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Engineering “Endothelialized” Microfluidics for Investigating Vascular and Hematologic Processes Using Non-Traditional Fabrication Techniques

Robert G. Mannino^{1,2,3,4,5}, Navaneeth KR Pandian⁶, Abhishek Jain, PhD⁶, and Wilbur A. Lam, MD, PhD^{1,2,3,4,5}

¹The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Atlanta, GA

²The Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA

³Emory University School of Medicine, Department of Pediatrics, Division of Pediatric Hematology/Oncology, Atlanta, GA

⁴Children’s Healthcare of Atlanta, Aflac Cancer & Blood Disorders Center

⁵Institute of Electronics and Nanotechnology, Georgia Institute of Technology, Atlanta, GA

⁶Department of Biomedical Engineering, College of Engineering, Texas A&M University, College Station, TX

Abstract

Investigating the complex interplay between blood cells and the endothelium is crucial in understanding the pathophysiology of many diseases. Observation of the *in vivo* vasculature is difficult due to the complexities of vessel geometry, limited visualization capability, as well as variability and complexity inherent to biologic systems. Therefore, *in vitro* systems serve as ideal tools to study these cellular interactions. Microfluidic technologies are an ideal tool for recapitulating the vasculature *in vivo* as they can be used to fabricate fluidic channels on the size scale capillaries using gas permeable, biologically inert, and optically transparent substrates. Microfluidic channels can be vascularized by coating the inner surface of the microchannels with a confluent monolayer of endothelial cells, representing a reductionist, tightly controlled, *in vitro* model of the microvasculature. In this review, we present advances in the field of “endothelialized” microfluidics, focusing specifically on non-traditional fabrication and endothelialization techniques. We then summarize the various applications of endothelialized microfluidics, and speculate on the future directions of the field, including the exciting applications to personalized medicine.

Corresponding Author: Wilbur A. Lam, MD, PhD, 412 Emory Children’s Center, 2015 Uppergate Drive, Atlanta, GA 30322, Tel: 404-727-7473, wilbur.lam@emory.edu.

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Introduction

Studying the cellular interactions which occur within the vasculature is of utmost importance in understanding the pathophysiology of various vascular etiologies such as sickle cell disease, malaria, and stroke[1–3]. These cellular interactions are controlled, in part, by the hemodynamic and rheological forces present within the vasculature[4]. For example, platelets have been shown to preferentially aggregate and activate in regions of high shear stress created by vascular abnormalities such as stenotic lesions[5, 6]. Furthermore, many underlying physiological complications that arise from these disease states have origins in the microvasculature, such as pain crisis and stroke in sickle cell disease[7]. Therefore, a complete investigation of the pathophysiology of various vascular diseases requires characterization of these hemodynamic forces and the impact that they have on cellular interactions within the microvasculature.

Due to the complexity of *in vivo* models, in which microvascular geometries and system inputs cannot be readily controlled, reductionist, *in vitro* representations of the microvasculature are necessary to fully characterize the pathophysiology of various diseases. To that end, microfluidic technologies have been developed to address this need (Fig. 1) [8–11]. In this review, we will present advances in the field of “endothelialized” microfluidics in which endothelial cells are seeded onto microchannels in order to recapitulate the *in vivo* microvasculature and investigate various vascular and hematological processes. We will particularly highlight recent advances in the field that utilize alternatives to the traditional photolithography-based microfluidic paradigm.

Microfluidic techniques for recapitulating *in vivo* vasculature

Microfluidics refers to the fabrication of small channels (channel width on the scale of microns) for the purpose of fluid flow[12]. These techniques are particularly useful for conducting biological assays and studying biological processes and phenomena for a number of reasons. First, the geometry of microfluidic devices can be very tightly controlled during fabrication. In the context of the microvasculature, tight geometric control facilitates investigation of biological interactions at specific geometric structures within the microvasculature (stenosis, aneurysm, vascular bifurcations, etc.) [13–15]. Importantly, microfluidic devices can be fabricated using microscopy compatible, transparent substrates, allowing for facile imaging of microvascular processes[16, 17]. Additionally, these devices may be constructed out of biologically inert materials, ensuring that the microvascular process of interest can be easily studied with minimal interference[18]. Finally and most importantly, microfluidic devices allow for control of the system inputs, which represent a significant improvement over *in vivo* models due to the inherent complexity of biological systems. These reductionist systems allow for isolation of specific microvascular processes for investigation.

