



## Engineering the multiscale complexity of vascular networks

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**Abstract** | The survival of vertebrate organisms depends on highly regulated delivery of oxygen and nutrients through vascular networks that pervade nearly all tissues in the body. Dysregulation of these vascular networks is implicated in many common human diseases such as hypertension, coronary artery disease, diabetes and cancer. Therefore, engineers have sought to create vascular networks within engineered tissues for applications such as regenerative therapies, human disease modelling and pharmacological testing. Yet engineering vascular networks has historically remained difficult, owing to both incomplete understanding of vascular structure and technical limitations for vascular fabrication. This Review highlights the materials advances that have enabled transformative progress in vascular engineering by ushering in new tools for both visualizing and building vasculature. New methods such as bioprinting, organoids and microfluidic systems are discussed, which have enabled the fabrication of 3D vascular topologies at a cellular scale with lumen perfusion. These approaches to vascular engineering are categorized into technology-driven and nature-driven approaches. Finally, the remaining knowledge gaps, emerging frontiers and opportunities for this field are highlighted, including the steps required to replicate the multiscale complexity of vascular networks found in nature.

Evolution of complex multicellular organisms requires the co-emergence of transport systems to support the metabolic needs of cells across a diverse range of organs, body types and body sizes. In vertebrates, these systems are typically characterized by a closed vascular system consisting of conduits for collection and distribution, connected by a central propulsive pump<sup>1</sup>. The complexity of vascular networks varies across and within organisms to meet the different metabolic needs of the tissues, with the most complex being hierarchical networks of branching tubular structures. In addition to delivering oxygen and blood, the vasculature regulates blood coagulation, transportation of inflammatory cells and critical cellular signalling interactions in an organ-specific manner<sup>2</sup>; thus, the vasculature plays diverse roles critical for life (BOX 1).

Vascular pathology is implicated in many human diseases, with cardiovascular disease being the top cause of death and disability in the United States<sup>3</sup>. Many cardiovascular diseases disproportionately affect groups of divergent ancestries, ethnicities and sociocultural backgrounds, with serious results<sup>4,5</sup>. For example, in the United States, African Americans are more likely than white Americans to have cardiovascular complications, such as strokes, congestive heart failure or end-stage renal disease, and experience lower life expectancy<sup>6,7</sup>.

To address the substantial clinical burden of cardiovascular disease, biomedical researchers and engineers began combining vascular biology and materials science to engineer blood vessels as early as the 1960s. The first wave of vascular engineering technologies focused on fabricating single blood vessels as vascular conduits for reconstructing or bypassing vascular occlusions or aneurysms<sup>8–12</sup>. The earliest such conduits were composed of synthetic membranes (made of materials such as silicone, collagen and polyethylene terephthalate) seeded with cells<sup>8,11</sup>, with more recent approaches diversifying to include wrapping cell sheets around a mandrel<sup>13,14</sup>, decellularizing an engineered construct after conditioning<sup>15</sup>, or using 3D printing<sup>16</sup>. Several such conduits have progressed to clinical trials, one of which has now been implanted as a haemodialysis conduit in over 240 patients with end-stage renal disease and is currently in phase III clinical trials<sup>17</sup>, as reviewed extensively elsewhere<sup>10,12</sup>.

Fabricating a single vascular conduit has achieved some success; however, in the past few years, replicating vascular networks — that is, the entire vascular hierarchy, from large-scale (~6 mm) to small-scale (<0.1 mm) vessels — has become a key challenge in the field of tissue engineering. This challenge came to the forefront as efforts to build engineered tissues that reproduce the

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**Box 1 | Emergence and general features of vascular networks**

During embryonic development, vascular networks emerge when clusters of angioblasts form blood islands, differentiate into endothelial cells and develop interior channels (lumens), then anastomose — that is, they become linked — to build an interconnected branching network called a primitive vascular plexus<sup>227,213</sup>. This coalescence and self-assembly of endothelial cells is known as vasculogenesis. The primitive vascular plexus then remodels to meet the metabolic needs of a given tissue, with new vessels sprouting from existing ones in tissues with high oxygen demand via angiogenesis<sup>214</sup>. The vascular plexus continues remodelling to make the network more efficient, with some vessels merging and/or enlarging, and some diminishing. Subsequent molecular signals and haemodynamic forces cooperatively interact to generate the arguably most prominent feature of mature vascular networks: vessel hierarchy at both the architectural and cellular levels<sup>215</sup> (FIG. 4a).

After its emergence, vessel hierarchy in highly metabolic solid tissues and organs has traditionally been divided into large vessels (arteries and veins, ~0.1–10.0 mm) and smaller vessels of the microvascular circulation (arterioles, venules and capillaries, ~8–100 µm)<sup>216</sup>. In general, large arteries and veins are responsible for transporting blood to and from the heart and are characterized by three structurally unique layers — an endothelialized inner layer, a muscular mid-layer and a collagenous outer (support) layer. Blood then enters the microvascular circulation in smaller arteries called arterioles, which regulate vascular resistance. Similar to arteries, arterioles also have three layers, albeit with lesser thickness. Vascular resistance is regulated by the muscular medial layer of arteries and arterioles, which constricts and dilates to match the rate of oxygen delivery with tissue demand<sup>217</sup>. Following the arterioles, the blood moves into capillaries where gas exchange with surrounding cells can occur through the single-layer endothelial wall. The oxygen-poor and carbon-dioxide-rich capillary blood then travels into small veins called venules, which are similar to arterioles, but have thinner walls and less elastic tissue. Finally, the blood moves into larger veins and is ultimately brought back to the heart<sup>217</sup>.

features and functions of native tissues became concerted in the 1980s to the present<sup>418–20</sup>. The field had early clinical success in replicating thin tissues such as skin; however, the construction of highly metabolic solid organs such as the liver, heart and kidney remains limited by insufficient nutrient and oxygen transport in thick tissues. This paradox is particularly important, as the largest impact on human health would be achieved by producing solid organs for clinical applications such as therapeutic treatment of end-stage organ disease<sup>21</sup>. Inspired by nature's solution of incorporating vascular networks into solid organs, biologists and engineers alike are now working intensely to reconstruct analogous engineered vascular networks within engineered tissues for solid organ repair and replacement.

This Review discusses approaches to fabricating an entire vascular network capable of sustaining engineered tissues and organs. We summarize how our knowledge of vascular network architecture, composition and heterogeneity has evolved, and how materials advances are leading to increasingly refined multiscale and multidimensional

vascular blueprints. We focus on how tissue engineers have harnessed knowledge of vascular biology to come closer to fabricating multiscale vascular networks within engineered organs and show how many of the greatest leaps towards this goal have stemmed from creative integration of diverse fields, including materials science. The focus of this Review is on engineering-driven approaches to create vascular networks, which we define broadly as all non-natural network-forming methods. We also more briefly review nature-driven approaches for vascular network assembly and highlight emerging efforts to bridge engineering and nature-driven assembly modes. Finally, we articulate some of the remaining steps required for therapeutic organ vascularization to become a mainstream clinical reality.

**Building vascular networks**

Our increasingly detailed knowledge of vascular network architecture and composition has made it clear that replicating the structural and biological complexities of vascular networks in engineered organs and tissues may require the integration of diverse technologies and fields of expertise.

**Engineering-driven approaches**

**Microfabrication.** The digital revolution and the subsequent rise of the personal computer industry in the 1970s and 1980s brought with it the demand for technologies that were more powerful, faster and smaller. With this demand came the microfabrication industry; the need to fabricate tiny microscale components for silicon computer chips became mainstream almost overnight. This revolution had great implications for the field of tissue engineering<sup>22</sup>, which at the time was integrating materials engineering with cell biology, but still could not achieve intricate spatial patterning of features such as those in vascular networks. Methods to spatially arrange cells and materials at the micro- and nanoscale were needed. The first publications to adapt microfabrication techniques for patterning cells both on 2D substrates and in engineered tissues emerged in the late 1990s<sup>23–26</sup>. This work quickly captured the interest and imagination of the community, giving rise to the intersection of microfabrication and tissue engineering in the vascular engineering field.

