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Bridging scales: Advances in multi-scale vasculature bioprinting for tissue engineering

Running title: Advances in Multi-scaled Vasculature Bioprinting

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Abstract

Three-dimensional (3D) Bioprinting has emerged as a groundbreaking approach for fabricating living tissues and organ analogues with spatial and structural fidelity. By integrating biomaterials, living cells, and bioactive molecules through computer-aided design (CAD), bioprinting allows for the construction of biologically relevant architectures that closely mimic native tissue organization. Despite significant progress, one of the most formidable barriers to the clinical translation of engineered tissues remains the challenge of vascularization. The successful integration of engineered constructs *in vivo* critically depends on the formation of functional vascularization. The human vascular system is a highly organized, multiscale network, comprising a variety of blood vessels, each with distinct morphological and physiological roles. Reproducing the multiscale architecture of the vascular system, from large arteries to microscopic capillaries, is therefore fundamental to the development of functional tissue and organ replacements. This review highlights the central role of vascularization in tissue engineering, discussing current strategies, emerging technologies, and future directions aimed at replicating the intricate multiscale vasculature necessary for the functional and morphological fidelity of engineered tissues.

Keywords: 3D Bioprinting; Multi-scale vasculature; Vascularized tissue; Tissue engineering

1. Introduction

Three-dimensional (3D) bioprinting has revolutionized tissue engineering, offering opportunities for creating complex biological structures, including vascular networks that span from macroscopic arteries and veins to microscopic capillaries. Engineered tissues with thickness greater than 1 mm are known to have difficulty in maintaining their normal cell viability without vascularization [1], as the cells must be sufficiently close for the biochemical exchange to occur beyond the diffusion limit (100 – 200 μm) [2]. Therefore, such engineered vascular systems are vital for organ-level tissue constructs, facilitating nutrient and oxygen transport, waste removal, and integration within the host circulatory system, which remains a challenge in the field [3].

Functional integration of engineered tissues requires a complete vascular hierarchy, spanning more than three orders of magnitude, from large arteries ($> 1\text{ mm}$) to capillaries with diameters of only 5 – 10 μm [2,4–6]. Reproducing this organization means addressing the different structural, cellular, and biomechanical roles that vessels play at each scale, since understanding the relationship between vascular dimensions and tissue health is paramount for the successful biofabrication of complex, multiscale vascularized tissues and organs [7]. This includes not only the macroscopic architecture of arteries and veins, but also the microvascular networks, vital for specialized organ functions and survival [2]. Macrovascular networks are essential for tissue perfusion and implantable macrovascular grafts that can replace or bypass damaged vessels through standard surgical anastomosis. However, larger conduits must withstand physiological blood pressure, remain patent over time, and mechanically integrate with the host artery. This makes their design and fabrication considerably more demanding. As a result, despite their clear clinical relevance, progress in developing functional macrovascular grafts has remained slower than advances seen in microvascular bioprinting [8,9]. Microvascular constructs, for instance, are essential for oxygen and nutrient delivery to cells, presenting a significant obstacle to overcome in the creation of physiologically relevant tissue constructs. However, the engineered microvasculature must allow for predictable flow patterns and have optimal minimum overall wall vessel porosity. While advances in geometric complexity are being made, it remains challenging to achieve complexity while simultaneously replicate true capillary size [10].

Reproducing the multiscale vascular hierarchy requires more than matching vessel size alone, but their anatomical structure, cellular composition, mechanical properties and function, which affects how they should be designed and assessed.

Successful multiscale vascular biofabrication requires not only reproducing structures across different length scales but also accommodating the distinct biological and mechanical requirements associated with each vascular compartment. This functional perspective explains why hybrid fabrication strategies and post-printing maturation have emerged as key components for achieving physiologically relevant vascularized tissues [11,12].

Despite significant progress in vascular bioprinting, current strategies remain largely constrained by their focus on isolated fabrication techniques or specific length scales, limiting their ability to generate fully integrated and functional vascular systems. While individual approaches have demonstrated success in replicating either macrovascular structures or microvascular networks, none of them alone can simultaneously address the requirements of structural stability, spatial resolution, and biological functionality. Moreover, the role of biomaterials is often discussed in terms of printability rather than their impact on vascular maturation and long-term performance.

In this context, this review provides an analysis of current vascular bioprinting strategies, with a particular focus on their multiscale capabilities and the interplay between fabrication methods and biomaterial design. By comparing different techniques in terms of resolution, scalability, and functional outcomes, and by highlighting key parameters such as permeability, burst pressure, and bioink adaptability, this work aims to identify the main limitations of current approaches and outline emerging directions toward the development of integrated, clinically relevant vascular constructs. In addition, this review proposes a practical design framework that links vascular function, target vascular scale, fabrication technology and biomaterial selection into a unified strategy for multiscale vascular biofabrication.

