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Detailed Terms
Fabrication of microchannels in methacrylated hyaluronic acid hydrogels

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Abstract— Culturing cells in a vascularized three-dimensional (3D) hydrogel scaffold has significant applications ranging from tissue engineering to drug discovery. In many large 3D scaffolds, mass transport and nutrient exchange leads to cell necrosis, limiting functionality. Here we present a technique for fabricating microfluidic channels in cell-laden methacrylated hyaluronic acid (MeHA) hydrogels. Using standard soft lithographic techniques, MeHA pre-polymer was molded against a PDMS master and cross-linked using UV light. A second UV cross-linking step generated sealed channels. Channels of different dimensions and geometric complexity demonstrated that MeHA, though highly porous, is a suitable material for microfluidics. Cells embedded within the hydrogel molds were well distributed and media pumped through the channels allowed the exchange of nutrients and waste products. Through repeated stacking and cross-linking steps, we were able to form multiple layers of 3D MeHA channels to form a highly perfuse microchannel network. Incorporating collagen into the MeHA to form a semi-interpenetrating network enabled endothelial cell attachment to the interior of the channels. Further development of this technique may lead to the generation of biomimetic synthetic vasculature for tissue engineering and drug screening.

I. INTRODUCTION

Fabricating robust biocompatible three-dimensional (3D) matrices that support cell growth and tissue formation is a prerequisite for many cell culture and tissue engineering applications [1]. Hydrogels are a potentially useful scaffold material for tissue engineering as well as for 3D tissue culture due to their biocompatibility, high water content, and 3D nature [2]. One disadvantage with many hydrogel molds is that they are not sufficiently diffusive to support large scale tissue engineering construct. Since mimicking the vasculature of natural tissues can be used to enhance the diffusivity of engineered tissues, the development of hydrogels with artificial vasculature may be beneficial for various biological and biomedical applications [3].

II. METHODS

A. MeHA Synthesis

MeHA was synthesized as previously described [4]. Briefly, methacrylic anhydride was reacted with a 1 wt% (% of total solution mass) solution of HA (MW=75 kDa; Lifecore Hyaluronan Division) for 24 h to produce methacrylated MeHA chains. The MeHA solution was dialyzed for 48 h and lyophilized for 72 h. The lyophilized product was then dissolved at a stock concentration of 10 wt% in phosphate buffered saline (PBS) and further diluted to prepare each sample.

B. Microfluidic Microchannel Fabrication

Using standard soft-lithographic techniques, a 100 micron diameter PDMS channel mold was created. MeHA pre-polymer was molded against the PDMS mold and cross-linked using a 5-10 second UV light exposure at 99 mW/cm². A 300 μm thick MeHA pre-polymer base was created using a different mold. Placing the two halves of the mold together, a 15-20 second UV cross-linking step generated sealed channels. Channels of different dimensions and geometric complexity were created demonstrating that MeHA, though highly porous and diffusive, is a suitable material for performing microfluidics.

Fig. 1. Schematic illustrating MeHA channel creation

Fig. 2. Top and profile view of two different channels (diameter 100 μm and 500 μm respectively) demonstrating the range of channel sizes that can be fabricated and the diffusivity observed in MeHA with fluorescent rhodamine and trypan blue dye respectively.
III. RESULTS

A. MeHA Microchannel Diffusion

By rigorously characterizing diffusion of both small and large molecules (Rhodamine B and FITC-BSA) we have a quantitative idea of the construct diffusion limit which will aid us in future design of engineered tissue constructs. As expected the FITC-BSA diffused much more slowly than the Rhodamine. In situ, the distance between blood vessels and mesenchymal cells are not larger than a few hundred microns [5]. Our channel networks were designed with this parameter in mind. Based on our diffusion results, we believe that the MeHA matrix will be able to sufficiently perfuse a similar region.

B. Microchannel Stacking

Using repeated channel crosslinking, formation steps, we have been able to stack four layers of channels on top of each other with 300 micron spacing between each layer of channels. This highly perfuse endothelial channel network resulted in high diffusion. Cell viability experiments are currently ongoing in these multi-level 3D microchannel networks.

C. Microchannel Endothelialization

Using a previously reported technique we incorporated collagen into the MeHA matrix to form a semi-interpenetrating collagen-MeHA network [6]. This network is highly conducive to cell surface adhesion. We made a channel using the techniques described above and seeded with a 15 million cells/mL suspension of human umbilical vein endothelial cells. The cells proliferated in the channel over the course of three days. We stained and imaged the resulting 3D channels and found a high degree of alignment along the channel length.

IV. CONCLUSION

We developed a new method to fabricate vascularized 3D MeHA hydrogels. It was shown that the channels were highly diffusive and capable of supporting microfluidic tissue culture. Incorporating collagen into the MeHA resulted in high cell adhesion and proliferation on the interior channel surfaces. In the future we aim to build on these initial findings to develop a large 3D hyaluronic acid hydrogel tissue with multiple channels leading to enhanced tissue function throughout the construct. It is anticipated that, given their biomimetic properties, these techniques and results will be of value in tissue engineering and 3D cell culture applications.

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REFERENCES