Microfabrication methods enabled the creation of relatively complex perfusable microfluidic networks, with increasingly refined networks being developed up to the present day<sup>27–29</sup> (FIG. 1a). To create branching vessel-like networks, some investigators patterned photoresist on silicon wafers to create positive features of microchannel conduits and then generated a poly(dimethyl siloxane) (PDMS) mould with perfusable microchannels<sup>29</sup>. This approach enabled the replication of some features of vascular topology (connectivity and geometry of the vascular network); however, PDMS is a synthetic material that fails to provide the extracellular cues found in native vessel matrices and does not support the encapsulation of functional cells of a tissue (parenchymal cells) around the networks. Thus, subsequent methods instead created vascular patterns in PDMS, and in turn used these PDMS patterns as moulds to create micromoulded channels

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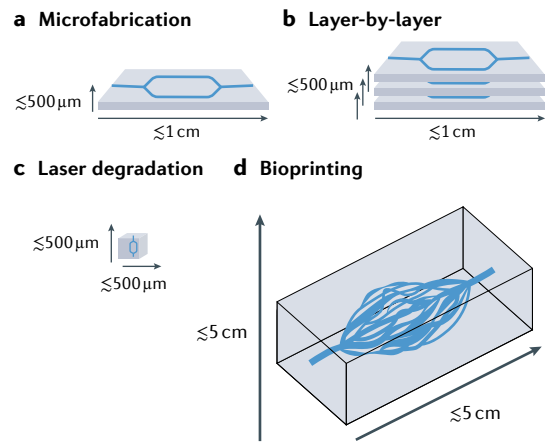
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within more biologically relevant materials<sup>30–32</sup>. For example, one group developed a multistep fabrication approach to incorporate a collagen-based microfluidic network within a perfusable chip<sup>30</sup>. The *in vitro* network of vessels formed by this approach was used to study vessel biology and disease, and was also incorporated into engineered tissues for *in vivo* therapeutic applications<sup>30,32</sup>.

One limitation inherent to using microfabrication for patterning is that the topological features used for moulding restrict patterns to a single 2D moulding plane. To overcome this limitation, layer-by-layer approaches were developed<sup>33–37</sup> (FIG. 1b). In these approaches, trenches or channels are typically first moulded in one layer, similar to standard microfabrication approaches (FIG. 1a). Subsequent layers are then stacked, aligned and laminated to form additional moulded layers in an iterative fashion (FIG. 1b). One such method used direct photolithography of a photopolymerizable hydrogel instead of indirectly moulding PDMS from a patterned photoresist<sup>38</sup>. Using this method, other groups assembled a cardiac tissue with an integrated vessel-like network that was surgically anastomosed by connecting the inlet and outlet channels to the femoral vein in rats<sup>39</sup>. Despite this and other important achievements, microfabrication and layer-by-layer assembly techniques remain slow and place substantial design constraints on the topologies and materials that can be used for fabrication, because patterning is performed on a planar substrate. Still, these microfabrication techniques continue to be useful, especially for *in vitro* studies of vessels and within the organ-on-a-chip field.

**Laser degradation and bioprinting.** To overcome the design constraints of microfabrication, other patterning methods have been developed that can volumetrically fabricate 3D vascular networks<sup>40</sup>. In one approach, a laser is rastered over a material to locally degrade vascular patterns within a bulk photodegradable hydrogel. Combined with multiphoton microscopy, this technique confines local degradation to a single point in 3D space, enabling the user to ‘write’ microchannel networks with complex and precise 3D features and curvature down to the capillary size scale in hydrogels<sup>40–43</sup> (FIG. 1c). This approach has already been used to fabricate channels as small as 5  $\mu\text{m}$  diameter, the scale of the smallest known blood vessels in the human body<sup>42</sup>. These channels were successfully seeded with endothelial cells through a photoablation-guided capillary ingrowth technique<sup>42</sup>. Although laser degradation is valuable for patterning relatively small regions of interest, generation of large vessels or scaled tissues with this technique alone remains prohibitively inefficient.

To address the need for scaling to create tissues of larger volume and size, 3D printing has garnered increasing attention because of its potential to rapidly fabricate volumetric vascular networks<sup>44–46</sup> (FIG. 1d). Early work in 3D printing to create engineered tissues (bioprinting) mixed cells within a viscous prepolymer, and printed both cells and materials through an extrusion nozzle to create tissue constructs<sup>10,47,48</sup>. An advantage of direct extrusion printing that uses cell-laden bioinks to assemble artificial tissues voxel by voxel is its compatibility with a variety of synthetic and natural materials<sup>49–52</sup>. However, this



**Fig. 1 | Engineering-driven approaches for vascular network fabrication.** **a** | Microfabrication enables the creation of complex perfusable microfluidic networks in a thin moulding plane by spatially arranging cells and materials at the microscale. **b** | Layer-by-layer approaches stack, align and laminate multiple microfabricated layers to increase tissue thickness compared with single-layer microfabricated networks. **c** | Laser degradation uses tools such as multiphoton microscopes to locally degrade photosensitive hydrogels, enabling the user to ‘write’ complex and precise 3D microchannel networks with lumens down to the capillary size scale. **d** | Bioprinting enables volumetric fabrication of vascular networks at greater scale and speed than laser degradation, albeit with lower resolution. Panel **d** adapted from REF.<sup>58</sup>, Springer Nature Limited.

technique has not yet led to the printing of refined vascular networks owing to limitations in both printing resolution and shear damage to cells as they are extruded. As the field of bioprinting matures, technological advances such as the ability to print with multiple materials simultaneously, development of natural and shear-thinning bioinks<sup>50,53</sup>, and improved methods for printing cells without damaging them<sup>54</sup> may allow engineers to replicate the architecture of the native vessel matrix more closely.

As a result of the early challenges faced when directly printing cells, strategies to print ‘sacrificial’ materials were developed to create vascular network architectures that are subsequently solidified and then embedded within a larger hydrogel<sup>54–57</sup>. This approach enables cells to be embedded in the larger encasing hydrogel, as opposed to in the printed network itself<sup>55</sup>. The sacrificial material is then dissolved to expose microfluidic vascular networks in the shape of the printed network, which can also later be seeded with cells<sup>57</sup>. Various sacrificial inks have been used for this approach, including carbohydrate-based glass<sup>57,58</sup>, the copolymer Pluronic F127 (REFS<sup>55,56</sup>) and gelatin<sup>54,59</sup>. Each of these materials offers tradeoffs in printability, print resolution and utility for fabricating complex 3D vascular topologies.

The limited print resolution of extrusion bioprinting has historically impeded its application for the construction of conduits smaller than large arterioles ( $\sim 200 \mu\text{m}$ ). However, a creative modification reversed the sacrificial printing process, in a technique called freeform reversible embedding of suspended hydrogels (FRESH), involving a bioink printed within a dissolvable

gelatin support bath. The second-generation version of this technology, FRESH 2.0, enabled printing of acellular collagen structures as small as 20  $\mu\text{m}$  in diameter<sup>60</sup>. This technique has been used to fabricate a perfusable multiscale vascular network to mimic coronary vasculature, with lumens as small as 100  $\mu\text{m}$  in diameter<sup>60</sup>. Although this was a considerable leap forward in the resolution of acellular extrusion bioprinting, direct printing of cells still requires substantially larger nozzle sizes and thus remains lower resolution (for example, approximately 10 times the diameter of a cell). Furthermore, a remaining challenge with extrusion printing for vascular network formation is that extrusion is most commonly performed by rastering across a volume, and thus is relatively slow, which makes it tedious to fabricate large networks. Although emerging technologies may ultimately address this constraint<sup>61</sup>, it currently necessitates tradeoffs between fabrication time and tissue scalability.

