MTT assay results confirming that the encapsulation helps in efficient transport of nutrients.

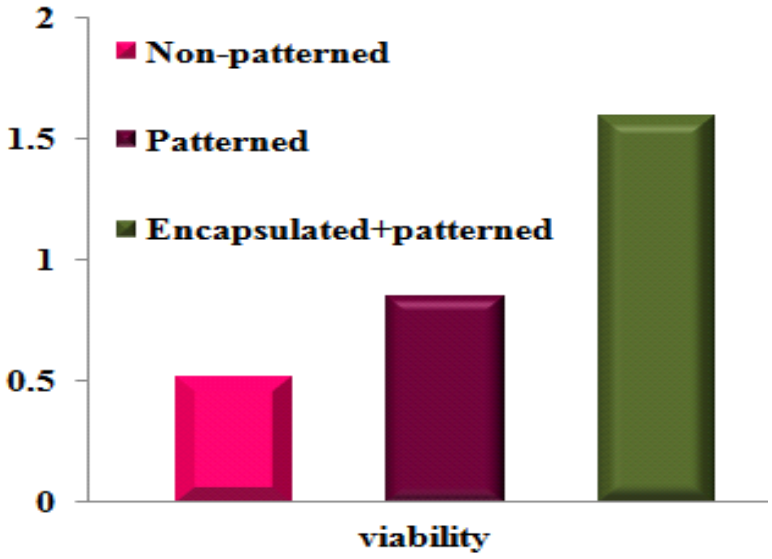

Figure 3. Viability trend for different pattern of culturing cells via MTT assay performed by calorimetric detection.

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