BIOINSPIRED VASCULAR STRUCTURES *VIA* 3D PRINTING AND SUSPENDED MICROFLUIDICS

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ABSTRACT

Accurate modeling of the complex three-dimensional (3D) microarchitectures of *in vivo* vascular structures has remained a formidable challenge for medical and biological communities. Although many platforms have been developed to mimic vascular microgeometry, difficulties associated with recreating *in vivo* characteristics such as permeability and tortuosity has impeded progress. To address these issues, here we present a novel strategy that combines additive manufacturing or "3D printing" with suspended microfluidics to fabricate semipermeable, tubular structures with tortuous architectures. Specifically, the approach entails: (i) using PolyJet 3D printing to create a tubular framework comprising local microcurvature and global tortuosity, and then (ii) employing a suspended microfluidics technique to coat the framework with a porous, biocompatible cellular scaffold. Fabricated proof-of-concept tubular scaffolds exhibited inner radii on the order of 100 μm. Experimental results revealed tubular rupture pressures in the range of approximately 30–65 kPa. Seeding of human umbilical vein endothelial cells (HUVECs) within the tubule suggests that the presented work could be extended to better model the micro/mesoscale architectures of vascular systems for applications including drug discovery, disease modeling, and personalized medicine.

INTRODUCTION

The human circulatory system has evolved to be physiologically and structurally complex in order to effectively transport oxygen, nutrients, and waste. Currently, platforms such as petri dishes and well plates represent the standard for investigating vascular cells; however, the flat substrate combined with quasi-static fluidic conditions bear little resemblance to their *in vivo* counterparts [1]. An ideal device to model these physiological conditions would match vascular constructs on the level of not only its 3D convolution, but also enable the perfusion of biochemicals through its boundaries to imitate an operational *in vivo* system.

Recent efforts to better mimic these physiological conditions have used microfabrication processes such as soft lithography [2]; however, such techniques are inherently monolithic, rendering it difficult or unfeasible to reproduce vascular microgeometry. Moreover, bulky stereolithographic PDMS casts limit permeability to biological components [3] and demonstrate an affinity for small hydrophobic molecules, substantially changing the concentration of solutions in microfluidic systems [4].

Consequently, researchers have recently investigated the potential for constructing vascular structures using 3D printing technologies. For example, researchers have 3D printed fugitive inks that can be encapsulated to form a cast, after which the fugitive ink can be evacuated [5, 6, 7]. Despite the potential of such approaches, challenges remain for simultaneously recreating both the thin, permeable lining of vascular networks as well as the complex micro/mesoscale 3D architectures. These methods incorporate a level of tortuosity to more closely mimic vascular architecture, though such devices are still impermeable to biological components and lack intervascular interaction.

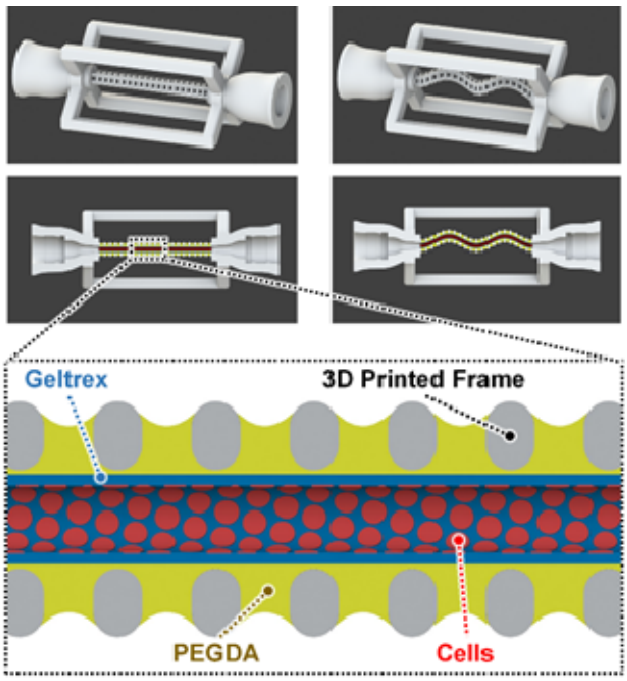

Figure 1: Illustrations of two 3D printing-enabled vascular-inspired scaffold concepts corresponding to a (left) straight tubular architecture, and (right) tortuous tubular architecture. The expanded view illustrates a fabricated device seeded with cells, which includes a PolyJet 3D printed outer framework, a coating of poly(ethylene glycol) diacrylate (PEGDA), an inner lining of extracellular matrix (ECM) in the form of Geltrex to support cellular attachment, and seeded HUVECs. In addition, the cylindrical access ports on each side serve as micro-to-macro interconnects to support the load biological fluids within the enclosed tubular structures in order to recreate the fluidic shear stresses associated with blood flow inside of vascular systems.