The most common microfluidic technique used to develop *in vitro* vascular models is known as soft lithography[19, 20]. Adapted from the semiconductor industry, soft lithography utilizes the patterned exposure of a photosensitive material to develop a master mold which serves as the geometric structure of the devices. Plasma bonding and replica molding of the

transparent, biologically inert, flexible silicone, gas permeable polymer polydimethylsiloxane (PDMS) is the most common technique used to create microfluidic channels[21]. These techniques are very robust and repeatable; however, the photolithography process requires specialized engineering training and expensive equipment. This labor intensive fabrication process precludes rapid iteration and testing of novel microvascular models. This represents a significant barrier to entry of the field to many researchers trying to study microvascular phenomena. Thus, a need exists for techniques utilizing *in vitro* microfluidics that avoid complex fabrication.

To that end, many innovative techniques have been developed that do not require photolithography to recapitulate microvascular environments. One such technique, 3D printing, allows for the development of 3 dimensional objects layer by layer. Once a desired geometry is designed in a computer aided design (CAD) program, microfluidic channels themselves may be constructed in one step, rather than fabrication of a master mold which must then be cast in PDMS[22]. Liu *et al.* describe a system in which a proprietary material was used to print channels which were subsequently combined with endothelialized inserts to study the interactions between blood vessel walls and red blood cells (RBCs) [23]. Gross *et al.* describe a method in which PDMS is coated on the inside of a 3D printed channel, which was subsequently used for endothelial cell culture, combining the benefits of PDMS with those of 3D printing[24]. Drolet *et al.* present a technique in which a 3D printer is modified to print a dissolvable sugar glass (Fig. 2), which may be encased in a biocompatible scaffold and dissolved, defining the microchannel geometry which can then be endothelialized[25]. Microfibers represent another technique used to create 3D structures for investigating microvascular cellular processes due to their ease of preparation and assembly, utilizing techniques such as multiple laminar flows and electrospinning[26]. Cheng *et al.* report a system in which hollow, bio-functional microfibers were formed using multiple laminar flows (a quick-gelling, biologically active alginate sheath flow surrounding a poly(vinyl alcohol) inner phase fluid) which were then endothelialized[27].

One non-traditional microfabrication technique of particular importance is the “do-it-yourself” method developed by Mannino *et al.*[28]. Rather than utilizing microfabrication facilities or expensive 3D printing equipment, this technique relies on off-the-shelf materials alone to generate robust, repeatable microfluidic channels. This technique utilizes poly(methyl methacrylate) (PMMA) optical fiber (500 μ m diameter encased in, and subsequently removed from, PDMS in order to form microchannels that mimic optical fiber can be made easily to create different geometries found in the environment of the microvasculature such as stenoses, aneurysms, and bifurcations (Fig. 3B). This technique represents a significant benefit over photolithography because it obviates the need for expensive microfabrication equipment and experience. Furthermore, the resultant structures have round lumens, a feature which is atypical of microfluidic devices and is more physiologically accurate[29, 30]. It is important to note that the choice of substrate material used and the microvascular geometries generated by these non-traditional fabrication processes is crucial to the success of these devices, as substrate mechanical properties and geometry-mediated fluid shear stress experienced by endothelial cells have been shown to significantly impact surface protein expression as well as permeability of the endothelial monolayer in the context of microfluidics[28, 31, 32].