2. Native vasculature complexity

2.1. Structural organization of the native vascular hierarchy

The native vasculature exhibits a hierarchical branching structure, with different types of vessels with different diameters, starting from aorta and vena cava (2.5 – 3 cm), arteries and veins (0.3 – 2.5 mm), arterioles and venules (10 – 300 μm) and capillaries (5 – 10 μm) [13,14]. Except capillaries, all blood vessels' walls are composed by three distinct concentric layers: tunica intima (inner), tunica media (middle) and tunica adventitia (outer). The tunica intima consist of a monolayer of endothelial cells (ECs), implicated in coagulation and thrombosis processes and regulation of blood cell and plasma proteins transmigration [15,16]; or perivascular cells [17], located between the ECs and the basal lamina and responsible for regulating capillary vasomotion and local tissue microcirculation; the basal lamina, made of collagen and elastin; and an internal elastic lamina, which provides flexibility and stability. The

tunica media is mainly composed of smooth muscle cells (SMCs) with contractile capacity in arterioles and capillaries regulating blood flow, but which remain passive in arteries, where they accommodate large volumes of blood cushioning pressure oscillations and allowing a homogeneous movement of blood to the rest of the body, protecting the integrity of the smallest blood vessels. An elastic lamina made of collagen and elastin surrounds this tunic, more prominent arteries closer to the heart, helping regulate blood flow. The tunica adventitia is formed by fibro-elastic or connective tissue with fibroblast cells, that protects and anchors the vessels to adjacent tissue, providing structural support. In larger blood vessels, it also has the nerve and lymphatic plexuses along with the *vaso vasorum*, a capillary network that nourishes the cells where the diffusion exchange does not reach [18,19].

Arteries are characterized by their thick elastic walls (1000 – 1500 μm thick), with a thick tunica media, designed to withstand high pulsatile pressures and regulate blood flow in tissues. As artery size decreases, the medial SMCs significantly increases (from 33.5% to 60.5%) while elastin concentration decreases (24.5% to 1.5%), reflecting the need for arterioles to actively control vessel calibre through SMC contraction. Veins, with thinner walls ($\sim 800 \mu\text{m}$ thick), function as low-resistance conduits for returning deoxygenated blood to the heart [20,21]. Wall thickness to radius ratio (h/r) varies considerably, ranging from 0.1 – 0.2 in arteries to 0.02 – 0.05 in veins [22]. In arterioles (125 – 800 μm wall thickness) wall thickness decreases as diameter increases, with overall thinner tunics. In venules, wall thickness ranges from 40 – 500 μm , with only a few SMC on the tunica media. Capillaries have extremely thin walls ($\sim 1 \mu\text{m}$ thick), with just tunica intima and optimized for efficient diffusion of biochemicals [21,23,24]. Depending on the continuity of the capillary's wall, capillaries can be: continuous, if they have an uninterrupted endothelium and basal layer, with abundant tight junctions between the ECs regulating the solutes' passage; fenestrated, with pores of 75 – 100 Å that limit the passage of proteins but allow free passage of water and solutes; and discontinuous or sinusoid, which have pores of 1500 – 2500 Å, allowing the passage of large cells and molecules on both sides of the cell wall. Each type of capillary is found in specific organs [13,25]. **Figure 1** summarizes general vessel information.