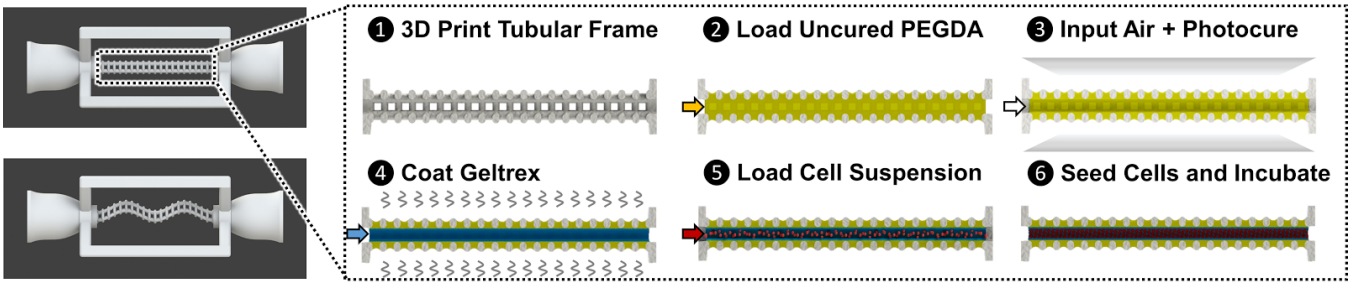

Figure 2: Conceptual illustrations of the methodology for coating the 3D printed framework with a porous outer biomaterial membrane to achieve an enclosed microfluidic channel, and then functionalizing the system with Geltrex to support seeding of vascular cells within the device.

CONCEPT

The concept of our 3D printing-based fabrication approach is presented in Figure 2. First, a tubular scaffold is constructed from evenly spaced concentric rings. PolyJet 3D printing serves as the method of construction, capable of producing hundreds of devices with complex geometries at microscale resolution *en masse* [8]. At this scale, such a structure allows the surface tension of liquids in the tube to be maintained within the unenclosed structure—a phenomenon known as "suspended microfluidics" [9]. A liquid-phase photocurable form of PEGDA is loaded into the framework by taking advantage of such phenomena. Then, pressurized air is inputted to resolve an inner channel (while PEGDA is maintained in contact with the outer framework) and then the device is simultaneously exposed to white light to photocure the PEGDA. This processes results in an enclosed PEGDA-based channel with its geometry dictated by the 3D printed frame. After that, liquid-phase Geltrex is injected into the newly formed tubule, and air is once again forced through to prevent occlusion. The Geltrex is then thermally cured in an incubator for ten minutes. Lastly, a suspension of cells can be loaded into the tubule for cell seeding within the tubular structure.

MATERIALS AND METHODS

Design

The original conceptual designs accounted for the limitations of the printer's resolution as well as the device dimensions needed for the application of suspended microfluidics. The devices were printed with the biocompatible material MED610 and designed with a 250 μm inner tubular radius, 125 μm-in-radius ring structures, and 250 μm ring-to-ring spacing. Initial trials conducted on different dimensions demonstrated that structural integrity was optimized through an elongated circular structure. Additionally, the open space between rings was maximized to allow for the exploitation of microfluidic techniques. The geometric design of the rings allows for the diffusion of PEGDA and an extracellular matrix (ECM) analogue into the unwalled spaces between the rings. The preparation of the devices with ECM analogues is followed by the seeding of human umbilical vein endothelial cells (HUVECs). The controllable feature of the design's specific dimensions allows for the construction of any desired size and tortuosity to accurately mimic the geometry of various vascular networks (Figure 1). Furthermore, this could allow for future constructs involving multiple tubules as part of a large vascular network to investigate diffusion interactions of adjacent tubules. Such a model would be optimal for assessing the complex diffusion that takes place in physiologically relevant areas such as the proximal tubule of a kidney nephron.

The design of the tubular structure employs a suspended microfluidics fabrication approach to exploit the surface tension of a fluid to draw it through a channel and to maintain it within an un-walled microstructure [9]. This allows for the creation of a layer of porous, biocompatible PEGDA based on the microgeometry of the device. This technique draws PEGDA from the device surroundings into the spacing between the rings of the device while a channel of air is forced through the device creating a tubule. The PEGDA layer is photocured onto the device, then coated in an extracellular matrix analogue. This process prepares a suitable surface of seeding of HUVECs [10].

