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Microfluidic approaches for engineering vasculature

Joe Tien[1,2]

Recent studies have validated a vascularization strategy that relies on microfluidic networks within biomaterials as templates to guide the formation of perfused vessels. This review discusses methods to form and vascularize microfluidic materials, physical principles that underlie stable vascularization, and computational models that seek to optimize the microfluidic design.

Addresses
[1] Department of Biomedical Engineering, Boston University, Boston, MA, United States
[2] Division of Materials Science and Engineering, Boston University, Brookline, MA, United States

Corresponding authors: Tien, Joe (jtien@bu.edu)

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Introduction

Vascularization of biomaterials — whether for envisioned applications in tissue engineering or for use in 'organ-on-a-chip' devices — remains a challenging problem, as a quick glance at many of the reviews in this issue reveals. Traditional methods of vascularization, based on the controlled release of angiogenic factors or on the self-organization of vascular cells into open tubes, have successfully elicited the growth of durable, functional vascular networks *in vitro* and *in vivo* [1–4]. Nevertheless, these methods all require at least three days for generation of a perfused vascular network; this waiting period may be a fundamental limit to vascularization strategies that rely in part on biologically-driven tubulogenesis and anastomosis. In addition, these methods cannot easily control the number and placement of vessels within a biomaterial, a goal that may be especially desirable in vascularized tissue arrays for high-throughput screening. Formation of networks on a faster time-scale and with better spatial control requires new strategies that can replace biological processes by other ones.

The geometric similarity between microvascular networks *in vivo* and many of the microfluidic networks that can be routinely created in the laboratory suggests that a microfluidic approach may be well-suited for creating vasculature. In particular, the presence of preexisting open channels within microfluidic materials can potentially eliminate the need for self-organized tubulogenesis and anastomosis, by providing a template that forces the growth of vascular cells into the desired open tubes and networks (Figure 1a). With a one-to-one scaling between the microfluidic channels and the subsequent microvessels, precise control over the sizes and locations of vessels may become possible.

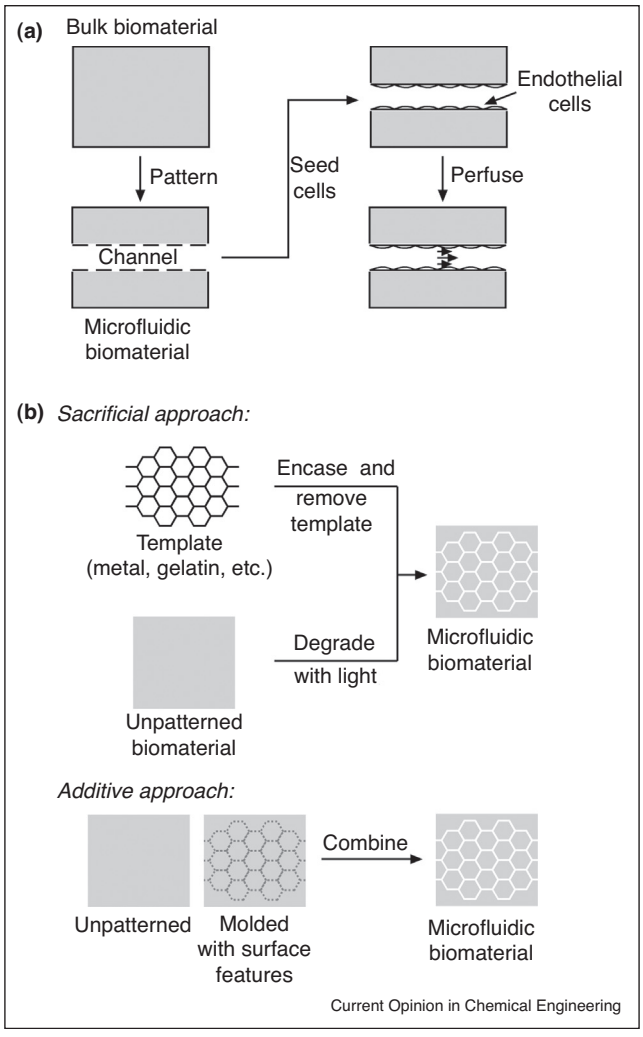
This review highlights recent progress in the realization of this microfluidic strategy for vascularizing biomaterials, and discusses possible future directions. It does not cover the much larger literature on vascularizing silicone (PDMS)-based microfluidic devices [5,6]. For a review of methods that generate vascular networks in microscale bulk gels, I refer the reader to the article by George in this issue.