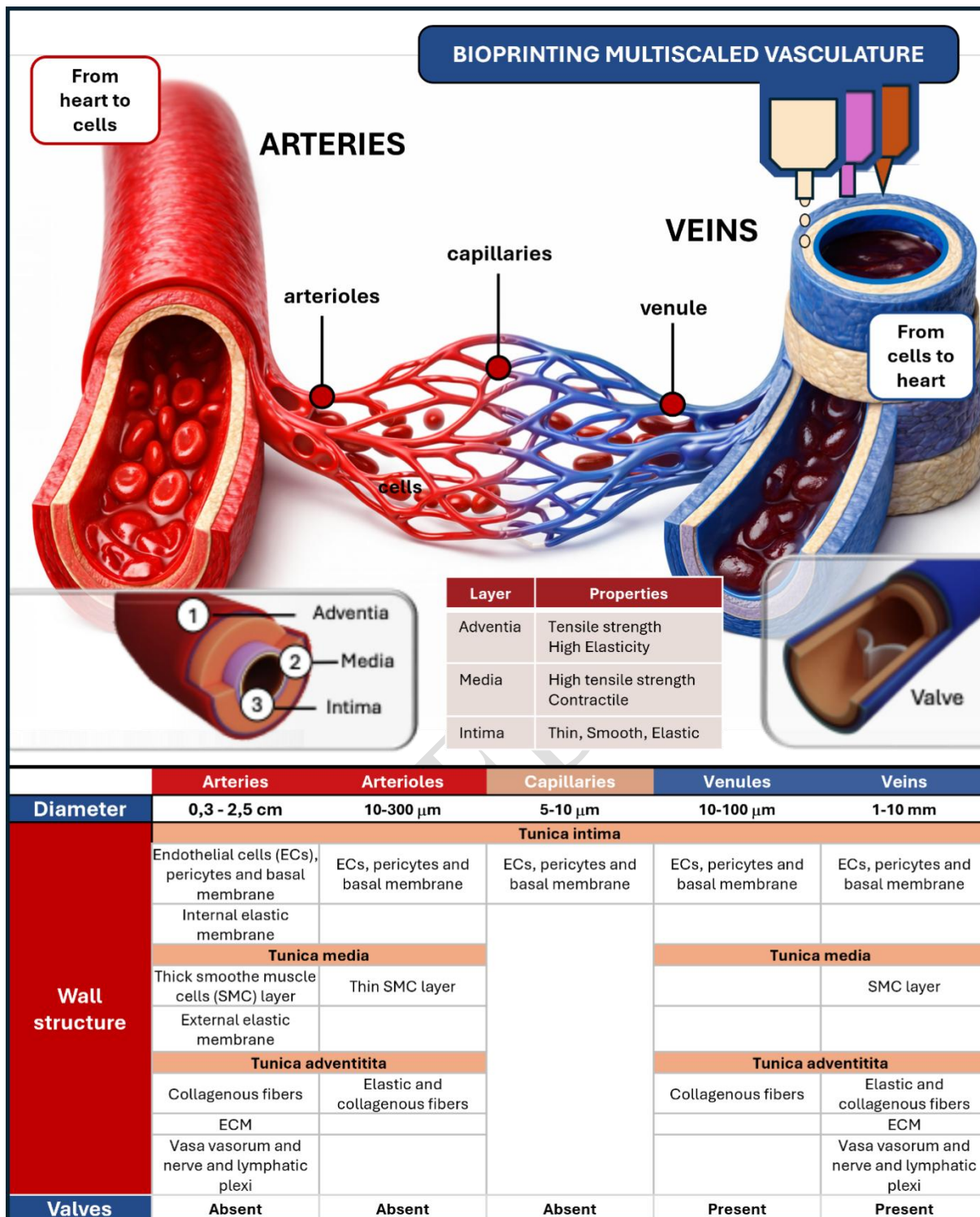


Figure 1: The typical diameters/wall thickness and cell types of blood vessel for humans. The smallest capillaries have diameters of a very few micrometers and a wall thickness of about one micrometer.

2.2. Structural complexity and functional requirements across vascular scales

The challenge of mimicking the complex structural and compositional hierarchy across different vascular layers remains a challenge, specially concerning the optimal distribution and

arrangement of cells within the printed constructs, since the interaction of cells with their physical environment is critical for correct function and mechanical properties [26,27]. Furthermore, the elastin and collagen content determine the interplay between the different layers and their specific mechanical properties, maintaining the physiological function of the vasculature. Consequently, bioprinting techniques must address precise control over biomaterial deposition and cell patterning to accurately reproduce the diverse mechanical properties inherent to different vascular regions [28].

Functional requirements are not the same across vascular length scales. Each scale needs its own evaluation criteria. For graft-scale constructs designed for surgical anastomosis, mechanical performance is the main concern. These grafts must tolerate high internal pressures, with reported burst pressures typically above 1500 – 2000 mmHg. Adequate suture retention is required, typically above 1 – 2 N, to allow safe implantation. Graft compliance should be close to that of native arteries (around 4 – 10% per 100 mmHg), since large mismatches can disturb flow and promote intimal hyperplasia [29–32]. At the mesoscale, printed channels mainly act as perfusable pathways within engineered tissues, not as standalone grafts. Therefore, functional assessment focuses less on mechanical strength and more on lumen patency, endothelial coverage, and hydraulic permeability. Commonly reported metrics include stable perfusion under physiological shear stresses ($1 - 10 \text{ dyn cm}^{-2}$) [33], continuous endothelial lining of the channel walls, and permeability levels that support nutrient transport while preserving barrier function [34]. For microvascular networks approaching capillary dimensions, functional success is defined less by mechanical strength and more by biological integration. Key indicators include permeability coefficients on the order of $10^{-6} - 10^{-7} \text{ cm s}^{-1}$, transendothelial electrical resistance (TEER) values comparable to native microvasculature, and rapid inosculation with host vessels, typically within 3 to 7 days post-implantation, to prevent hypoxia and necrosis in thick constructs [35,36]. Together, these scale-dependent criteria highlight that “functionality” in vascular bioprinting cannot be defined by a single metric but must be contextualized according to the intended vessel size and application [36–38].

2.3. Biomechanical considerations for engineered vasculature

Bioprinting techniques for macrovascular grafts, at the artery-scale vessels ($\geq 1 \text{ mm}$) [39], face distinct challenges compared to microvascular networks, primarily due to their substantial wall thickness, higher mechanical load and the necessity for long-term patency [40], requiring robust mechanical properties and complex trilayered structures able to withstand physiological pressures maintaining functionality over extended periods. The mechanical assessment mainly

focuses on burst pressure, suture retention, compliance, and fatigue resistance, since these factors affect surgical handling and long-term performance under arterial pressure [41], and determine whether a graft can tolerate arterial pressures or withstand the forces involved in surgical handling. Recent work on tissue-engineered and composite grafts has reported burst pressures exceeding 1000 mmHg and, in some cases, approaching the physiological range or native arteries, although both compliance and suture pull-out strength remain highly dependent on scaffold design and on how the grafts are conditioned in bioreactors [42]. Even with these advances, many engineered conduits still fall short of the consistency and reliability seen in clinically used autologous vessels such as the great saphenous vein [43]. These limitations have encouraged the development of more detailed design guidelines and characterization frameworks that connect wall composition, reinforcement strategies, and physiologic loading conditions in a more systematic way [44]. By contrast, microvascular vessels ($\approx 5 - 100 \mu\text{m}$) operate under much lower pressures [41], and their function is therefore more closely linked to permeability, barrier integrity, matrix stiffness, and shear-dependent endothelial behaviour. As a result, measures such as burst pressure or suture retention are not relevant at the microvascular scale. Studies using tuneable 3D hydrogels demonstrate that ECs are highly sensitive to matrix stiffness within a narrow physiologic range: softer matrices ($< 1 - 2 \text{ kPa}$) promote lumen formation and sprouting, whereas stiffer environments enhance mechanotransduction but may destabilize microvessel architecture or promote inflammatory activation under flow [44,45]. Furthermore, substrate stiffness and shear stress act synergistically to regulate junctional integrity and endothelial phenotype, emphasizing that functional evaluation of microvessel constructs should prioritize permeability, junction protein expression, and mechanosensitivity rather than metrics such as burst pressure or suture retention, which are relevant mainly to macro-scale conduits [46–48].