Manufacturing

The devices were printed on a Stratasys Objet500 Connex3 PolyJet Printer. Figure 3 depicts progressive images of the printing process and the freshly printed devices. The SUP706 support material was removed by dissolution with stirring in a 2% NaOH/2% Na_2SiO_3 solution for four hours, and the bare devices were air-dried overnight before continuing processing.

Solution of 5% Irgacure in PEGDA was infused into the devices *via* suspended microfluidics in a dark environment at room temperature with minimized human handling. A Fluigent MAESFLO system pumped air through the tubules to ensure a clear channel, a Kimwipe was used to prevent accumulation of material at the ports, and the material was photocured over a two-minute duration. Next, thawed Geltrex was similarly infused into the devices at about 10°C, excess material was similarly expelled with the MAESFLO, and the devices were set to incubate at 37°C for ten minutes. Devices were stored at room temperature and sterilized under UV light for 30 minutes prior to cell culturing.

A confluent monolayer of HUVECs from a 25 cm^2 culture flask was first treated with trypsin. A suspension was created from these cells by resuspension in approximately 5–7 mL of culture medium (Vasculife Basal Medium, 10% FBS). The highly concentrated suspension of cells was then pumped into the tubule using

a syringe. To ensure uniform coating of the interior, devices were incubated at room temperature and rotated 180° every 30 minutes for two hours. The devices were incubated overnight. They were then flushed with fresh media to prevent cell detachment. Finished devices were then incubated for an additional 24–72 hours.

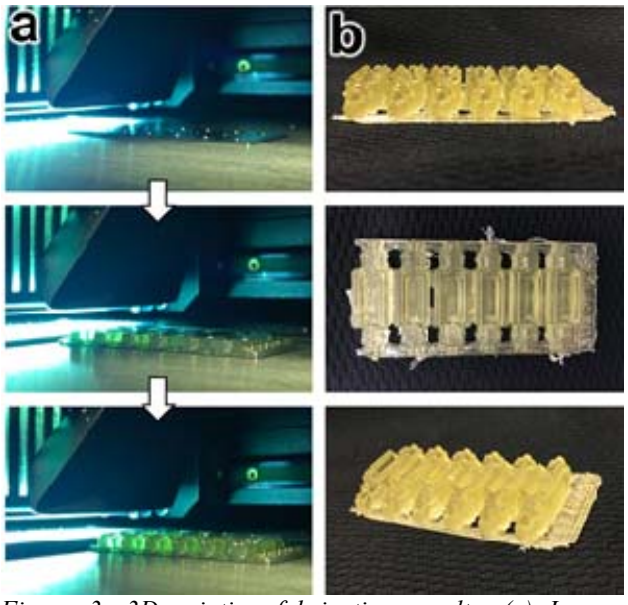

Figure 3: 3D printing fabrication results. (a) Images captured from a video of the PolyJet printing process inside the Objet500 Connex3 printer. (b) Devices after completion of the 3D printing process.

RESULTS AND DISCUSSION

Fabrication

Numerous devices of identical dimensions were fabricated for device characterization and analysis. Figure 4 depicts the post-printing preparation process and fluid being pumped into the device to mimic the shear forces of flowing blood. Red dye was used to visually establish a sealed, unobstructed fluid path through the tubule.

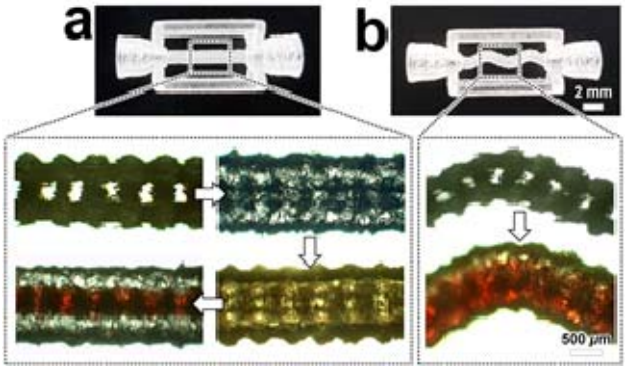

Figure 4: Fabrication results for the biomaterial (PEGDA) coating process for (a) straight device architecture and (b) architecture with tortuosity. Dyed water is inserted into each device as a demonstrative example. Scale bars = 2 mm (top), 500 μm (bottom)

SEM images of devices post–PEGDA infusion reveal successful PEGDA coating and inner tubule clearance (Figure 5). Furthermore, it provides characterization of the surface of the finished interior of the structure. Smooth, contiguous surfaces are most likely to be encountered in vascular structures and therefore is the more desired surface characteristic. The radius of the device post–PEGDA infusion is on the order of 100 μm with a wall thickness of about 150 μm.