Microfluidic vascularization of biomaterials

Several additive and subtractive methods have been developed to construct and vascularize microfluidic networks directly within biomaterials (Figure 1b) [6]. 'Vascularization' in these methods, with rare exception, refers only to the formation of an endothelial lining on the channels. Most of these methods focus on patterning hydrogels, both natural (e.g. collagens, fibrin) and synthetic [e.g. polyethylene glycol (PEG)]. In 2006, my colleagues and I described a simple process for generating single microvessels in extracellular matrix (ECM) gels [7]; this process uses a thin cylindrical rod as a template. Because the process relies on physical removal of the rod to generate a channel within the gel, it can be extended to a wide variety of materials, including silk and photocurable polymers, as recent studies have shown [8,9]. Seeding endothelial cells as a suspension into the channels is straightforward, as long as the diameter of the channels is above ~50 μm; cells tend to clog narrower channels [7]. Given that ECM gels are outstanding substrata for cell adhesion, spreading, and growth, it is not surprising that seeded cells attach to the surface of the channels and proliferate to form open, tubular monolayers.

Recent work has extended this single-channel process to form microfluidic configurations of greater complexity. For instance, it is possible to incorporate empty channels next to the endothelial tubes by using multiple rods as templates in a single scaffold (Figure 2a). These channels have been used to independently modulate the fluid pressures within the vessel and scaffold, thereby enabling the generation of lymphatic-like drainage [10•]. They have also been used to control the composition of the

Figure 1



Microfluidic strategy of vascularization. **(a)** Patterned biomaterials serve as templates that guide the formation of vessels; non-vascular cells can be incorporated during the patterning step or after vascularization. Perfusion can follow immediately after vascularization. **(b)** Examples of different techniques to form microfluidic scaffolds.

interstitial fluid, in particular, to generate gradients of cytokines for *in vitro* studies of angiogenesis [11].

Microfluidic biomaterials that contain interconnected networks rather than disjoint channels have required fabrication methods based on photolithographic and soft lithographic processing. Additive photopatterning of synthetic PEG gels, in which exposure to light induces the formation of a gel, is now well-established [12,13]. Recent studies have also shown that subtractive processing of synthetic gels is possible, through incorporation of photolabile bonds within the gel [14]. For these materials, the advantage of well-defined chemistries that tailor the photosensitivity is tempered by the need to incorporate adhesion groups into the resulting material to ensure that endothelial cells can attach to the channels. In principle, direct photoablation of ECM gels to form channels is also possible, although only the generation of internal cavities has been described [15].