Stereolithography (SLA) printing offers an entirely different bioprinting approach that confers additional advantages for scalability<sup>62–64</sup>. By projecting an  $x$ - $y$  plane of light within a photopolymerizable hydrogel precursor, SLA printers rapidly construct volumes, since they print volumetric pixels (voxels) plane by plane, as opposed to voxel by voxel. The utility of SLA for bioprinting was initially constrained by the penetration of light through sequential printed layers, which prevented the printing of many details and topologies characteristic of volumetric vascular networks<sup>65</sup>. The discovery that a biocompatible food-safe dye could be added to the prepolymer bath to limit light penetration has enabled the printing of thousands of layers and fabrication of perfusable vascular networks with complex entangled topologies. SLA printing has been used to model the interconnected 3D architecture of a lung alveolus and the integrated branching tubular network of an engineered liver tissue, demonstrating the ability of this approach to recapitulate complex natural topologies<sup>62</sup>. Yet, despite considerable progress in the fabrication of vascular topologies, this approach currently remains limited to  $\sim 300 \mu\text{m}$  print resolution. Further innovations might overcome this limitation.

Advances in volumetric fabrication methods such as laser degradation and bioprinting have energized the community and brought us closer to the possibility of fabricating vascular networks within '3D-printed organs'. Although each technology still requires tradeoffs in terms of materials, resolution, print time or scalability, even in their current forms these technologies can be useful for organ-on-a-chip technologies. The rapid rate of progress in this field and the potential to combine different technologies make us optimistic that technological developments will lead to the fabrication of multiscale vascular networks in the future.

**Materials considerations.** The compatibility of the materials used in each engineering technology is an important consideration when fabricating vascular networks. These materials have historically been categorized by whether they are naturally derived or synthetic hydrogels, although notably the latest work focuses on developing new materials with both natural and synthetic elements. Accordingly, these categories represent the

far ends of a spectrum, between which lie a wide range of hybrid materials for fabrication.

Natural matrices such as collagen, fibrin, decellularized extracellular matrix (ECM) or Matrigel provide cell-adhesion sites, contain native signalling capabilities and can be degraded by cellular processes to permit the remodelling of the hydrogel. Microfabrication<sup>30,65</sup> and extrusion bioprinting<sup>60,66</sup> have been used to create perfusable vessels in some natural matrices, although the material properties define how compatible a given natural polymer is with each technology. For example, microfabrication techniques such as micromoulding necessitate that materials retain topographic features after the mould is removed, as well as during subsequent culture and under pressure or shear. Thus, stiffer materials (those with higher elastic moduli) are more commonly used for these applications (such as  $>10 \text{ mg ml}^{-1}$  fibrin or  $5\text{--}6 \text{ mg ml}^{-1}$  collagen). Similarly, the printability, or ability of a material to both be printed and retain the intended form, known as shape fidelity, affects the choice of material for extrusion printing. Material parameters that affect printability primarily include deformation and flow behaviour determined by a material's viscosity, elastic recovery and shear stress<sup>67,68</sup>. Physical properties such as contact angle and surface tension will also affect printability<sup>68,69</sup>. Many materials used in extrusion printing either naturally have, or are designed to have, shear-thinning properties, such that material viscosity decreases under shear stress as it travels through the printing needle.

Conversely, light-based patterning methods such as layer-by-layer photolithography, multiphoton laser ablation and SLA bioprinting require that materials are photoactive. Therefore, traditionally materials developed for use with these techniques were largely based on synthetic polymers. For example, the early photopatterning field used synthetic poly(ethylene glycol)-based materials with photo-crosslinkable moieties, such as poly(ethylene glycol) diacrylate (PEGDA)<sup>62–64</sup>. When exposed to light in the presence of a photoinitiator, these materials become covalently and physically crosslinked<sup>70–72</sup>. Both the choice and concentration of photoinitiator are critical to the material's cytocompatibility and cell survival<sup>73</sup>.

Although PEGDA and similar synthetic polymers are photoactive, they lack the bioactive signals provided by natural ECM and thus poorly support living cells in engineered vascular networks or the bulk tissue surrounding these networks. To address this limitation, materials scientists functionalized synthetic polymers to include some natural ECM features, which became some of the earliest hybrid biomaterials<sup>74</sup>. These features include the addition of enzyme-degradable linkages<sup>75</sup> and peptide moieties such as the Arg-Gly-Asp (RGD) cell-adhesion recognition signal<sup>76</sup>, the principal epitope found in the most abundant ECM molecules during embryonic vascular development<sup>77</sup>. Incorporating bioactive molecules within synthetic matrices aids the survival, functionality and remodelling of diverse cellular populations, including endothelial cells that line the blood vessels and mural cells that support them<sup>78</sup>.

Notably, natural materials have also been modified to incorporate non-natural control features associated with synthetic materials, such as photoactivity, to render

them compatible with various biofabrication strategies. For example, to equip the natural material gelatin with photoactivity, gelatin macromers can be synthetically modified with methacrylate functionalities to produce gelatin methacrylate (GelMA). When exposed to light in the presence of a photoinitiator, GelMA polymerizes to form a crosslinked hydrogel<sup>79</sup>.

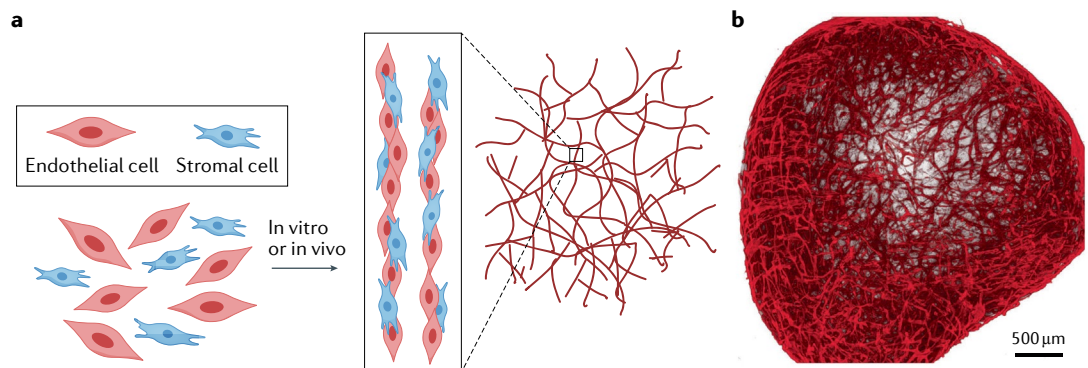
Innovative materials have been critical for many of the technological advances in engineering vascular networks. Often, such materials have been borrowed and creatively adapted for tissue engineering from other industries, such as the food industry. This includes the introduction of gelatin, which is produced by the partial breakdown of the triple-helical structure of collagen and was originally a by-product from the meat processing industry<sup>34,79–82</sup>. Other examples of materials coopted from the food industry include carbohydrate glasses (such as sugar formulations) for 3D printing sacrificial vascular networks<sup>57,58</sup> and the addition of food-safe dyes to hydrogel precursors to block the transmission of unwanted (out of plane) light in multilayer SLA printing<sup>62</sup>.

Despite these groundbreaking technological advances and the materials that have enabled them, engineering-driven technologies still fall short of replicating the composition of native vessels. Arguably one of the most important remaining challenges is the need to replicate vascular hierarchy at the cellular and molecular levels, not only the dimensional and architectural levels that have so far been the focus of most engineering-driven approaches. At present, most engineered vascular networks contain only a single population of endothelial cells that form a single confluent layer on the inner surface of the channels. Some groups have added stromal cells or pericytes to the matrix surrounding the vascular channels, but a perivascular coating that functions to control vascular tone has not yet been achieved<sup>83,84</sup>. We expect that new materials will continue to drive progress in engineering-driven construction of vascular networks. Meanwhile, to more closely replicate the cellular aspects of vascular networks in human tissues, others have taken an entirely different nature-driven approach.

### Nature-driven approaches

**Self-assembled vascular networks.** In contrast to engineering-driven methods, biology-driven approaches rely on the inherent capacity of cellular communities to undergo morphogenesis and self-assemble into vascular networks. This field was propelled by the observation in 1988 that endothelial cells cultured in a 3D laminin and collagen matrix have the capacity to self-assemble into vessel-like networks *in vitro*<sup>85</sup>. Since then, other matrices, including fibrin<sup>86</sup>, collagen<sup>87</sup> and hyaluronic acid<sup>88</sup> hydrogels, have been exploited to support the self-assembly of endothelial cells into vessel-like networks. Remarkably, this process can also be induced *in vivo* by seeding endothelial cells in natural, namely fibronectin and collagen<sup>89</sup>, or synthetic, namely poly(lactic-co-glycolic acid) (PLGA) and poly(L-lactic acid) (PLLA)<sup>90</sup>, hydrogel scaffolds implanted in immunodeficient mice. The resultant self-assembled endothelial networks anastomosed with host vessels and became perfused with blood<sup>89,90</sup>.