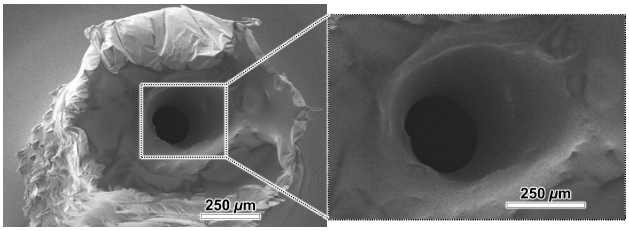

Figure 5: SEM micrographs showing fabrication results of a cross-section of PEGDA-coated device.

Burst Pressure

To assess the maximum pressure the PEGDA-infused devices could withstand without rupture, several devices were connected to a Fluigent MAESFLO system using catheter couplers and Tygon microbore tubing at one end. The devices were then pressurized with deionized water while the input pressures and flow rate were monitored using the MAESFLO's Microfluidic Flow Control System (MFCS), the FLOWELL microfluidic flow sensor and the Sensirion SLI-1000 flow sensor. Pressure was steadily increased until the wall of the device burst (Figure 6).

The devices maintained a sealed vessel free of leaks when subjected to excessive pressures. The rupture points on the PEGDA-infused devices ranges from approximately 30 kPa to 65 kPa.

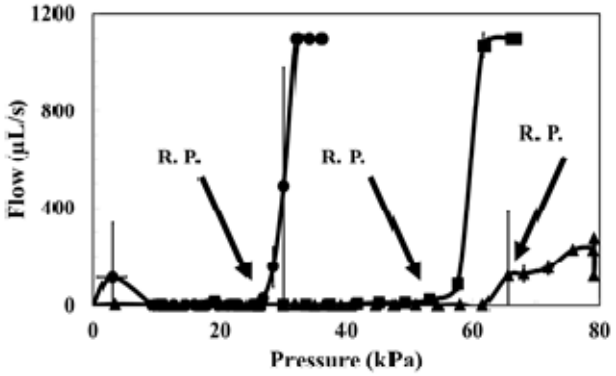

Figure 6: Experimental results for burst pressure testing of PEGDA-coated devices, which entailed using a microfluidic pressure regulator to increase the input water pressure until leakage. Increases in fluid flow indicate rupture of the tubular membrane. Error bars denote standard deviation. Rupture points are denoted by "R.P."

Cellular Assay

After HUVECs were seeded into a finished device, a fluorescent viability assay was used to determine the viability of the cells that have been seeded into the device. A solution of 2 μM calcein acetoxymethyl and 4 μM ethidium homodimer-1 was prepared, then diluted in 4 mL of cell culture media. This dilution was then pumped into the device with a syringe and then incubated for one

hour before imaging.

The assay determined that a majority of the cells within the tubule were viable (Figure 7). Live cells are displayed in green, while dead cells are displayed in red. From the photos obtained, it was difficult to ascertain accurate counts for both live and dead cells because the device itself absorbed larger amounts of fluorescent dye than anticipated. These preliminary results suggest the feasibility of producing a uniform monolayer of cells within the tubule.

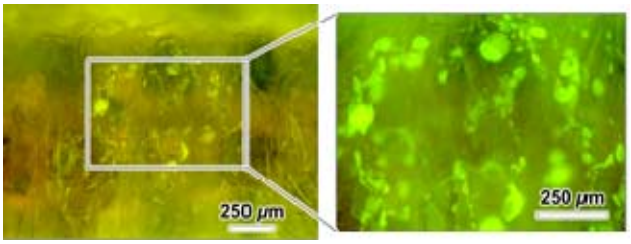

Figure 7: Fluorescent images of HUVECs from Live/Dead Cytotoxicity Assay seeded into a finished device.

CONCLUSIONS

In this work, we demonstrated the use of PolyJet 3D printing and suspended microfluidics to create a novel approach for fabricating 3D devices that better imitate *in vivo* microarchitecture and tortuosity of vascular networks. The appearance of the device from the SEM images reveals the formation of an enclosed microchannel with microcurvature. Continuous streams of dye and burst pressure data demonstrated that the devices are sealed vessels that can maintain structural integrity under pressure; however, the vessels demonstrated a substantial range of maximum pressure, this variation suggests the difficulty of and necessity for an optimized, consistent fabrication process. Live HUVECs were found on the devices after HUVEC seeding, revealing these fabricated devices coated with ECM analogue are capable of supporting live cells. The ability to support living cells suggests that this approach may be extended to achieve a closer model to *in vivo* vasculature.

We demonstrated that additive 3D printing can be successfully applied to model microscale vasculature with the inclusion of viable cells. Further adjustments towards the structure and design of the device with the incorporation of additional helices and 3D geometries will produce a more relevant tubular model of vascular networks throughout the human body.

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