Endothelialization techniques within microfluidic devices

While creating physical channels is a crucial step in the development of *in vitro* microvascular models, these models must seamlessly incorporate the biological components of blood vessels found *in vivo* to accurately recapitulate the microvasculature. Endothelialization is the key element of any *in vitro* microfluidic vasculature model. Endothelialization refers to the process of lining the microchannel lumen with a 3-dimensional monolayer of endothelial cells. Endothelial cells line the inner surface of blood vessels and play a key role in the barrier function of the microvasculature. Additionally, endothelial cells are a primary contributor to the cellular interactions that occur within the microvasculature [33–35]. Endothelialization of microfluidic channels typically involves the deposition of an extracellular matrix (ECM) protein, along the inner surface of the microchannels to provide the endothelial cells with a basement layer to adhere to [36, 37]. The primary ECM proteins collagen, fibronectin, and laminin have all been used to promote endothelial attachment and healthy endothelial phenotype in microfluidic devices fabricated from multiple substrates (e.g. Polystyrene and PDMS) [38, 39]. After the basement ECM layer is deposited within the microchannels, endothelial cells are perfused through the microchannels, where they are allowed to adhere to, and spread across the microchannel walls. The cells are cultured until they spread and form a monolayer uniformly across the entire inner surface of the microchannels.

Several unique approaches have been developed to endothelialise microfluidic devices. Rotational seeding was utilized by Mannino *et al.* to endothelialize larger microfluidic devices (500+ μm diameter) [28]. Typically, in microfluidic systems, gravity plays a smaller role in endothelial spreading than capillary action (i.e. cells will spread in all directions within channels regardless of orientation with respect to gravity) [40]. However, in larger microfluidic devices (on the order of 500 μm diameter channels) gravity plays a larger role, and rotation about the central axis of the microchannel becomes crucial to ensure successful seeding of endothelial cells and formation of the endothelial monolayer. Hewes *et al.* reports a technique in which an inkjet printer was modified to bioprint free-standing micro vessels by continuously printing endothelial cell-laden alginate drops into a cross-linker bath in a circular pattern [41]. This technique represents a novel microfluidic fabrication method, as well as a novel endothelialization technique, as the endothelial cells required to recapitulate the microvasculature are already present in the structural material used to create the microfluidic device.

Applications of endothelialized microfluidics

Endothelialized microfluidic techniques have a wide variety of applications in biomedical engineering and medicine. In addition to the examples previously presented, endothelialized microfluidic technology has been used to study a variety of cellular interactions that occur in the microvasculature, such as leukocyte-endothelial and RBC-endothelial interactions *in vitro* [42, 43]. Furthermore, endothelialized microfluidic technology has been utilized to investigate microvascular phenomena implicated in diverse pathologies. Jain *et al.* report a novel microfluidic device containing a chemically preserved endothelium that can be used to evaluate platelet aggregation and thrombosis under different physiological and

pharmacological conditions after prolonged storage[44]. The ability to successfully evaluate thrombosis after prolonged storage potentially allows this technology to function in point-of-care settings. Furthermore, endothelial monolayers have been developed within ECM-based hydrogels to study a variety of microvascular processes. In order to study the spatiotemporal effects of chemotherapy delivery within a tumor, “do-it-yourself” endothelialized microfluidics have been utilized by fabricating a micro vessel within a tumor cell laden hydrogel (Fig. 4A–C) [45]. Chemotherapy can then be perfused through this “do-it-yourself” tumor-on-a-chip and the effects of the drug on the cancer cells within the model can be visualized in real time, something that was previously impossible with typical *in vivo* tumor models. Additionally, Bischel et al. report the development of a hydrogel-based microfluidic device that can be used to study angiogenesis *in vitro*, enabling the creation of new microchannels within microfluidic devices as well as the observation of this process (Fig. 4D–E) [46].