Comparing engineered vascular constructs and native vessels reveals still a long way to go. Clinically used autologous arteries and veins typically show burst pressures on the order of 2.000 mmHg or higher, together with compliance values typically ranging from approximately 4 – 10% per 100 mmHg, depending on vessel type and anatomical location [49]. These mechanical characteristics contribute to their excellent long-term performance under physiological cyclic loading. In contrast, although many engineered macrovascular grafts reach arterial-level burst pressures, their compliance and fatigue resistance remain highly variable and often inferior to those of native tissue. Engineered microvasculature struggles to achieve high cell density and close cell-cell interactions, crucial for robust microvessel formation and maturation, resulting in functionally inadequate structures for long-term integration and

viability [50,51]. Additionally, advanced fabrication techniques capable of achieving feature sizes smaller than 100 μm are required in order to effectively mimic native microvascular structures and ensuring adequate nutrient and oxygen supply [52,53]. Achieving the typically 10 mm diameter of a capillary remains a significant challenge.

2.4. Design requirements for multiscale vascular biofabrication

Reproducing a vascular system that truly reflects the layered structure and behaviour of native vessels is still very difficult to achieve, which has limited the growth of thick tissue or organ-level constructs [54–56], as replication of the complex structural and functional properties of native vasculature, including appropriate mechanical properties are paramount for successful clinical translation [57]. Bioprinting vascularized constructs in macroscale scaffolds improve cell viability, yet these printed networks fall short of reproducing the complex and well-organized architecture seen in native vessels [58–60]. Other approaches have achieved micro-scale network bioprinting, or angiogenesis on the printed construct, but with limited applications in larger-scale bioprinting or host transplantation [61]. However, the ability to engineer an intra-organ hierarchical vascular network, ranging from arteries and veins to capillaries, is paramount for the survival of biofabricated tissues, ensuring sufficient cell density within the critical diffusion distance to maintain viability, particularly in high vascularized organs, and allowing anastomosis with the host circulatory system [62–65]. Advanced bioprinting techniques must also account for the heterogeneous cellular and extracellular matrix compositions of the various vascular layers, including all three tunics, critical for structural integrity and biological function [26,66]. The structural distinction among vessel types underscores the necessity for bioprinting strategies to accurately replicate these varying scales and anatomical and mechanical properties to achieve functional vascular networks [67]. Overall, despite significant technological improvements to bioprinting blood vessels, the fabrication of functional multiscale vessel networks from capillaries to large vessels remains a challenge.

Therefore, engineered vascular constructs must satisfy specific criteria (see figure 2):

- a) **Barrier integrity (micro-vessels):** The endothelial lining must form a tight, semi-permeable barrier. This is quantitatively assessed by measuring restricted diffusion of high-molecular weight markers (e.g., FITC-dextran) and the expression of tight junction proteins like VE-Cadherin [68,69]. Barrier function has been successfully maintained *in vitro* for over one month in hydrogel-based microdevices [70].
- b) **Mechanical robustness (macro-vessels):** large grafts must withstand physiological burst pressure and exhibit sufficient suture retention for surgical anastomosis [42]. Furthermore, they

require compliance matching to the host vessel to prevent intimal hyperplasia and thrombosis, often requiring elastic moduli closer to native aorta [71,72].

c) Blood flow responses (all scales): The endothelium must sense and respond to fluid shear stress (0.1 to 100 kPa reported in extrusion) [5,72]. Controlled shear stress is crucial for maintaining a quiescent, anti-inflammatory EC phenotype, promoting arterial specification, and ensuring long-term vessel patency and stability [5,69].

d) Resolution, scale integration and perfusion: Printing functional capillaries (5 – 10 μm) and smoothly connecting them with macro-grafts (> 1 mm) for adequate anastomosis within a single automated process remains a challenge [5,73,74]. Current techniques often require secondary assemble stems or manual removing/seeding, compromising scalability [2,75], and thick construct viability, since maintaining cell viability throughout large constructs post-printing requires continuous, controlled perfusion to avoid hypoxic necrosis.

The pronounced differences in mechanical and functional requirements across vascular scales present a major engineering challenge. Addressing this challenge has led to a wide range of fabrication and bioprinting approaches designed to generate multiscale vascular networks.

Although vessel diameter provides a convenient engineering classification, native vascular organization is fundamentally hierarchical in terms of function. Arteries, arterioles, capillaries and veins collectively regulate blood transport, pressure distribution, molecular exchange and tissue homeostasis through coordinated interactions [76–78]. Consequently, successful multiscale biofabrication should reproduce not only anatomical dimensions but also the functional continuity existing between vascular compartments.

