The Development of Hydrogel Microwells for Perfused 3D Culture of Hepatocytes

J.A. Shepard[1], V. Chan[2], M.G. Rhoads[1], M.S. Raredon[1], R.L. Dyer[1], P.T. Hammond[1], R. Bashir[2], L.G. Griffith[1]
[1]Massachusetts Institute of Technology, Cambridge, MA, [2]University of Illinois at Urbana-Champaign, Urbana, IL

Statement of Purpose: More sophisticated *in vitro* models of the liver that better predict *in vivo* physiological responses are important for drug discovery. While animal models remain costly and time-consuming, many 3D culture models fail to reconstruct the *in vivo* human physiology. In the liver, oxygen concentration gradients are important for establishing cell types and survival of the highly metabolic hepatocytes. This has led to the development of perfused bioreactors for 3D tissue culture to more closely recapitulate the cellular microenvironment *in vivo* by controlling oxygen concentration gradients and providing biomechanical stimuli [1]. Hydrogels are attractive biomaterial scaffolds for perfused 3D tissue culture because they enable the transport of oxygen and nutrients and their mechanical properties more closely resemble that of soft tissue [2]. Synthetic hydrogels such as poly(ethylene glycol) diacrylate (PEGDA) are well-defined matrices that can be readily tailored to incorporate bioactive groups to target cellular functions and patterned to create complex architectures. Herein, we report the development of patterned PEGDA hydrogel microwells that are fabricated onto polycarbonate (PC) filters to create a hydrogel scaffold for perfused cell culture within a multiwell bioreactor (Figure. 1). We demonstrate the ability of this hydrogel scaffold to withstand perfusion flow and support the viability of primary hepatocytes.

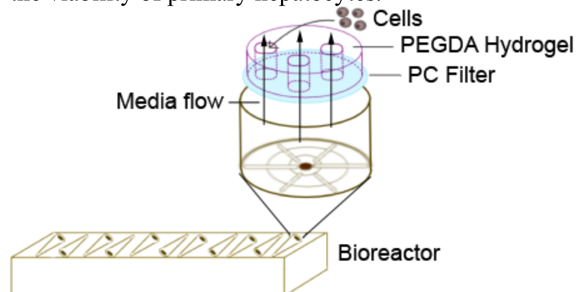

Figure. 1. Schematic representing the components of the perfused bioreactor to recapitulate the liver microenvironment. The bioreactor recirculates media upward through the pores of the PC filter to access cells cultured within the PEGDA microwells.

Methods: PEGDA hydrogels having open microwells were crosslinked onto functionalized polycarbonate filters using a stereolithography apparatus and the photoinitiator 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone. Track-etched polycarbonate filters with 5 µm pore size were functionalized by animation using ethylene diamine and subsequent acrylation with acrylic acid N-hydroxy-succinimide ester. CAD models of the desired soft scaffold design were generated in AutoCAD (Figure. 2a) and exported to stereolithograpy format. Following fabrication, hydrogel scaffolds were sterilized with ethanol and stored in PBS. Hepatocyte-rich cell populations isolated from Fischer rats were seeded above the hydrogel scaffold in the bioreactor well. Bioreactor flow was maintained throughout the culture inside a humidified 5% CO_2 incubator at 37 °C. Cell viability was assessed with a live/dead assay kit, and cell nuclei were stained with Hoechst. Photoinitiator concentration and stereolithography energy dose were investigated for their impact on achieving hydrogel scaffolds that remain intact during perfused culture while maintaining cell viability.

Results: Using stereolithography, open, well-defined PEGDA hydrogel microwells were polymerized on PC filters (Figure. 2b). Modulation of the polymerization conditions led to robust hydrogel scaffolds that enable the culture of hepatocytes under perfusion flow (Figure. 2c). After 3 days of perfused culture, primarily live cells are located within the open microwells of the PEGDA hydrogels placed in the bioreactor (Figure. 2d).

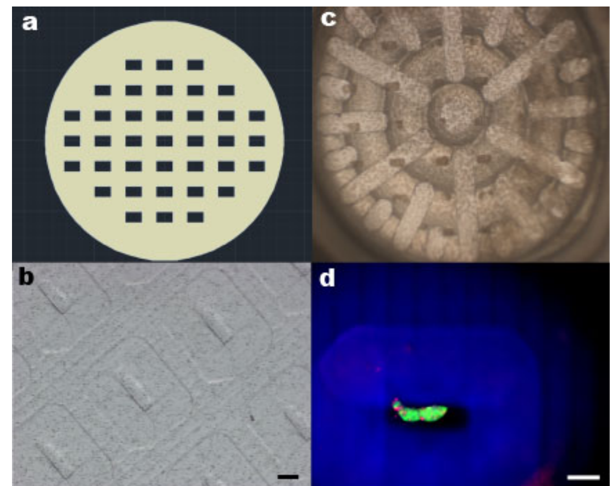

Figure. 2. Hydrogel microwells within the bioreactor are generated from a CAD file (a) to generate well-define microwells in PEGDA that withstand perfusion flow during culture (b). Primary hepatocytes cultured in microwells (c) are primarily viable at the end of the culture (green = live cells, red = dead cells, blue = cell nuclei) (d). Scale bars represent 100 µm.

Conclusions: New designs of perfused bioreactors aim to more closely resemble *in vivo* models to create better tools for drug safety applications. Here, we developed a hydrogel microwell array for perfused 3D culture of primary hepatocytes. Although this system was developed for cells of the liver, it can be used for perfusion culture to study drug toxicity of other cell types such as lung, liver, or heart cells.

References: [1] Domanksy K. Lab Chip. 2010;10:51-58.
[2] Peppas N. Eur J Pharm Biopharm. 2000;50:27-46.

©2013 Society For Biomaterials