The composition of the endothelial monolayer may also be varied in order to study the vasculature of specific regions in the body. In this case, endothelial cells may be co-cultured with organ-specific cells to investigate organ-specific microvascular cellular interactions. Chonan *et al.* report a microfluidic system in which glioma initiating cells are co-cultured with endothelial cells in order to study the invasive properties of glioblastoma[47]. Wang et al. and Pradhakarpanian et al. report co-cultured microfluidic systems that employ co-culture to simulate the highly selective barrier function of the blood brain barrier (BBB) [48, 49]. Jain *et al.* have recently demonstrated co-culture of vascular lumen and alveolar epithelial cells under whole blood perfusion, and shown organ-level responses to pulmonary injury, vascular inflammation and thrombus formation [50]. As these studies indicate, co-culture of endothelial cells with organ specific cells alters endothelial function to more accurately recapitulate the organ of interest. For example, co-culture of endothelial cells with alveolar epithelial cells as well as astrocytes and pericytes had a significant impact on the permeability of the endothelial monolayer[48, 50]. When co-cultured with astrocytes and pericytes, endothelial permeability decreased, due to the fact that these cell types maintain the highly impermeable function of the blood-brain barrier[48]. When co-cultured with alveolar epithelial cells in the presence of lipopolysaccharide endotoxin, endothelial permeability increased due to activation of the epithelial cells and subsequent interaction with the endothelium rather than interaction with the endothelium alone[50]. Overall, as the pathophysiology of many disease states profoundly impacts the vasculature, these models serve as key test beds for elucidating the complex role of the microvasculature in disease.

Conclusion and Future Directions

Endothelialized microfluidics offer advantages over traditional *in vivo* and *in vitro* approaches to studying the microvasculature, including the ability to isolate the system of interest from the variability and confounding factors found *in vivo*, as well as the ability to tightly control the geometry of the desired system. These methods have recently been adapted for a myriad of applications in the microvascular space, by utilizing cutting edge advances in microfabrication technology. While traditional photolithography-based approaches to developing these microfluidic models offer the ability to accurately and repeatably generate channels with tightly controlled geometries, they suffer from the time,

training, cost, and equipment requirements necessary to develop them. In order to minimize the impact of these drawbacks, non-traditional fabrication techniques utilizing diverse tools such as 3D-printing, electrospinning, inkjet printing, and “do-it-yourself” replica molding have been used to generate endothelialized microfluidic. While these techniques do not require specialized cleanroom equipment and training like traditional photolithography, they are not without drawbacks. These techniques also come with their own associated costs and specialized equipment, and suffer from channel resolution and repeatability issues with respect to traditional photolithography. Overall, these methods represent a crucial tool in improving our understanding of microvascular processes and phenomena.

Going forward, endothelialized microfluidic technology is being adapted to address a wide range of problems. As fabrication methods used to develop these technologies improve, fabrication of more complex vascular geometries (e.g. complex tortuosity of blood vessels found *in vivo*) will be enabled[51]. Fabrication of complex vascular geometries enables more accurate recapitulation of the *in vivo* environment, which further enables more physiologically relevant research. Furthermore, the increased sophistication of fabrication techniques may enable microfluidic advances into the field of personalized medicine. For example, endothelialized microfluidic techniques (e.g. 3D printing) may be used in conjunction with clinical imaging (e.g. computed tomography, magnetic resonance imaging, and angiography) to develop patient specific microvascular models which can then be used as a test-bed for potential therapies[52, 53]. Additionally, these technologies may be personalized via culture with patient-derived cells in order to study the patient-specific impact of treatment approaches[54]. Finally, the addition of multi-cell co-cultures increases the number of vascular environments and pathophysiology that may be studied using these techniques[55, 56]. Overall, culturing the inner surface of microfluidic devices with endothelial cells has enabled researchers to observe and investigate microvascular phenomena which were previously inaccessible via traditional *in vivo* and *in vitro* models. As this field continues to grow and mature using sophisticated, yet non-traditional, fabrication techniques, researchers will have unparalleled access to investigate disease pathophysiology and test potential clinical therapies *in vitro*.

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(•) – of special interest (••) – of outstanding interest

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Highlights

- Microfluidics can be endothelialized to recapitulate the microvasculature *in vitro*.
- Fabrication techniques advances have increased the accessibility of microfluidics.
- Varied endothelialization techniques enable the study of a multiple diseases.
- These techniques have exciting implications in personalized medicine.