Specific criteria of bioprinted vascular networks

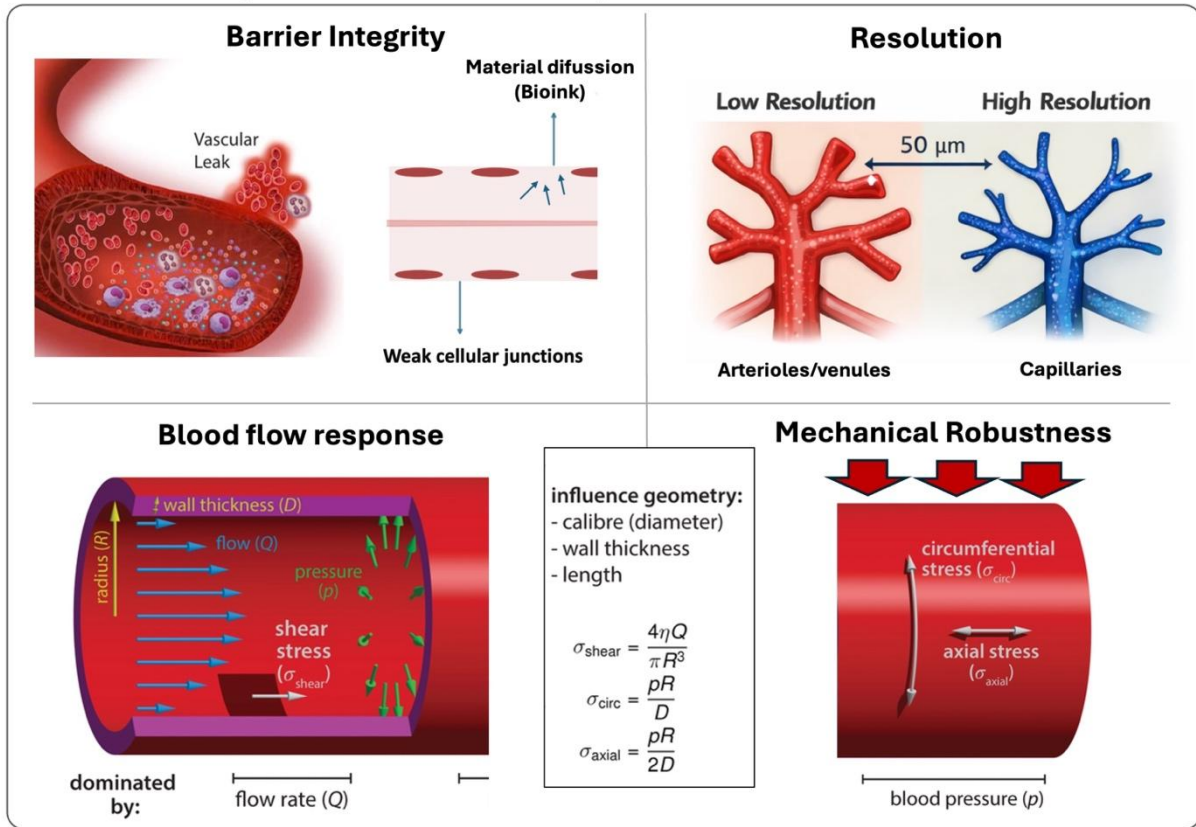


Figure 2: Major challenges associated with bioprinting vascular networks, which may adversely affect the structural integrity and functional performance of the bioprinted construct.

3. Fabrication strategies for engineered vasculature

Because the structural and functional requirements of engineered vasculature vary considerably across vascular scales, no single biofabrication technology is currently able to reproduce the entire vascular hierarchy [79–82]. Instead, each printing modality offers distinct advantages in terms of resolution, material compatibility, fabrication throughput and achievable vessel architecture, making them more suitable for particular stages of vascular reconstruction rather than universal solutions. Therefore, the choice of fabrication strategy should be guided not only by the target vessel dimensions, but also by the intended biological function and the level of structural complexity required.

Accordingly, current vascular biofabrication relies on several complementary printing technologies, each characterized by different capabilities in terms of resolution, printable biomaterials and structural complexity. As previously reported, extrusion-based bioprinting (EBB), droplet-based bioprinting (DBB), laser-assisted printing (LAB) and 3D printing by photopolymerization are the main methods used for the fabrication of blood vessels. However, recent advances in embedded bioprinting, volumetric-based bioprinting (VBB), and freeform

microfluidic approaches have begun to address the multiscale vasculature challenge and offer new possibilities for constructing integrated vascular networks [83–85].

As summarized in Table 1, different bioprinting techniques are naturally suited to different vascular size ranges. EBB is best matched to macro- and mesoscale channels, from just over 100 μm up to several millimetres, largely because it can deposit highly viscous bioinks and produce mechanically stable structures [7,86,87]. DBB, by comparison, typically generates features in the 50 – 300 μm range, which makes it well suited for creating patterned mesoscale vascular beds [87–89]. Light-based bioprinting methods, including LAB, stereolithography (SLA), digital light processing (DLP) and VBB techniques, can reach resolutions as low as 10 – 50 μm , allowing microvascular architectures to be produced at dimensions close to those of native capillaries or bigger scales up to a few centimetres, due to light use versatility [90–92]. Before discussing individual fabrication technologies, it is important to distinguish between printed vascular architecture and mature vascular function. Although macrovascular conduits can often be fabricated with their final geometry, most microvascular networks are not directly printed as functional capillary beds. Instead, they progressively emerge through endothelial self-assembly, sprouting, extracellular matrix (ECM) remodelling and perfusion-induced maturation. Consequently, bioprinting should be regarded as the initial step in vascular tissue formation, while biological maturation is essential for achieving stable and functional vascular networks. This distinction provides an appropriate framework for comparing the different biofabrication strategies discussed below according to both their printing capabilities and their ability to support subsequent vascular development.

The following technologies should be interpreted as complementary rather than competing approaches, each contributing differently to multiscale vascular reconstruction. Their integration into a unified design strategy is discussed in Section 5.1.

Table 1. Correspondence among main bioprinting techniques and vascular scale.

