Cell-Encapsulated 3D Hydrogels with Micro-pores and Micro-channels

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ABSTRACT

In this project, an approach for engineering microporous 3D cell-laden hydrogel scaffolds was developed to improve the physical properties of microfluidic hydrogels such as high diffusivity and permeability. To enhance the diffusivity of macromolecules with the hydrogels, micropores were engineered by dissolution of sugar crystals. Microengineered scaffolds offer several advantages such as enhanced diffusion which could be beneficial for long term cell-culturing. Furthermore, it provides space for the settlement and proliferation of cells. Finally, microengineered pores provide biomimetic conditions similar to natural and porous tissues.

KEYWORDS: Biomaterial, Hydrogel, 3D cell culture, Microporous, Biomimetic

INTRODUCTION

Recently, tissue engineering has generated much attention towards creating artificial tissues for regenerative human therapy. In particular, cell-laden hydrogel microfluidic devices have been developed to generate artificial microvasculature that mimic the vasculature of natural tissues [1-6] (Figure 1). The integration of microfabricated devices and biocompatible hydrogels can also be used to study the diffusion of macromolecules within microengineered materials. We have previously developed a cell-laden hydrogel microfluidic system [1] by encapsulating cells within agarose and analyzing diffusion profiles from an engineered microchannels within the hydrogel. It was demonstrated that only cells cultured near microchannels maintained their viability due to continuous perfusion of medium through the channels. The limitations with this system were the high gelation temperature (46℃) and that it was difficult to bond the agarose mold to the substrate (71℃, 3 sec). To address these limitations and to minimize cell death for cells that are far from the channels,

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we hypothesized that microporous structures could offer potential improvements in biomolecular diffusion and oxygen transport in microfluidic biomaterials.

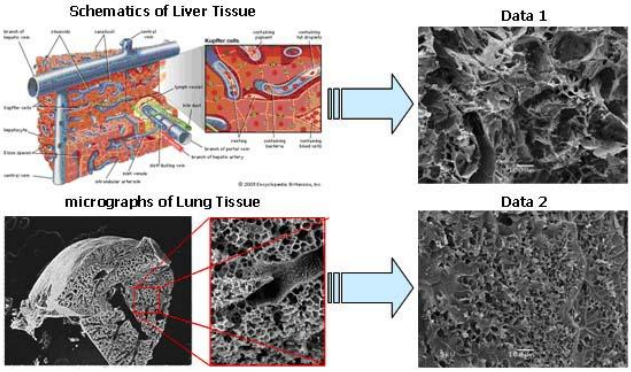

Figure 1: Representative porous tissues in nature and SEM images of our engineered macroporous hydrogels.

EXPERIMENTAL, RESULTS AND DISCUSSION

In this research, 3D cell-laden bio-scaffolds were utilized as a platform for culturing liver cells. To engineer hydrogel scaffolds containing micropores and microchannels for tissue engineering applications, 3% wt agarose hydrogel (which is FDA approved) was used as a suitable bio-scaffold material due to its biocompatibility and biodegradability. To engineer microchannels in the scaffolds, we used microneedles as templates within the hydrogel scaffold. The hydrogel was poured around the microneedle and upon removal of the needle could generate a fluidic channel that was the same size as the needle's outer diameter (Figure 2). Furthermore, to engineer the micropores within the agarose hydrogel, we used sucrose crystals with concentrations ranging from 0 wt% to 300 wt%.

To enable the formation of pores by sucrose leaching within the gels, it is important to reach saturation levels of concentration. To determine the minimum concentration of sucrose crystals that resulted in the formation of macropores in engineered hydrogels, sucrose leaching was analyzed at various concentrations. The saturation of sucrose in 3 wt% agarose was observed at 50 wt% to 75 wt% (Figure 3). After polymerization of supersaturated agarose hydrogel containing sucrose crystals was immersed into sterilized DPBS solution to be dissolved. The required time for complete sucrose dissolution was about 2 hours in the 2 cm (diameter) by 1 cm (height) agarose specimen. We also determined that uniform pores were generated in sucrose concentrations that ranged from 100 wt% to 150 wt%. In subsequent experiments, homogeneity of pores around the channel was evaluated at 100 wt% to be about 13.5 %. We have also shown that cells can remain viable within the engineered hydrogels with controlled porosity (Figure 4). Cells that were encapsulated within gels made by using various concentrations of sucrose crystals, cells remained viable as shown by the expression of calcein AM dye.