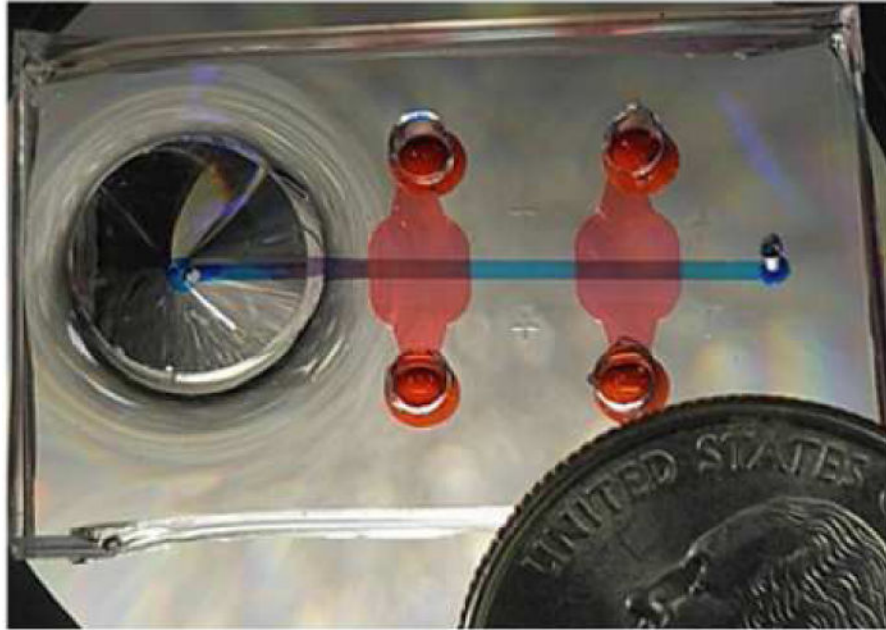


Fig. 1. Top view of a microfluidic device filled with dye to accentuate channels
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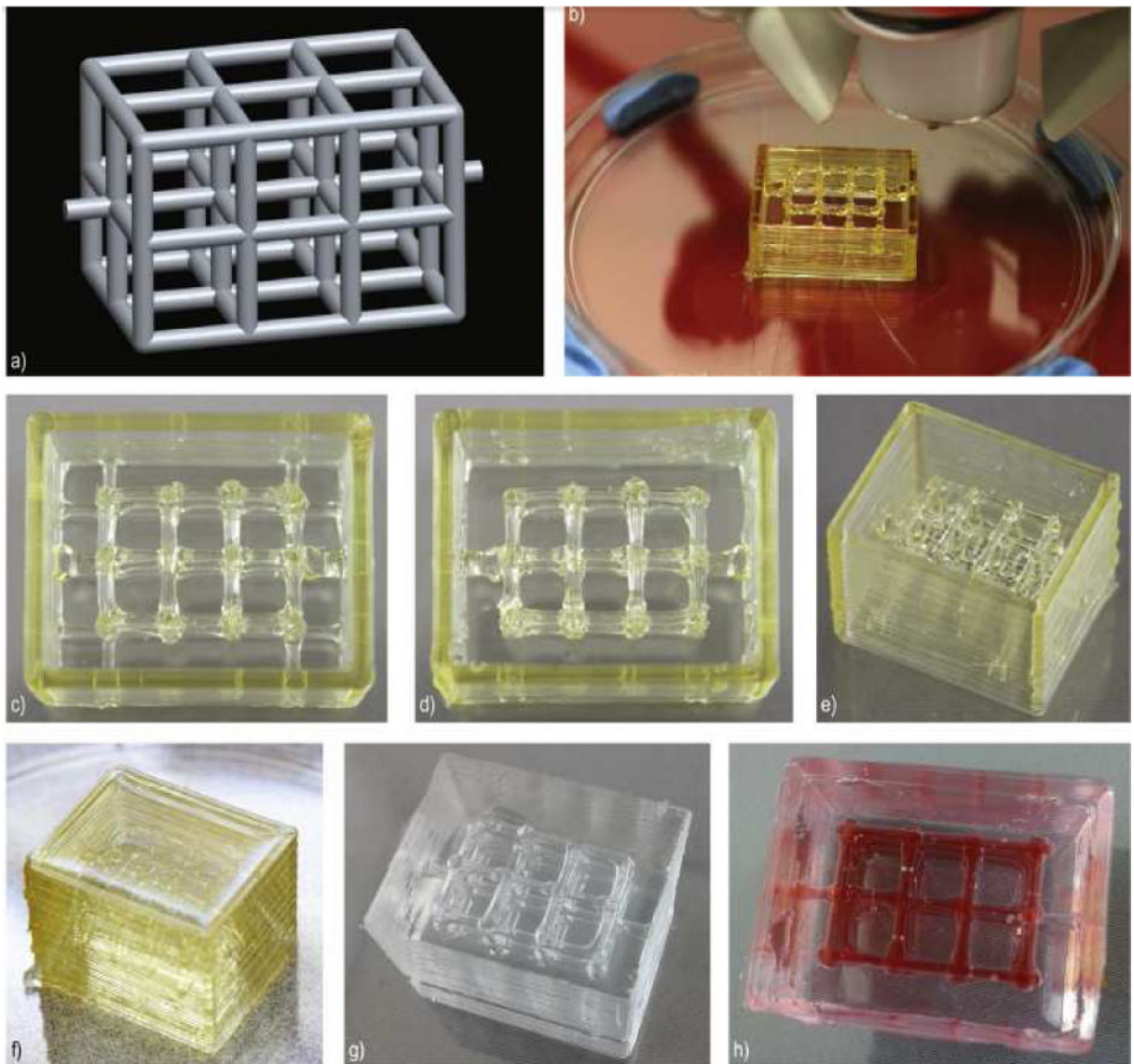