Bioprinting approach	Typical feature size/vessel diameter	Relevant vascular scale	Scale-based justification	Contribution to multiscale vascular integration	Ref.
EBB	~100 μm to several mm (<i>TRACE/FRESH based bioprinting can reach 10-20 μm</i>).	Macro- and mesoscale vessels (<i>TRACE/FRESH microscale</i>)	Enables deposition of highly viscous, cell-laden bioinks and fabrication of mechanically stable, self-supporting channels suitable	Provides mechanically robust conduits that can be integrated with sacrificial or microfluidic approaches to generate	[63,93]

			for large-diameter conduits	hierarchical vascular trees.	
DBB	~30 – 300 μm	Mesoscale vascular beds	High spatial resolution droplet deposition supports patterned vascular beds, although limited mechanical robustness restricts use at larger diameters	Suitable for patterned vascular beds and EC deposition, although generally requires complementary techniques for structural support	[94–96]
Light-based bioprinting	~10 mm to several cm	Macro-microvascular scale	Optical patterning allows high-resolution fabrication of microchannels approaching native capillary dimensions and scalability potential	Enables precise microvascular architectures and can complement extrusion-based fabrication in hybrid constructs	[93,97]
Indirect biofabrication strategies	Template-dependent	Micro- to macrovascular scale	Sacrificial materials, moulding and embedded approaches facilitate hollow channels and complex geometries that are difficult to print directly	Enable interconnected perfusable vascular networks and are frequently used together with direct bioprinting technologies to increase architectural complexity.	[3,72,98–106]
<i>In situ</i> bioprinting	Tissue-dependent	Defect-specific	Direct printing onto the implantation site facilitates anatomical adaptation and immediate integration with host tissues	Represents a promising strategy for translating vascularized constructs by reducing handling steps and improving anatomical conformity during implantation.	[76–78]
<i>Complementary approaches</i>	Cell-scale microvascular to	Mainly microvascular	Includes acoustic patterning and cell-driven vascular self-assembly, which complement rather than replace direct printing	Provides complementary biological organization, cellular patterning and post-printing maturation that enhance hybrid multiscale vascular fabrication.	[107,108]

3.1. Extrusion-based bioprinting (EBB)

EBB is a most widely adopted modality, valued for its capability to print high-viscosity cell-laden hydrogels and create mechanically robust centimeter-scale structures [71,92]. EBB technology allows multimaterial and vertical printing, offering control over the spatial distribution of cells and the overall architecture of vascular networks, making it suitable for engineering conduits with defined luminal diameters and wall thicknesses [109–111].

However, a primary limitation of EBB is the lower resolution, limited to nozzle diameter, making it challenging to fabricate intricate microvascular networks or achieve high cell patterning precision [78]. Additionally, the relatively slow speed and the need for bioinks with specific rheological properties further restrict its application in generating complex, highly branched microvascular systems [62].

Despite these limitations, EBB remains valuable for creating macrovascular components and serves as a foundational approach for multiscale strategies. Direct EBB can be utilized for fabrication of macrovascular grafts, replicating key aspects of native vessel architecture and maintaining high cell viability and phenotypic stability [112]. However, these grafts often lack sufficient printing fidelity or mechanical stability. For smaller-scale constructs, EBB is commonly combined with other fabrication methods, such as molding or sacrificial materials, to enable multiscale vascularization and perfusion [58]. Nevertheless, multi-step fabrication processes can compromise cell viability and often require post-fabrication cell seeding, leading to non-specific cell adhesion [112,113].

To expand the capabilities of EBB, novel approaches such as Continuously Extruded Variable Internal Channeling (CEVIC) have been developed, enabling the fabrication of structured hydrogel sheets with internal striations and improved resolution. When combined with fugitive inks, this strategy allows the creation of perfusable microvascular channels. However, further optimization is required to improve structural stability and biological performance [114].

(Figure 3.a)

Other extrusion-derived methods have also been proposed. Coaxial bioprinting enables the fabrication of perfusable tubular structures through the simultaneous extrusion of core-shell materials, supporting the generation of multilayered vascular constructs in a single step. Despite its advantages, challenges remain in maintaining concentricity, controlling lumen geometry, and preserving cell viability due to shear stress [115–118].

Embedded bioprinting approaches, such as Freeform reversible embedding of suspended hydrogels (FRESH), use temporary support baths to prevent structural collapse and improve printing

fidelity, allowing the use of low-viscosity, ECM-based bioinks [78,119–122]. These strategies enable the fabrication of complex and perfusable vascular structures with improved resolution. FRESH approach enabled printing of acetic collagen at concentrations up to 20 mg mL⁻¹ with feature sizes as low as ~ 20 µm. However, the final resolution and performance remain dependent on the overall printing strategy and material properties rather than the extrusion process alone [123]. (Figure 3. i)

From a translational perspective, extrusion-based bioprinting remains one of the most versatile and scalable techniques, particularly for generating mechanically stable, large-diameter vascular constructs. However, its limited resolution restricts the fabrication of capillary-scale features. As a result, this approach is well-suited for macrovascular structures and structural support, but requires integration with higher-resolution techniques to achieve fully functional multiscale vascular systems.

3.2. Droplet-based bioprinting (DBB)

DBB, also known as inkjet bioprinting, is one of the most common 3D printing technologies for biological applications, based on the automated delivery of controlled volumes of bioink in a droplet fashion following a predefined pattern [124,125].

One of its main advantages is its high resolution (typically < 100 µm), attributed to the generation of small droplets [124,126–128]. It also enables the creation of gradients of biomaterials, cells, and growth factors, while maintaining high cell viability [125,129].

However, limitations include restricted biomaterial viscosity, potential cellular damage during jetting, and nozzle clogging [7]. These factors hinder the fabrication of complex, perfusable vascular networks with sufficient structural integrity [130,131]. Although DBB can generate microvascular models, its application to larger and mechanically robust constructs remains limited.