Although early studies demonstrated the potential power of self-assembly, vessels derived solely from endothelial cells were unstable and prone to regression over time<sup>91</sup>. Natural blood vessels are formed and stabilized through the association of smooth muscle cells or pericytes with the endothelium, and, similarly, perivascular support cells can stabilize implanted engineered vascular networks *in vivo*<sup>91,92</sup> (FIG. 2a). Co-seeding mesenchymal cells and endothelial cells in a fibronectin and collagen scaffold enabled the formation of a stably perfused graft-derived vessel upon scaffold implantation<sup>91</sup>. Stromal cells also support and even enhance vascular network self-assembly both *in vitro* and upon implantation in other material settings, such as in fibrin<sup>93</sup> or PLGA and PLLA hydrogels<sup>94</sup>, and even in ‘scaffold-free’<sup>95</sup> situations, in which tissues are composed only of the cells and the matrix these cells secrete. Self-assembling networks supported by stromal cell inclusion have also been used to support parenchymal cells for a variety of translational organ fabrication applications, including for skeletal muscle<sup>94</sup>, cardiac<sup>96</sup> and hepatic<sup>93</sup> tissue engineering.



**Fig. 2 | Nature-driven approaches for vascular network fabrication. a** | Nature-driven approaches rely on the inherent capacity of cellular communities to undergo morphogenesis (that is, the biological process that causes a cell or tissue to develop its characteristic shape) and self-assemble into vascular networks. For example, co-cultures of endothelial cells and stromal cells can spontaneously self-assemble into vascular networks. **b** | A blood-vessel organoid self-assembled from induced pluripotent stem cell (iPSC)-derived endothelial cells *in vitro*. After implantation into mice, such organoids undergo remodelling *in vivo* to form a perfused hierarchical vascular network. Panel **b** reprinted from REF.<sup>112</sup>, Springer Nature Limited.

Over the past decade, much of the self-assembly field has turned its focus towards creating more physiologically relevant vascular networks *in vitro* by incorporating microenvironmental factors such as controlled perfusion. Most of this work has been performed within microfluidic platforms<sup>29,97</sup>. For example, incorporating angiogenic growth factors or stromal cells with endothelial cells in microfluidic chips can induce endothelial sprouting or self-assembly to generate stable and perfusable vascular networks with patent lumens<sup>98–100</sup>. Formation of vascular plexi can be enhanced by transiently reactivating transcription factors associated with a foetal cell state in endothelial cells before their incorporation in microfluidic platforms<sup>101</sup>. Such models have been used to screen therapeutic strategies for treating diseases, including cancer<sup>97,102</sup>, and for penetrating highly selective endothelia such as the blood–brain barrier of the cerebral vasculature<sup>103</sup>.

**Organoids.** An emerging area that also makes use of self-assembly is the creation of vascularized organoids, 3D mini-organs up to ~150 µm in diameter that replicate many of the developmental, structural and functional properties of their full-sized counterparts<sup>104,105</sup>. Organoid vascularization is particularly interesting, as during embryonic development organogenesis of many organs (such as the liver<sup>106</sup>, pancreas<sup>107</sup> and lungs<sup>108,109</sup>) occurs concordantly with vascularization. Most efforts to vascularize organoids *in vitro* have taken either a co-culture approach of mixing endothelial cells with organ-specific cells<sup>110,111</sup> or a co-differentiation approach using mesodermal precursors that can differentiate into various vascular cell types during organoid maturation<sup>112,113</sup>. Although both of these methods enable the formation of endothelial tubular networks during organoid culture, perfusion of these self-assembled networks surrounding organoids *in vitro* remains challenging<sup>105</sup>. Multiple groups have demonstrated that *in vivo* implantation of these ‘prevascularized’ organoids leads to rapid perfusion of the preassembled vascular network and further organoid maturation<sup>111,112,114</sup>.

Most methods of creating self-assembled networks have generated vessel sizes that range from capillary to arteriole equivalents (diameter 5–50 µm)<sup>115</sup>. Producing the multiple levels of the vascular structural hierarchy through these approaches has remained challenging. One report suggests that this vascular scaling and structural limitation could be overcome by creating blood-vessel organoids<sup>112</sup>. For example, blood-vessel organoids have been created from induced pluripotent stem cell (iPSC)-derived endothelial cells *in vitro* (FIG. 2b). On implantation in the kidney capsule of immunodeficient mice, these organoids self-assembled *in vivo* to form a perfused hierarchical network that included arteries, arterioles, venules and capillaries. The success of this approach suggests that self-organized systems based on vascular organoids have the potential to replicate vessel hierarchy<sup>112</sup>. As these studies required that the tissues be implanted *in vivo* for full maturation of self-assembled vascular networks, elucidating these cues and replicating them *in vitro* would be a major step towards vascularized systems that replicate the vascular hierarchy *in vitro*.

In addition to their potential utility in artificial tissues, vascular networks created from human blood-vessel organoids could present new opportunities for disease modelling and drug discovery. Such organoids have been used to identify new therapeutics in both diabetes<sup>112</sup> and SARS-CoV-2 infection<sup>116</sup>. In these two examples, human blood-vessel organoids reproduced features of the human disease and were used to test potential compounds for future clinical use.

**Materials considerations.** The choice of scaffold material in which to embed endothelial cells is essential for aiding the self-assembly of vascular networks in both randomly dispersed endothelial cell suspensions and within organoids. Contrary to technology-driven approaches, most early matrices used in this field were derived from natural polymers such as Matrigel<sup>85</sup>, fibrin<sup>117</sup> and collagen<sup>118</sup>. The main advantage of such natural polymers is that they contain adhesive ligands and have remodeling capabilities, which collectively support vascular morphogenesis and self-assembly<sup>119</sup>.

Despite their biological advantages, natural hydrogels can be difficult to manufacture and are subject to natural lot-to-lot variability. In particular, culture of organoids is frequently reliant on Matrigel, a chemically undefined ECM mixture derived from mouse tumour cells, which contributes to issues of replicability and translatability<sup>120</sup>. Additionally, the complexity of many natural polymers makes their chemical or mechanical modification more challenging, restricting the ability to further tune these materials for improved performance. Nevertheless, natural materials still have great utility and remain routinely used today, especially for vascular self-assembly.

To address some of the challenges with natural materials, several groups have developed fully synthetic or hybrid materials that mimic at least some of the angiogenic features of the native matrix. For example, the tunable and unique synthetic environment of hyaluronic acid-based hydrogels, combined with soluble angiogenic factors, promotes the vascular differentiation of human embryonic stem cells<sup>121</sup>. Subsequent studies demonstrated the success of differentiating pluripotent stem cells and creating vascular networks via self-assembly within implantable hyaluronic acid hydrogels<sup>122</sup>.

The elastic modulus of the matrix within engineered tissues also affects cellular behaviour, including the self-assembly of endothelial cells. One method used to tune hydrogel stiffness relies on increasing the density of the matrix, although notably both stiffness and ligand density change using this approach. Interestingly, increasing the density inhibits endothelial sprouting in 3D hydrogels<sup>123</sup>, whereas increasing the stiffness independent of density has the opposite effect<sup>124</sup>. These results suggest that more work may be needed to isolate the effects of material stiffness from other properties such as ligand density. Beyond effects on angiogenic sprouting behaviour, tuning the matrix stiffness may also improve the control of vessel structure. For example, altering the matrix stiffness independent of pore size or density controls the lumen size of self-assembled

vessels in a collagen gel<sup>125</sup>. Stiff gels (~20,000 Pa) promoted the formation of larger, multicellular lumens, whereas soft gels (~5,000 Pa) had dense, thin vessel networks<sup>125</sup>.

