Rapid Printing of Vascular Networks for Large-Scale 3D Tissue Culture

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Statement of Purpose: The major remaining barrier to the creation of functional tissues is a lack of general materials and methods to rapidly construct vascular networks throughout 3D scaffolds. Large constructs containing living cells develop necrotic cores due to nutrient and waste diffusion limitations. To address this challenge, we created a cytocompatible carbohydrate glass and then thermally extruded the material with a 3D printer to form an interconnected filamentous lattice that serves as a sacrificial element for vascular network molding. The filament network exhibits smooth surface finish and interfilament fusions, optical clarity at visible wavelengths, and sufficient stiffness to be mechanically self-supporting. The network can be encapsulated in a variety of extracellular matrix materials along with living cells, yet dissolves controllably in normal cell media to create vascular networks without damage to embedded cells (greater than 95% cell viability). We demonstrate the generation of 3D vasculature in many types of matrices including collagen, fibrin, agarose, alginate, and PEG-based hydrogels. Cell-based luciferase reporters demonstrate direct control of cellular homeostasis and metabolism by the vascular architecture patterned into the gels. We will also present data on how this method can enable scaling of engineered tissues to arbitrary size. The speed, efficiency, flexibility, and highly automated nature of this vascular molding technique, as well as the cellular responses observed, open new possibilities for 3D tissue culture and implantable, vascularized scaffolds.

Methods: Carbohydrate glass was extruded at 100 ºC with a RepRap Mendel 3D printer (http://reprap.org/) to reproducibly create 3D multiscale interconnected filamentous lattices. The material and network were characterized by optical microscopy, scanning electron microscopy, and UV/Vis spectrophotometry. After post-processing of the lattice, the network was encapsulated in ECMs such as PEG hydrogels or fibrin gels containing living cells. After gel formation the carbohydrate glass was dissolved in PBS for 10 min and the gels were transferred to cell culture media for time course studies. Lentiviral reporters for Luc2P (luciferase) under the CMV promoter enabled non-invasive imaging of cell survival and metabolism.

Results: Carbohydrate glass networks are reproducibly extruded using a 3D printer in minutes (Figure 1). Individual filaments are cylindrical, extremely smooth on their surface, and likewise form smooth interfilament fusions. The material is mechanically stiff and optically clear. Cross-section views through fibrin gels containing HUVEC:10T$^{1/2}$ co-cultures demonstrate monolithic gels and greater than 95% cell viability near channels (Figure 2). HEK cells expressing luciferase reporter show dramatic metabolic differences that scales directly with the vascular network architecture.

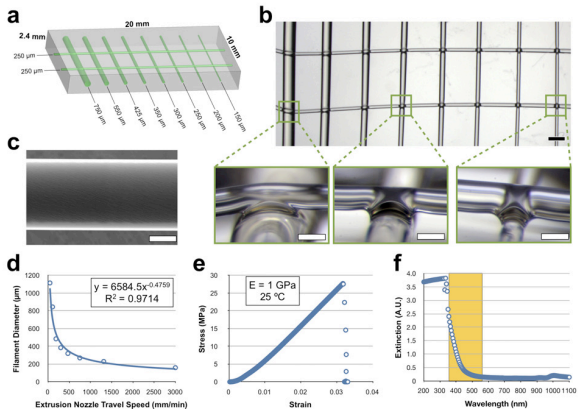

Figure 1. a) design of carbohydrate glass vascular network (green). b) 3D printed filamentous networks (bar = 1 mm) have smooth junctions (insets, bar = 200 µm) and c) sub-micron surface roughness. d) filament diameter is controlled by 3D printing parameters. e) carbohydrate glass is mechanically stiff and f) amenable to use with photopolymerizable ECM due to its optical clarity at visible wavelengths.

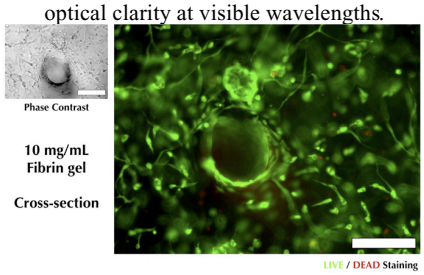

Figure 2. HUVECs and 10T$^{1/2}$ cells survive (> 95%) and spread in monolithic fibrin gels containing interconnected vessel networks molded from carbohydrate glass filaments as seen by phase contrast and live/dead staining. bars = 200 µm.

Conclusions: We present a new, rapid, facile, and flexible 3D printing approach to generate vascular networks in a variety of extracellular matrices used in 2D and 3D cell culture. Importantly, the vasculature can be generated in the presence of living cells and can modulate cellular metabolism in thick, densely populated 3D constructs. These studies are enabling a wide range of quantitative insight into the role for vascular patterning in large scale 3D tissue culture in a plurality of cell and ECM systems and are broadly applicable both to cell biology and tissue engineering.