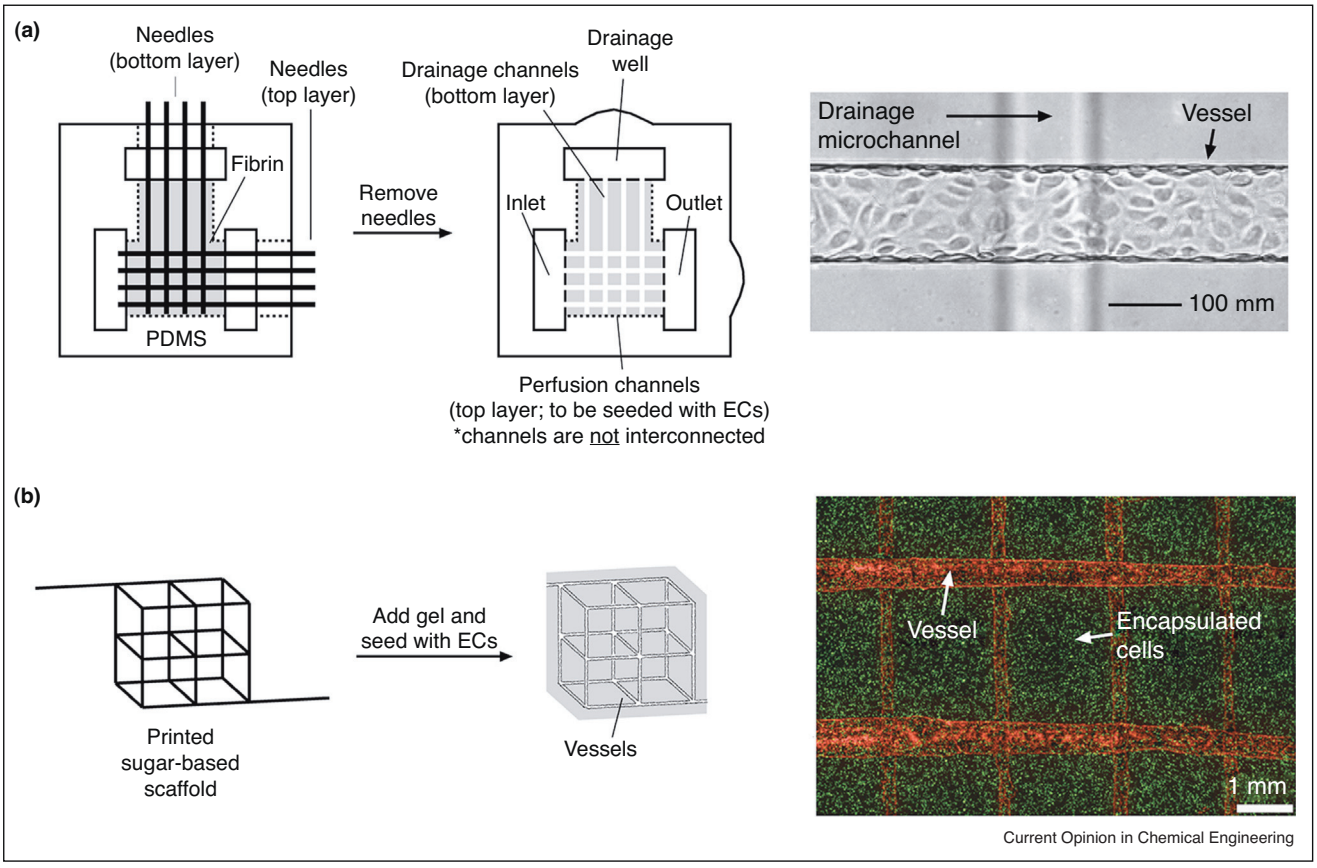
For patterning interconnected networks into ECM gels, soft lithography has emerged as the method-of-choice, and recent studies have taken advantage of the gentle, physical nature of micromolding to generate such networks. Driven by the finding that separately molded alginate gels could be fused into a single entity with the molded features defining microfluidic networks [16], several studies have shown that a similar additive approach can be used to form microfluidic networks within ECM gels [17,18•]. Alternatively, a micromolded mesh of gelatin can serve as a sacrificial template around which ECM gels are formed [19]. An interesting pair of studies by Chen and colleagues and by Lewis and colleagues recently showed that direct writing of concentrated sugar or Pluronic solutions can generate free-standing, three-dimensional sacrificial templates for the formation of large-scale, complex networks within a variety of natural and synthetic hydrogels (Figure 2b) [20•,21]. 'Viscous fingering' of a liquid collagen gel has also been used to generate channels and networks [22]. In these studies, the inherently adhesive nature of the ECM induced formation of open, interconnected endothelial networks that followed the design of the original microfluidic patterns.

Taken together, these studies demonstrate the remarkable diversity of microfluidic structures and biomaterials that can now be vascularized. As originally envisioned, the unique ability of microfluidic materials to support fluid flow has provided a means to distribute endothelial cells throughout the channels and to provide perfusion after seeding.