In conclusion, we have developed a method of creating pores around microengineered channels in hydrogels. It is anticipated that the method that is developed here will be of benefit for microengineered tissue scaffolds to provide anchoring sites for cell adhesion as well as for enhancing cell viability by increasing the macromolecular diffusion properties away from the channels.

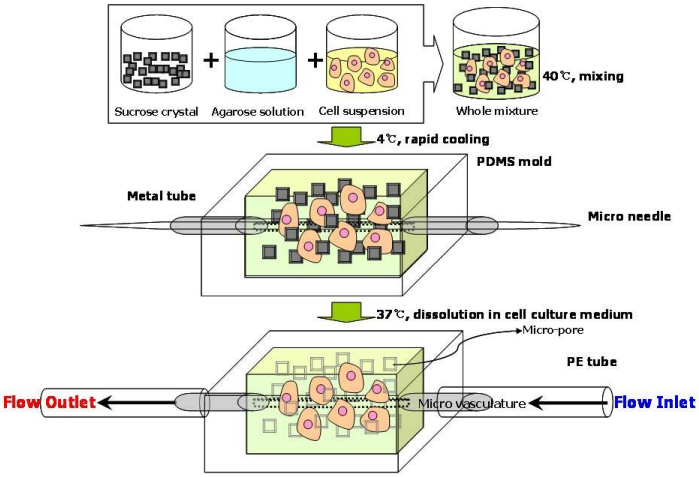

Figure 2. Fabrication process of the 3-D cell-laden tissue architecture with micro-pores and a micro-vasculature.

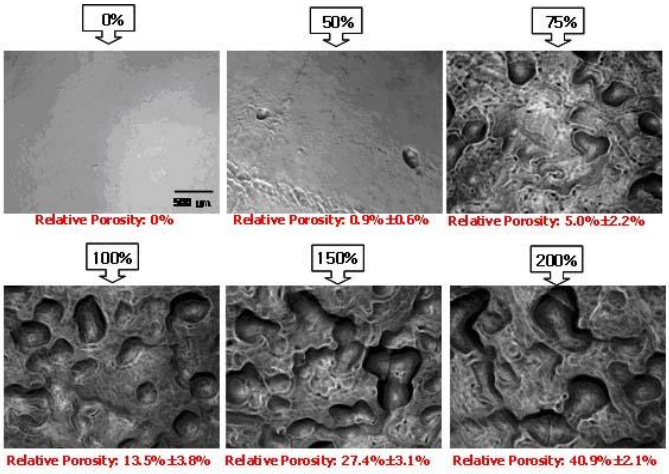

Figure 3. Micrographs of micro-pores derived from sucrose crystals in agarose hydrogels according to sucrose concentration

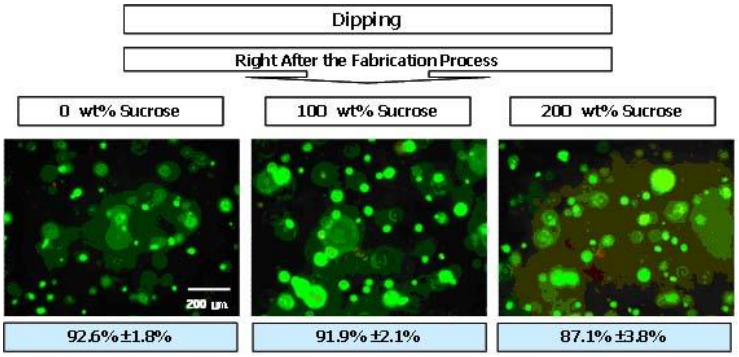

Figure 4. Fluorescent micrographs of hepatocyte cell viability at 500 μm distance form the surface right after fabrication process. Green staining indicates live cells and red staining indicates dead cells.

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