Electrohydrodynamic (EHD) jetting further improves resolution by using electric fields to generate smaller droplets. This approach has enabled the fabrication of microvascular networks with capillary-scale features when combined with sacrificial templates and suitable hydrogels [132]. (Figure 3. d)

From a translational perspective, DBB offers high precision and cell viability, making it suitable for controlled cell patterning and micro-scale applications. However, limitations in viscosity range and structural stability restrict its use in fabricating robust, load-bearing vascular constructs. Its primary potential therefore lies in *in vitro* models and microvascular patterning rather than clinically relevant vascular grafts.

3.3. Light-based bioprinting

3.3.1. Laser-assisted bioprinting (LAB)

LAB is a non-contact technique in which laser pulses propel bioink droplets onto a substrate, enabling precise material deposition and control over cell placement [124,125,133]. It supports a wide range of viscosities and minimizes shear stress on cells [119,134–137].

Despite these advantages, LAB presents challenges including long processing times, potential thermal damage, and limited scalability [20,63]. Resolution depends on droplet size, with smaller droplets enabling microvascular structures while larger ones reduce reproducibility [138].

Variants such as Laser-Induced Forward Transfer (LIFT) (Figure 3. k) and Two-Photon Polymerization (TPP) further improve resolution and cell viability, enabling the fabrication of complex microvascular structures [24,139–141].

3.3.2. Stereolithography (SLA)

SLA uses photopolymerization to fabricate complex 3D structures with high resolution, making it suitable for vascular networks [109,125,134]. It enables rapid fabrication independent of structural complexity and supports high cell viability [1,59]. However, it requires photopolymerizable bioinks and post-processing steps, and UV exposure may affect cell viability. Material selection is limited, and photoinitiators may introduce cytotoxic effects [59,133,142,143].

SLA has been widely used to fabricate vascular structures, including perfusable and multimaterial constructs, with recent approaches improving cell viability and structural fidelity [41,144,145]. (Figure 3. e) A Fast Hydrogel Stereolithography Printing (FLOAT) was proposed to support the continuous replenishment of the prepolymer solution or bioink below the curing part, reducing deformation and cellular injury caused by the prolonged exposure to environmental stress in the layer-by-layer process of SLA printing. This method achieved a multiscale embedded vascular network which allowed culture media perfusion, helping to support high cell viability and metabolic functions in the deep core of a large-size model, avoiding hypoxia necrosis [144].

3.3.3. Digital Light Processing (DLP)

DLP enables rapid fabrication through projection-based photopolymerization, allowing high-resolution and multiscale structures [146–148]. It supports the fabrication of complex vascular architectures with controlled mechanical properties and spatial heterogeneity [137,149,150]. (Figure 3. c) Limitations include the requirement for photocurable, low viscosity bioinks and potential cytotoxic effects from UV exposure and photoinitiators. Cell sedimentation during printing can also affect construct homogeneity [92].

Advanced approaches such as microscale continuous optical printing (mCOP) further enhance resolution and enable precise cell patterning in complex microarchitectures [151,152].

3.3.4. Volumetric bioprinting (VBP)

VBP enables rapid fabrication of entire 3D constructs without layer-by-layer deposition, improving cell viability and structural homogeneity [153–155]. It supports the formation of large, continuous vascular architectures and promotes uniform cell distribution.

However, limitations include restricted material availability and reduced control over fine structural features. Despite this, it represents a promising complementary approach for large-scale vascular fabrication [156,157]. (Figure 3. g)

From a translational perspective, light-based bioprinting provides high resolution and rapid fabrication, enabling precise control over microvascular architectures. Despite these advantages, its application is often limited by the availability of suitable photopolymerizable biomaterials that combine biocompatibility with adequate mechanical strength. This technique shows strong potential for microvascular networks and multiscale integration when combined with complementary approaches.

3.4. Indirect bioprinting

Indirect bioprinting relies on sacrificial templates that are later removed to generate hollow, perfusable channels. This approach enables the fabrication of vascular networks within bulk hydrogels.

Sacrificial material

Sacrificial materials such as Pluronic F127, agarose, and gelatin provide temporary structural support and can be removed to create channels with good structural integrity and resolution [3,98]. (Figure 3. f)

Molding or casting

Molding and casting techniques allow reproducible tubular structures but offer limited control over cell placement and vessel architecture [99,100]. (Figure 3. b)

Microfluidics

Microfluidic systems provide precise control over flow conditions and cellular microenvironments, enabling the development of physiologically relevant vascular models. However, scaling these systems to larger, clinically relevant constructs remains challenging due to limitations in geometry control, perfusion, and integration [72,101–106]. (Figure 3. j)

From a translational perspective, indirect bioprinting approaches are highly effective for generating perfusable vascular networks with complex geometries. However, these methods often depend on multi-step processes and may lack mechanical robustness, limiting their

scalability and clinical applicability. They are particularly well-suited for *in vitro* vascularized models where perfusion and reproducibility are prioritized.

3.5. *In situ* bioprinting

In situ bioprinting is increasingly considered in the context of vascular regeneration, especially in applications aiming for closer clinical relevance. *In vitro* engineered constructs usually require time expensive maturation in bioreactors, and their handling and transplant introduces sterility and contamination risks, compromising the safety and success of the procedure. Rather than fabricating constructs ex-vivo, *in situ* bioprinting strategy works within the native tissue environment, which may help the printed structures to be a better match to patient-specific geometries and local biological conditions, while also limiting the need for extensive handling after fabrication. Recent advances in 3D bioprinting include hand-held and *in situ* robotic bioprinters that can directly print on the body's surface or injured site, most commonly using EBB or DBB technologies. Although the technology is still developing and remains technically demanding, *in situ* bioprinting offers a practical direction for addressing complex or irregular vascular defects that are difficult to treat using prefabricated constructs [76–78,94].