Fig. 2. Production of a microfluidic device using 3D printing of sugar glass
a) CAD model of the desired microfluidic channel. **b)** Device in the process of printing. **c–e)** Completed view of the sugar glass structure. **f)** Sugar glass structure after casting in PDMS **g)** microfluidic chambers after casting with PDMS and dissolution of sugar glass channels. **h)** Red Dye added for channel visualization. Image reproduced, with permission, from reference 25.

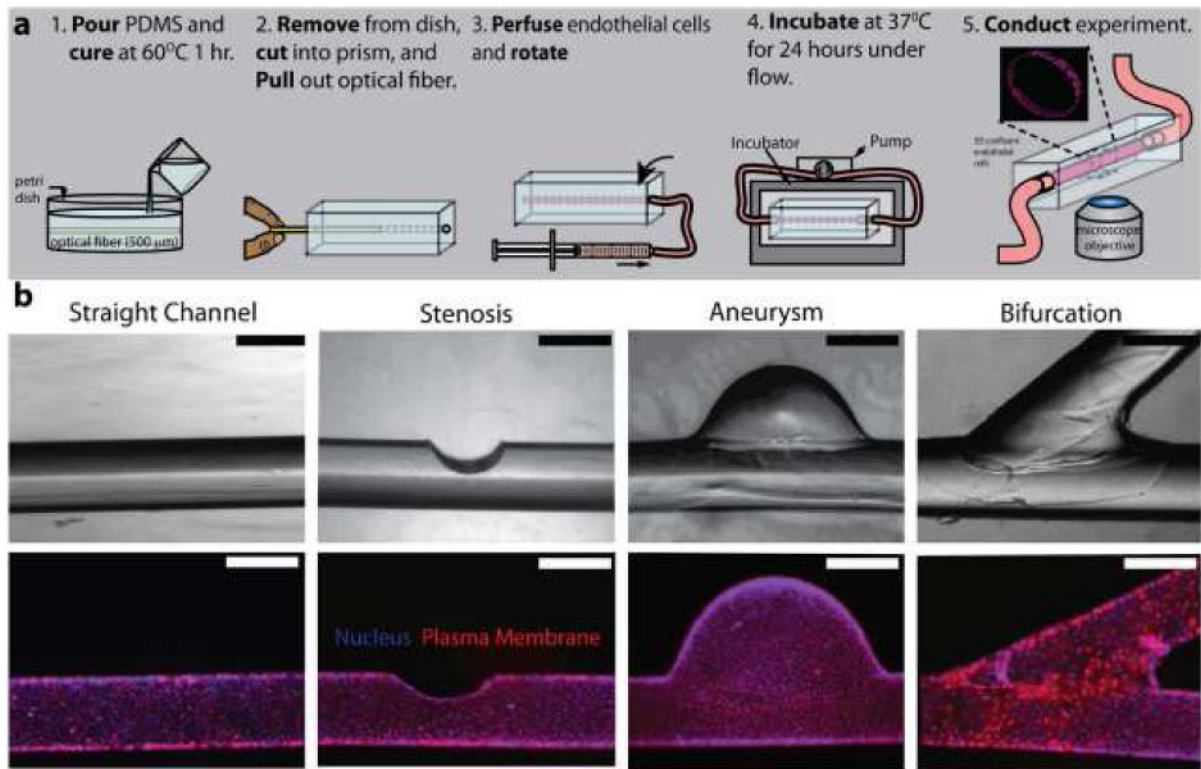


Fig. 3. Endothelialized microfluidics can be developed using off-the-shelf laboratory materials