An interesting development is the emergence of dynamic hydrogels that can rapidly change their viscoelastic properties in response to the traction forces exerted by encapsulated cells, thereby mimicking the mechanosensitive properties of natural tissues<sup>126</sup>. These cell-responsive, dynamic hydrogels aid the formation of self-assembled vascular networks *in vitro* and *in vivo* by promoting contraction-induced integrin clustering<sup>126</sup>, findings that point to the importance of two-way communication between cells and their extracellular environment.

Despite these efforts to identify an optimal material for supporting the formation of vascular networks, cells also secrete matrix molecules and modifiers that degrade and replace the synthetic or natural matrices over time. Thus, the initial matrix material will differ from that which evolves in the absence of intentional control not just of the ECM, but also of the cells themselves. Future developments for engineering self-assembled vascular networks may seek to aid cell–ECM communication by not only providing appropriate biochemical cues, but also adapting their physical and chemical properties in response to cell-provided cues.

### Emerging frontiers

In the past few decades, considerable progress has been made in vascular engineering. Yet, even with these transformative advances, achieving the hierarchy, heterogeneity and function of vascular networks remains out of reach. Here we articulate the limitations of current technologies, what future developments are needed to advance the field, and what knowledge and tools are required.

#### **Dimensional and cellular hierarchies**

Fabricating vascular networks that replicate the hierarchical organization of native vasculature has become one of the pre-eminent goals of vascular engineering. So far, most technology-driven approaches have focused on achieving the dimensional hierarchy of the vasculature (FIG. 3a,b), with the goal of constructing branching networks that seamlessly connect ~5 mm (artery-sized) to ~10 μm (capillary-sized) channels. Although this goal remains elusive, laser degradation and bioprinting have taken the field closer to creating hierarchies of dimensional scales<sup>41,45</sup>.

In addition to recreating hierarchies of scale, we are likely to need to simultaneously recreate hierarchies of vessel cellular composition, morphology, phenotype and function (FIG. 3a,c). For example, arteries are defined not only by their size (>0.1 mm), but also by their medial layer, rich in elastin and smooth muscle cells, which can withstand high pulsatile pressures. Capillaries, by contrast, require single-layer endothelial walls to allow gas exchange<sup>127</sup>. Most bioprinted channels have dimensions of >0.1 mm, similar to the dimensions of an artery or large arteriole. However, these channels are typically lined with only a single

layer of endothelial cells, which resembles the structure of a capillary. Such vessels may ultimately fail to perform the physiological function of either arteries or capillaries. Thus, although methods such as bioprinting can spatially pattern endothelial cells to take up specific positions relative to other cell types, work to generate multilayer vessels at appropriate size scales remains largely in its infancy<sup>128</sup>.

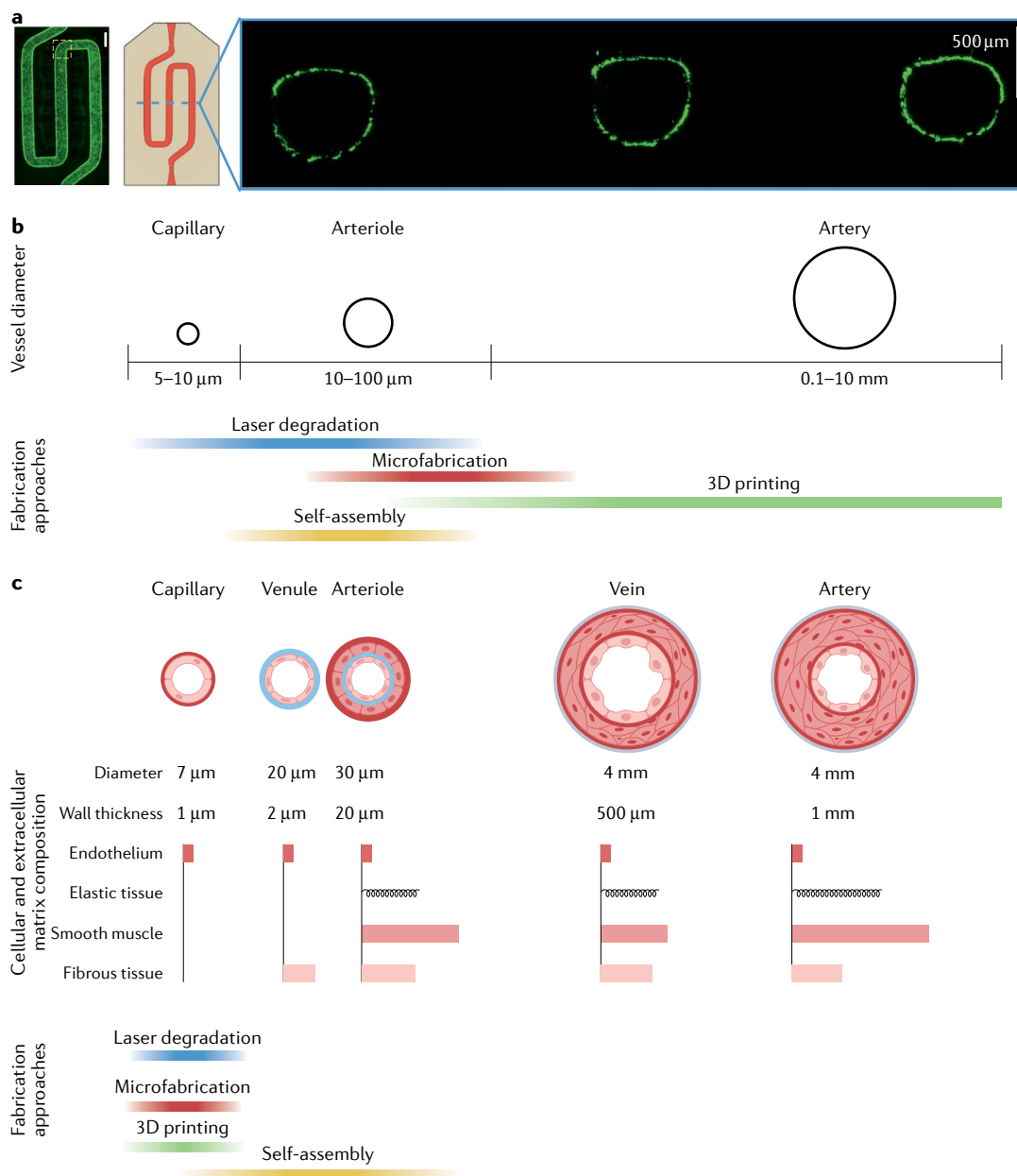
On the other hand, self-assembled vascular networks and blood-vessel organoids more closely replicate native vessel structure, especially for microvessels, than do networks built with technology-driven approaches<sup>112</sup>. Yet these self-assembly methods do not easily allow the control of hierarchical dimensions or topology, leading to irregular network geometries that bear little topological resemblance to healthy vascular beds. Although implanted self-assembled vessels rapidly anastomose with the host following implantation, the often tortuous geometries of these vessels can make them prone to premature thrombosis<sup>129,130</sup>.

In the future, the field will need to address these limitations. We envisage approaches that combine various technological tools with self-assembly. To this end, one emerging body of hybrid approaches seeks to exert some level of exogenous control over biology-driven network assembly. These strategies typically use material or architectural cues to guide or direct vessel formation<sup>131–136</sup>. One such method uses micropatterning to spatially deposit endothelial cells and collagen fibres to create preformed ‘cords’ within engineered tissues *in vitro*<sup>137</sup>. On implantation, these cords act as guides for the assembly of perfused, chimeric host–graft vessels along the axis of each cord<sup>31</sup>. As the vessels assemble around these cords *in vivo*, the first structures to appear resemble those occurring in embryonic lumenogenesis<sup>138</sup>. Endothelial cells also respond to the topographic features of the underlying basement membrane<sup>139–141</sup>. As this field moves forward, engineers should consider in what situations vascular networks should be specified or guided using engineering technology, and in what situations they should relinquish control to biology-driven processes. Hybrid strategies that harness the power of biology while maintaining control over vessel architecture may empower the field to address this problem.