Physical principles of microfluidic vascularization

Although microfluidic channels have successfully directed the initial formation of open vascular tubes and networks, less is known about the long-term stability of these vessels. As noted above, most studies to date have relied solely on endothelial cells to generate the vascular lining. Numerous studies *in vivo* have established that mural cells, such as pericytes and smooth muscle cells, are required to ensure survival and quiescence of the endothelium. Perhaps not surprisingly, the stability of vessels in microfluidic materials appears to be highly sensitive to the perfusion conditions. In the absence of any special treatment, these vessels can degrade over the span of weeks, often sloughing off as a sheet or denuding as individual cells; in such cases, the perfusion rate can decrease substantially.

Figure 2



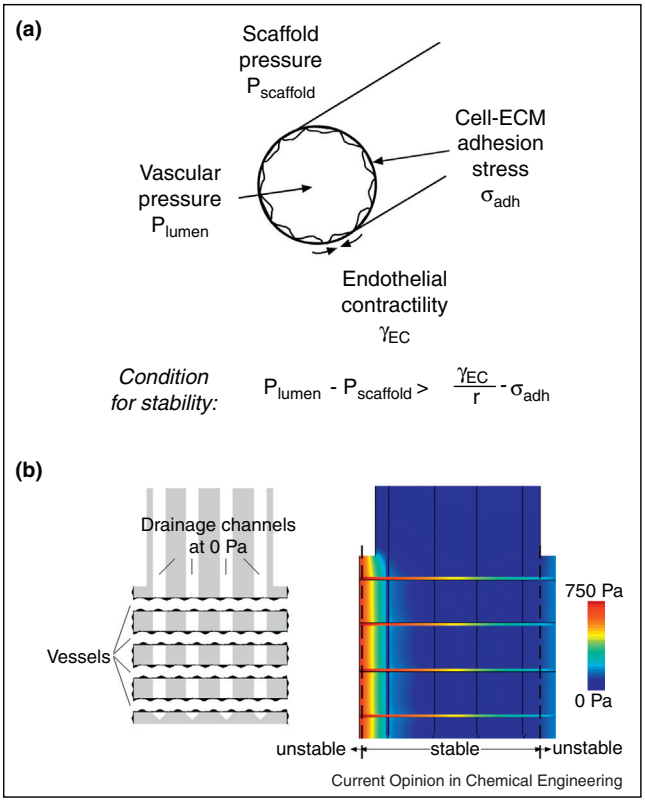
Examples of microfluidic configurations that allow tailoring of vascular geometries. **(a)** Multiplexed vessels and bare channels in fibrin gel; the latter provided a lymphatic-like drainage function [10•]. **(b)** Vascular network in fibrin gel that was formed using printed sugar as a sacrificial material [20•]. Reprinted with permission from John Wiley and Sons and the Nature Publishing Group.

Recent studies have shown that several perfusion-related signals can independently stabilize the vessels against degradation. These signals include: dibutyryl cyclic AMP, high-molecular-weight polymers (such as dextran and hydroxyethyl starch), shear stress, and transmural pressure [10•,23–25]. Several of these signals also induce a tightening of the endothelial barrier, and these findings suggest that the simultaneous changes in vascular permeability and stability are not mere coincidence. Computational models of the pressure distribution in the presence of vascular leaks found that leaks of a sufficient size and density could cause the transmural pressure to diminish as fluid escapes from a high-pressure vessel to a low-pressure scaffold [23].

These findings support a model of vascular stability in which a delicate balance of stabilizing and destabilizing mechanical stresses determines whether or not the endothelium will remain adherent to the scaffold (Figure 3a). Stabilizing stresses consist of the transmural pressure and the effective adhesion stress from integrin-ECM binding. Destabilizing stress results solely from the contractility of the endothelium. According to this model, molecules that relax the endothelium (such as dibutyryl cyclic AMP) will promote vascular stability. It also predicts that manipulations that increase transmural pressure will stabilize vessels. These manipulations can be direct (as in the use of artificial lymphatic-like channels to lower the scaffold fluid pressure and thereby increase transmural pressure) or indirect (through changes in vascular permeability, as described above). Recent studies support these predictions and imply that vascular stability is primarily a mechanical, rather than biochemical, issue [10•].