a) Fabrication process flow of this “do-it-yourself” endothelialized microfluidic device. **b)** Different microvascular geometries created via slight alterations in the fabrication protocol. Scale bars represent 500 μm . Image reproduced, with permission, from reference 28.

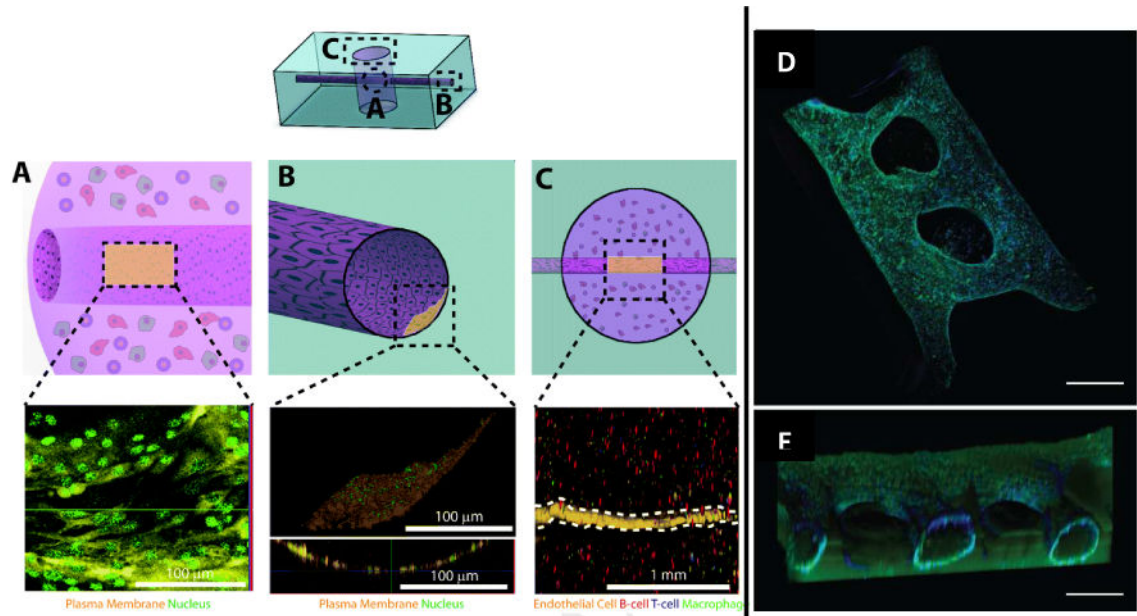


Fig. 4. Vascular networks can be generated within hydrogels

A–C) Endothelial cells can be successfully cultured within microchannels traversing a tumor cell-laden hydrogel. Image reproduced, with permission, from reference 45 D–E) In vitro angiogenesis assay featuring endothelial channels which are encouraged to invade the surrounding hydrogel. Scale bars represent 500 μm. Image reproduced, with permission, from reference 46.