#### **Organ-specific endothelial subtypes**

Many groups in the field of engineering vascular networks, especially those using technology-driven approaches, have relied on readily available primary endothelial cells such as human umbilical vein endothelial cells to provide the cell source for engineered vessels. However, an increasing appreciation of endothelial cell heterogeneity is motivating engineers to seek biologically more appropriate cell sources for engineering vascular networks (FIG. 4). This includes the need to obtain different endothelial cell subtypes that compose a given vascular bed (such as arterial and venous endothelial cells), as well as obtaining further organ-specific endothelial cells that vary in both phenotype and function (BOX 2).

Unfortunately, organ-specific primary human endothelial cells remain difficult to obtain, and their



**Fig. 3 | Vascular network fabrication requires both dimensional and cellular hierarchy.** Fabricating vascular networks that replicate the hierarchy of native vasculature is one of the leading goals of vascular engineering. This field has mostly focused on dimensional hierarchy so far but will almost certainly need to simultaneously achieve hierarchy of vessel composition (cell types and extracellular matrix), morphology, phenotype and function to successfully recreate the vasculature. **a** | 3D-printed vascular networks (left and centre images) often have lumen diameters similar to those of arteries or large arterioles, but are lined with only an endothelial monolayer, as is characteristic of capillaries. Endothelialized lumens are shown in the cross-section view, as acquired by confocal microscopy (right image). **b** | Different fabrication approaches can achieve different lumen diameters, which determine the achievable dimensional hierarchy in engineered vascular networks. **c** | Different blood-vessel types vary considerably in cellular and matrix composition. Current approaches used for engineering vascular networks can achieve dimensional and compositional hierarchies resembling vascular organization at the capillary and/or arteriole level. Panel **a** adapted from REF.<sup>169</sup>, Springer Nature Limited. Panel **c** adapted with permission from REF.<sup>211</sup>, The American Physiological Society.

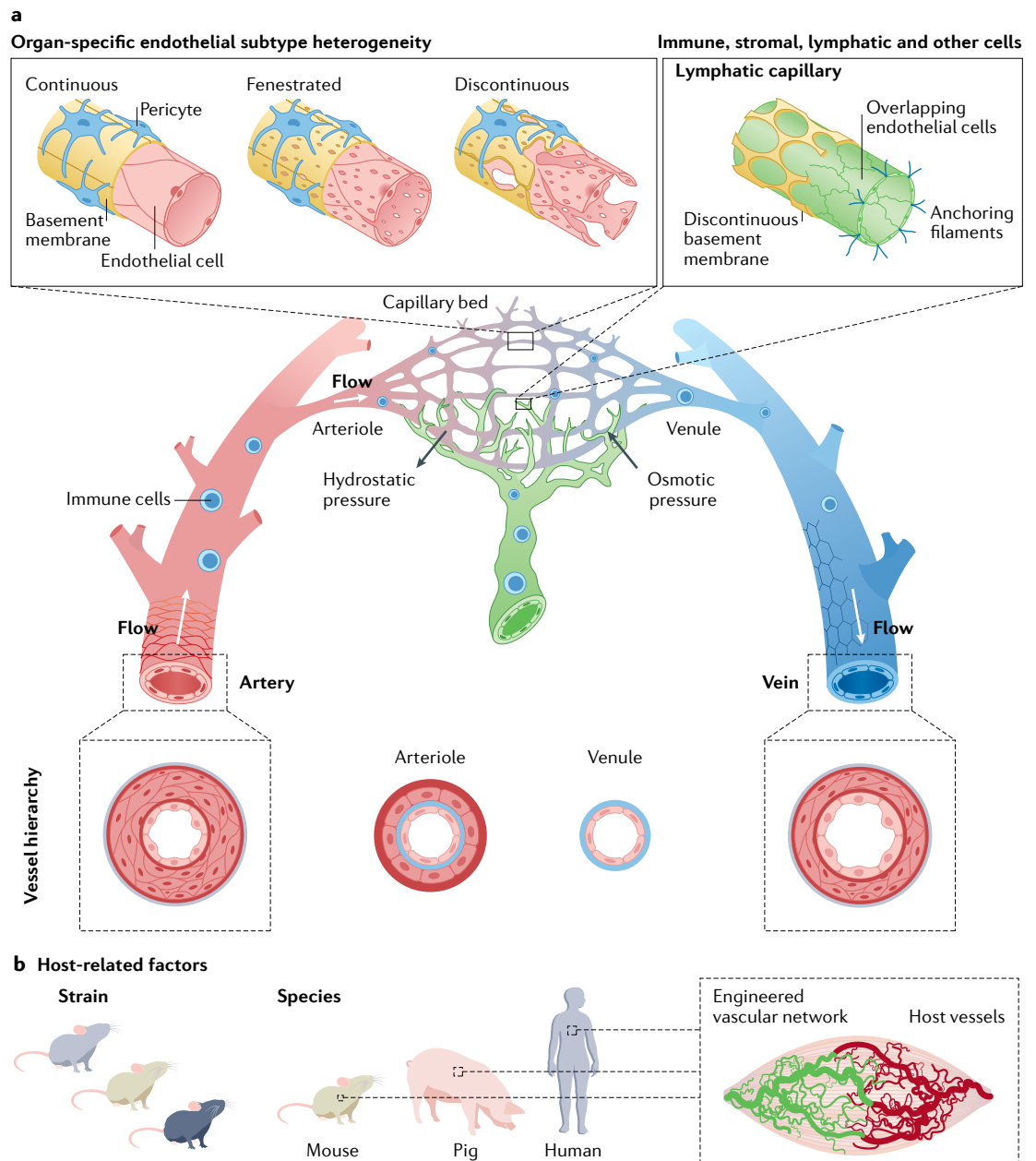
clinical relevance is limited. Moreover, organ-specific primary endothelial cells exhibit large variability in key abilities, including in their capacity to self-assemble. These cells also have considerable plasticity, meaning that they are capable of readily changing phenotype in response to their microenvironment<sup>43</sup>. More work is needed to identify environments that support the

maintenance of particular endothelial subtypes<sup>142</sup>. A potential source of organ-specific human cells may be endothelial cells derived from human iPSCs with varying subtype identities that can be scaled for product development. An emerging body of work has offered protocols to differentiate cells that exhibit some tissue-specific functions and organotypic phenotypes,



through *in vitro* directed differentiation protocols or *in vivo* specification within an appropriate tissue environment<sup>143–146</sup>. An alternative approach transiently reprogrammes normally quiescent adult endothelial cells to acquire an angiogenic, more foetal-like, phenotype<sup>101</sup>. This approach can be used with adult endothelial cells from a variety of organs, although it remains to be seen whether these reprogrammed cells fully retain their organ-specific phenotype.

Moreover, iPSC-derived endothelial cells that are not themselves organ-specific have also been co-cultured with other organ-specific cell populations. Several groups have worked to mimic aspects of the brain microcirculation (for example, the blood–brain barrier), by co-culturing iPSC-derived endothelial cells and human brain cells, such as pericytes and astrocytes, within perfusable microfluidic platforms<sup>136,147–150</sup>. Engineers of the future will almost certainly need to consider including



**Fig. 4 | Emerging frontiers in engineering vascular networks. a** | Matching the complexity of healthy vascular networks requires a combination of engineering approaches capable of generating a hierarchical network of vessels that corresponds to the healthy vasculature in cellular composition, function and architecture. A diverse population of cellular components is needed, including endothelial cells, smooth muscle cells, and immune, stromal and lymphatic cells. Non-cellular components include the basement membrane and elastic fibres. Additionally, mechanical aspects such as fluidic flow, hydrostatic pressure and osmotic pressure must be considered. **b** | Improved understanding of host-related factors such as the effect of immune, neuronal and endocrine interactions, as well as host species and strain, is also needed. Panel **a** adapted from REF.<sup>212</sup>, Springer Nature Limited.