This mechanical model of vascular stability further predicts that increasing the adhesion strength between endothelium and scaffold should be beneficial. Consideration of the energies of delamination suggests that the adhesion stress scales as $\sqrt{G\Gamma/r}$, where G is the shear modulus of the scaffold, Γ is the adhesive surface energy between cells and scaffold, and r is the vessel radius (JW

Figure 3



Physical modeling of the mechanical state of a vascularized microfluidic scaffold. **(a)** Free-body diagram of a vessel under flow, with vascular stability determined by the balance between transmural pressure and the stresses induced by endothelial cell-ECM adhesion and endothelial cell contractility [37]. **(b)** Computed vascular and scaffold fluid pressures, for a vascular design that consists of arrays of vessels and drainage channels [10•]. The pressure fields allowed prediction of where vessels would be stable. Reprinted with permission from John Wiley and Sons.

Hutchinson, personal communication). Thus, stiffening scaffolds should promote vascular stability, and my colleagues and I have very recently found this prediction to be correct [26•].

Despite the ability of a physical theory to explain changes in vascular stability in microfluidic scaffolds, not all studies are in concordance. Stroock and coworkers noted that vessels in their collagen scaffolds did not detach from the channels, despite the presence of low flow and a leaky vascular barrier [18•]. They suggested that various differences in endothelial cell type and perfusate composition may account for the differences in vascular stability. The unsteady flow in their study versus the steady flows in other studies may also partially account for the differences. A common theme appears to be that actively sprouting endothelium is more resistant to delamination, perhaps because the sprouts serve to 'anchor' the endothelium firmly into the scaffolds [11,18•,25]. More detailed studies, especially ones that can measure the forces exerted by quiescent and angiogenic endothelium in microfluidic scaffolds, will be needed to clarify these issues.

Optimal design of the microfluidic network

With methods for making microfluidic biomaterials now at a relatively mature stage of development, it is appropriate to ask if particular microfluidic designs are better than others for a given application. Several recent studies have used patterned channels to perfuse a cell-laden tissue [9,20•,27–29], but the cell densities have been modest; in almost all of these studies, the channels were not vascularized (i.e. they remained barren). To generate tissues of *in vivo*-like cell densities, optimization of the network geometries will be necessary to ensure that the appropriate transport capacities exist without excessive vascular resistance or volume.

Computational models are well-suited for such optimization of designs, since the underlying transport equations are known. For optimization, it is necessary to define a minimization (or maximization) function and one or more constraints. Most of the studies in this area have focused on minimization of perfusion parameters, such as the mechanical work of perfusion, for a given geometric constraint [30–32]. Not surprisingly, these studies found that analogs of Murray's Law (which describes the relationship between proximal and distal vascular radii at a junction, and minimizes a transport cost function) apply to optimal microfluidic design. Other studies have considered the efficiency of extravascular transport [33,34]; for computational ease, these studies examined perfusion through parallel arrays. A recent study determined the vascular geometry that minimized the volume fraction occupied by vessels, while maintaining the extravascular oxygen concentration above a given value [34]. This study found that the optimal diameters and spacings are on the order of 100 μm, and provided analytical expressions for these optima. Similar models can be used to obtain optimal designs that maintain vascular stability by maximizing transmural pressure (Figure 3b) [35].

A separate issue relates to optimal design of the cross-sectional shape of channels within microfluidic biomaterials. Rectangular cross-sections are often more convenient to form than rounded ones, but may lead to non-uniform shear stress across the cross-section. Studies by Stroock, Fischbach, and colleagues have suggested that rectangular features may remain so [36], or may become rounded over time [18•]. Whether sharp angles adversely affect vascular function in microfluidic scaffolds remains unclear.

Conclusions

The last few years have provided several examples of the unique potential of microfluidic biomaterials in vascularization. These materials can support perfusion at all

times, and thus can potentially enable the maintenance of large, densely cellularized constructs before and after implantation. Microfluidic materials also allow routine control over vascular geometry, which raises the possibility of rational scaffold design for a desired vascular outcome. The microfluidic approach to vascularization has emerged as a viable alternative to growth factor-dependent and self-organization-dependent strategies, and combination of these vascularization strategies will likely yield even better outcomes.

Acknowledgment

I thank John Hutchinson for discussing the application of thin-film mechanics to endothelial delamination.

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- •• of outstanding interest

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