**Box 2 | Vascular networks vary between and even within organs**

Although vascular hierarchy is a prominent feature in most solid organs, the nuances of vascular architecture, cellular and molecular composition, and subtype identity vary greatly across and even within organs and affect both transport and communication<sup>218</sup>. One example of vascular subtype heterogeneity is evident at the capillary level, where differences in capillary barrier function regulate the passage of substances through the vessel wall<sup>219</sup>. For example, blood capillary endothelium can be continuous, fenestrated or discontinuous. Continuous capillaries are characterized by tight junctions and are found in tissues such as the brain microvasculature, forming the highly impermeable blood–brain barrier. Fenestrated capillaries are found in tissues such as the kidney glomeruli<sup>219</sup>, enabling filtration and secretion. Discontinuous capillaries, characterized by the largest fenestrations, are found in liver sinusoids and enable the mixing of oxygen-rich blood with nutrient-rich blood<sup>219</sup>. Similarly, lymphatic capillaries have overlapping epithelial cells that act as valves promoting the ingress of fluid, macromolecules and immune cells (FIG. 4a).

In the past few years, breakthroughs in molecular biology, such as those that led to an explosion of single-cell transcriptomics studies, have revealed a plethora of organ-specific and even intra-organ differences in endothelial cell subtype identity and function<sup>199,219</sup>. Transcriptomic heterogeneity has also been correlated with functional heterogeneity in paracrine signalling<sup>2</sup>, metabolism<sup>218</sup> and immune regulation<sup>220</sup>, emphasizing how, beyond barrier functions, the microvascular endothelium engages in molecular crosstalk with the surrounding cells including the parenchymal and immune cells, in an organ-specific manner.

heterogeneous populations of both endothelial cells and support cells to achieve vascular networks that support organ-specific functions.

**Crosstalk between cell types**

Although most cellular work in vascular network engineering has focused on endothelial cells, replicating the phenotypic heterogeneity of non-endothelial cell types is also likely to be important. For example, stromal or mural cells surrounding the vasculature are of critical importance to vascular functions, and their specialization also contributes to tissue-specific microvascular identity<sup>151</sup>. In addition, parenchymal cells, the functional cells of the tissue, often interact with vascular cells to influence vascular structure and functions during both development and homeostasis. The parenchyma supports vascular identity, generates angiogenic factors and drives vascular ingrowth<sup>152,153</sup>. At the same time, vascular cells generate tissue-specific angiocrine factors that support the development and function of parenchymal cells<sup>2</sup>. Thus, for vascular networks to retain their structure and function, they may need to be engineered concordantly with the parenchymal functional tissue elements of a given organ. Defining and obtaining appropriate perivascular and parenchymal cells will be necessary to capture the complete cellular diversity of natural vessels in different tissues.

Endothelial cells lining the vasculature also interact constantly with circulating cells, and their interactions with platelets, leukocytes and even red blood cells are key events in inflammation and immunity<sup>154</sup> (FIG. 4a). Circulating platelets carefully guard endothelial cell integrity; when stimuli are present, platelets adhere to activated endothelial cells and trigger inflammation, leading to consequences such as leukocyte extravasation, change in vessel permeability or vascular tone, thrombosis, degradation of the ECM, and angiogenesis<sup>154</sup>. Some of these functions have also been demonstrated in engineered microvessels<sup>30,155</sup>. In inflammation, endothelial

cells can control the number and types of immune cells that extravasate into the interstitium. Organ-specific endothelial cells carry different adhesion molecules that enable homing of immune cells into specific vascular beds.

Some immune cell types, including neutrophils, macrophages, T cells and even B cells, play a role in orchestrating vessel organization and the emergence of hierarchical structure during embryonic development, making them potentially important for vascular self-assembly. For example, yolk-sac-derived macrophages are associated with vessel formation in organogenesis of testes<sup>156</sup> and brain development<sup>157,158</sup>. These macrophages associate with endothelial cells and promote the formation of the vascular plexus. They also mediate vascular pruning, connection and reorganization<sup>156</sup>. Immune cells also contribute to vascular anastomosis by serving as endothelial tip cell-chaperones<sup>157</sup>, adopting pericyte-level interactions<sup>159</sup> and remodelling vessel structures<sup>160</sup>. However, in addition to the supportive role that immune cells can perform in vascular formation, we note that immune cell presence and surveillance also govern vessel anastomosis and graft rejection. Thus, although we must consider the source of each cell type used to construct engineered vascular networks, we must also consider the interactions of antigen-presenting cells and T cells within the vasculature. Immunological activation and/or clearance of incompatible cells is likely to occur if the cell sources are not immunologically compatible.

Further, when modelling vasculature in vitro, one essential but often missing component is the circulating plasma and blood cells. These missing components are likely to be responsible for the gap between engineered vasculature in vitro and in vivo. Supplementation with blood proteins and/or blood cell types (including white blood cells and platelets) would be important to promote vascular maturation<sup>158</sup> and better mimic vasculature in vivo. Including these circulating cells will be important when engineering models in the future to better shape the vascular hierarchical structure and promote vessel maturation.

Finally, most vascular network engineering so far has overlooked lymphatic vessels (FIG. 4a), which return interstitial arterial fluid and tissue metabolites into the circulation<sup>161</sup>. Increased understanding of the role of lymphatic vessels in development, homeostasis and disease will be important for engineering vascular networks. Emerging work is already looking towards building human lymphatic vessel networks using both technological and self-assembly-based approaches<sup>162,163</sup>.

**Accounting for flow and pressure**

The fact that vascular perfusion is needed for the delivery of nutrients and oxygen in vivo is a reminder that vessels in the body are also subjected to constant haemodynamic forces that are sensed by mechanoreceptors on the surface of endothelial cells<sup>164</sup>. The response of endothelial cells to blood flow within the vessel regulates many important vascular functions, including vessel stability and angiogenesis<sup>164</sup>. For example, persistent high blood flow may induce endothelial remodelling to

enlarge lumens, and conversely, persistent decreased flow can induce vascular network remodelling, with some vessels regressing or closing<sup>165</sup>. Furthermore, interstitial fluid flow, driven primarily by plasma leaving the capillary walls and draining into the lymphatics, plays a critical role in regulating normal tissue function and homeostasis<sup>166,167</sup>. Indeed, the formation of both blood and lymphatic capillaries can be enhanced by the presence of interstitial flow<sup>167</sup>.

Progress in microfabrication and bioprinted vascular networks has enabled the methodical introduction of fluid flow into the networks so that the role of perfusion in vascular biology can be explored<sup>32,168,169</sup>. For example, controlled perfusion of microfabricated vessels showed that laminar flow improves endothelial barrier function and vessel stability by signalling through the mechanosensor NOTCH1 (REFS<sup>164,170,171</sup>).

In addition to being used to develop our understanding of the regulation of vessel permeability, microfabricated vascular networks have also been used to study how flow and shear stress influence angiogenic sprouting<sup>172</sup>. Controlling the force and direction of flow within vascular networks can induce or inhibit sprouting and direct the orientation of resultant sprouts<sup>172,173</sup>. Strategic control of flow direction within engineered networks has even been used to pattern angiogenic growth. Studies demonstrating that endothelial cells respond to torsion within helical vessel constructs highlight the need to model complicated physiological flow patterns *in vitro*<sup>174</sup>. These studies suggest that replicating physiological flow in engineered networks *in vitro* (such as for organ-on-a-chip applications) will improve endothelial function and more closely approximate the biology of healthy vessels<sup>164,170</sup>.

Another external force that cells regularly experience is cyclic stretch and relaxation. Although predominantly exposed to blood shear stress in the direction of blood flow, endothelial cells are also subjected to cyclic circumferential stretch caused primarily by pulsatile pressure tangential to the direction of flow. Cells in 2D *in vitro* environments can respond to mechanical stretching by reorienting themselves in well defined alignments that are dependent on the mechanical stimuli<sup>175–177</sup>. Under cyclic stretch in a 3D biomaterial construct, vascular networks orient themselves perpendicular to the stretching direction, whereas under static stretch, networks orient themselves parallel to the stretching direction<sup>177</sup>. Harnessing tissue-level mechanosensitivity may thus aid the generation of 3D vascular networks with a well defined orientation.

In addition to *in vitro* applications, exposing engineered vessel networks to flow before implantation may have important translational implications for the use of engineered tissue therapies in organ repair. In one study, perfusing microfabricated vessel networks in engineered tissues *in vitro*, prior to their implantation, improved the functional self-assembly and perfusion of these vascular networks after they had become integrated on the hearts of rats<sup>32</sup>. One of the main challenges in this field will be to develop refined methods to introduce, ensure and measure vascular perfusion in engineered tissues, both *in vitro* and after tissue implantation.

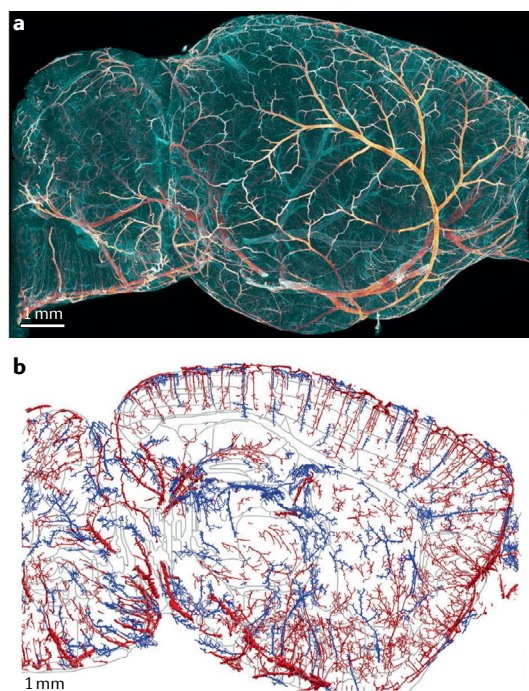
### The role of the host

Harnessing the biology of the host — that is, the animal into which an engineered tissue is surgically implanted — has been a major element of engineering vascular networks since the beginning of this field. Some of the earliest strategies to vascularize engineered tissues relied on stimulating vascular ingrowth from the host into a tissue graft by releasing angiogenic growth factors that were crosslinked or embedded in particles in the engineered tissue<sup>132–136</sup>. Later systems made further use of stimuli-responsive materials engineered to release growth factor payloads in response to environmental cues (such as pH and enzymatic activity) or external cues (such as small molecules, ultrasound<sup>178,179</sup>, heat<sup>180</sup> or magnetic fields<sup>181</sup>). In the past 20 years, the tissue engineering field has often used the intrinsic capacity of grafted human endothelial cells to self-organize into blood vessels that anastomose with host vessels, to carry blood upon tissue implantation<sup>31,32,94</sup>. This process of host-graft vascular integration is thought to occur through a mechanism in which the graft-derived endothelial cells wrap around host vessels and locally degrade the basement membrane by secreting matrix metalloproteinases, which enables graft-derived endothelial cells to infiltrate the host vessel and divert blood to the graft<sup>182</sup>.

Such host-driven technologies have been enormously powerful for the field, and much of the progress in engineering vascular networks depends on the host biology to do some of the engineering work. We caution that emerging work demonstrating variability in graft–host vascular anastomosis across different hosts<sup>183</sup> suggests that the field may need to better understand the role of the host, including details like immune, neuronal and endocrine interactions, as well as host species, strain and model, in these processes (FIG. 4b). In other words, just because a host-driven technology works well in one host does not mean it will work the same way in a different host model system.

The issue of host variability will become increasingly important as more engineered tissue strategies progress to the clinic. Translation requires that therapeutic tissues are first tested in animal species with increasing similarity to humans, thus introducing variables such as host species, strain and immune status. Translation is also likely to entail adapting tissues with engineered vascular networks for various disease settings. Unlike the relatively young animals commonly used for the initial development of engineered vascular networks, the patient populations most in need of tissue therapies are likely to have ageing and diseased vasculatures<sup>184–186</sup>, and in many cases pathological host environments such as scarring caused by myocardial infarction<sup>187,188</sup>. The field will benefit from careful consideration of animal models and host factors at early stages of technology development, so that these strategies can be adapted for varying host environments.

Relying entirely on vascular infiltration by the host to achieve anastomosis is also inherently limited by a delay in perfusion that leaves vulnerable cells deprived of oxygen. An alternative is to achieve instantaneous



**Fig. 5 | Multiscale mapping of vascular networks.**  
**a** | 3D projection of deep immunolabelled and imaged mouse brain microvasculature using clearing techniques and light-sheet microscopy. Vasculature of the perfused brain is immunolabelled against CD31, podocalyxin (endothelial cells) and ACTA2 (arteries). **b** | Slice through a vascular brain graph depicting only arteries (red) and veins (blue). Panels **a** and **b** reprinted with permission from REF.<sup>199</sup>, Elsevier.

perfusion through surgical anastomosis between graft and host vessels, such as by constructing multiscale vascular networks with larger conduits that can be surgically anastomosed to host vasculature<sup>189</sup>. This approach has been used with some success to achieve instantaneous perfusion within engineered tissues, although more work in this area is needed<sup>189,190</sup>. Further development of this approach may allow the convergence of technologies used in large tissue-engineered blood vessels with advances from the engineered microvascular field. We also envisage future hybrid technologies that blend progress in controlled release, nanotechnology and synthetic biology<sup>191,192</sup> with technology-driven approaches for engineering vascular networks, to move towards controlling host biology to assemble vascular networks in engineered tissues.

#### Developing multidimensional blueprints

The architectural, cellular and molecular features of vascular networks are spatially patterned at scales ranging from subcellular to whole organ levels, with known heterogeneity across different organs. Yet even the most advanced maps of human organ vasculature still do not incorporate the vasculature's full scale, dimensionality or heterogeneity. The lack of comprehensive organ-specific vascular blueprints makes it challenging for engineers to build and rigorously evaluate engineered vascular networks.

Many vascular network features have been uncovered using fluorescence, light or electron microscopy coupled with 2D histology. Other methods that capture 3D architectural features, such as magnetic resonance imaging and microcomputed tomography, often have limited fields of view and do not visualize the diverse populations of cells that make up the vasculature<sup>193,194</sup>. Recent breakthroughs that conflate materials science with tissue imaging could address these gaps. For example, advances in tissue clearing reagents and methods that remove lipids, pigments and/or calcium phosphate from tissues and match refractive indices<sup>195–197</sup> render the tissue nearly transparent, enabling microscopes to 'see' deeper into tissues<sup>198</sup>. Combined with confocal or light-sheet microscopy, tissue clearing has enabled 3D visualization of the vasculature of entire mouse organs and large swaths of human organs with unprecedented cellular and even subcellular detail<sup>199</sup> (FIG. 5). To further push the resolution boundaries of these maps, other materials-driven solutions such as expansion microscopy aid in superresolution of structures on the nanoscale<sup>200</sup>, by using an expandable polymer to physically increase sample size before imaging.

In addition to vascular architecture, the recent growth of transcriptomics technologies such as single-cell sequencing has ushered in a flood of new molecular data<sup>201–204</sup>. Such studies have led to an increased appreciation for the complex heterogeneity of vessels across organs and shed further light on how vascular cells exist in constant two-way communication with other cells, matrix components and small molecules in their local environment<sup>205</sup>. Further advances in materials have led to remarkable progress towards integrating this molecular information into maps of tissues, such as spatial transcriptomic approaches using nucleic-acid barcoding<sup>160,206–210</sup>. Continued refinement of these technologies and development of analogous methods for spatially resolved proteomics, matrisomics and metabolomics will push the field closer to achieving comprehensive molecular blueprints of vascular networks across organ systems. Ultimately, multiscale and multidimensional maps of vascular networks across organs that incorporate different types of architectural, cellular and molecular information — from subcellular to the dimensional scale of organs — will equip engineers with the blueprints needed to build refined human vascular networks.

#### Conclusions

From its beginning, the engineering of vascular networks has risen by integrating diverse and often disparate disciplines. This field has made substantial progress over recent decades, yet much remains to be done. By intentionally converging diverse expertise and experience, we will generate innovative science that will ultimately enable us to reach the elusive goal of engineering the complexity of vascular networks. Transformative progress over the past few years shows us that what was once thought to be science fiction is now becoming increasingly achievable.

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**Author contributions**

C.O.C., E.B. and E.M. researched data for the article. All authors contributed substantially to discussion of the content, wrote the article, and reviewed and/or edited the manuscript before submission.

**Competing interests**

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