From a translational perspective, *in situ* bioprinting offers unique potential for direct clinical application by enabling on-site tissue reconstruction. However, current limitations in precision, material control, and vascular complexity restrict its ability to generate fully functional vascular networks. Further development is required before it can be considered a viable strategy for multiscale vascular fabrication.

3.6. Complementary biofabrication approaches

Acoustic patterning provides a non-contact strategy for organizing cells with high spatial precision, making it well suited for microvascular and capillary-scale applications. By using acoustic fields to position endothelial and supporting cells within hydrogels, it enables controlled cellular arrangement without exposing cells to shear stress or nozzle-induced damage [107,108]. (Figure 3. 1)

This approach facilitates lumen formation, enhanced network connectivity, and hierarchical organization at the microscale, making it a valuable complement to bioprinting strategies.

From a translational perspective, acoustic patterning enables contactless and rapid organization of cells into predefined structures, offering unique advantages for microvascular assembly. However, the lack of structural stability and reliance on secondary stabilization steps limit its direct application in fabricating standalone vascular constructs. Its most relevant application is therefore in enhancing cellular organization within hybrid biofabrication strategies.

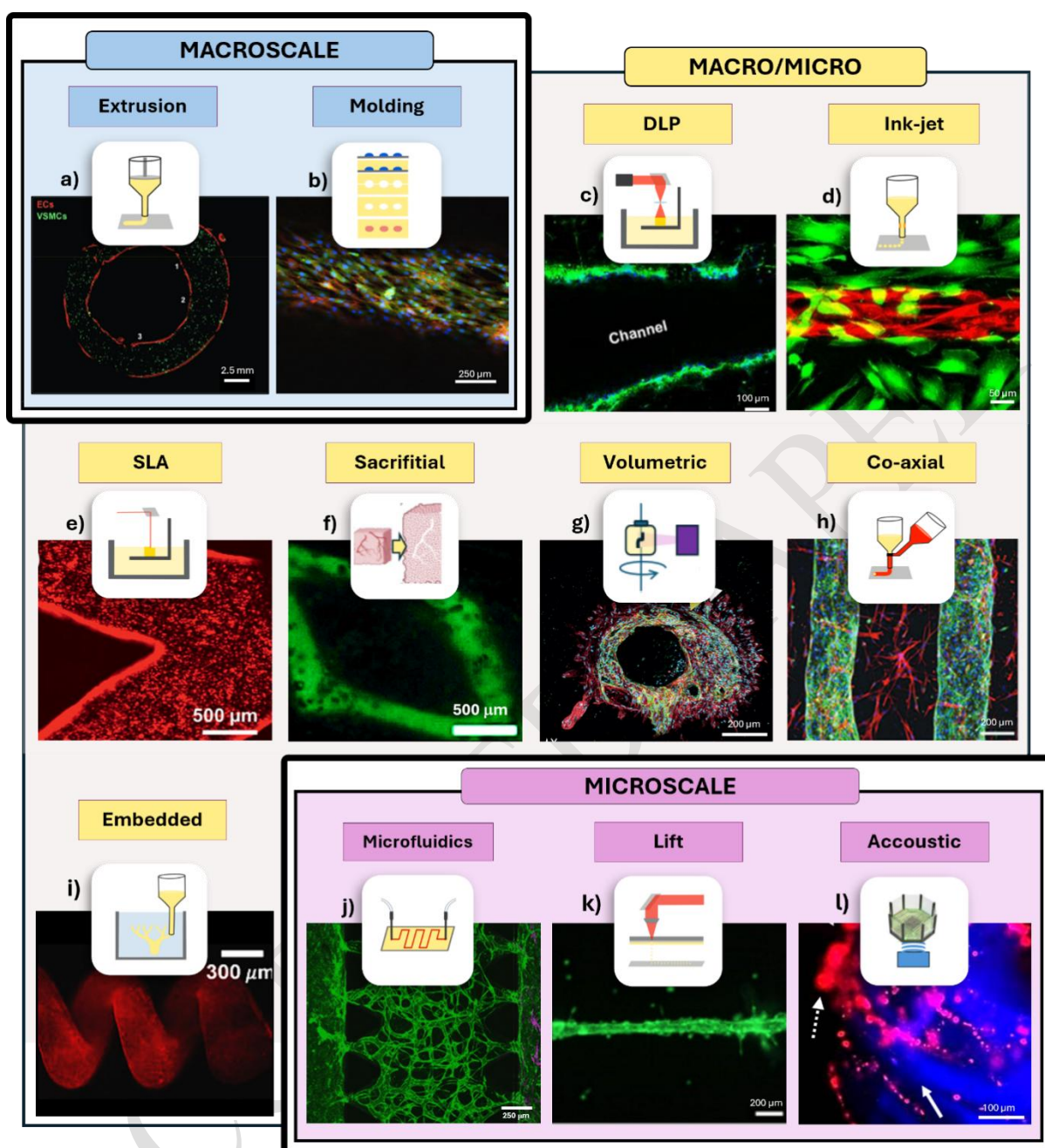


Figure 3. Representative vascular structures fabricated using the principal biofabrication strategies across different vascular scales. Current biofabrication approaches are largely scale-dependent and are therefore grouped according to the vascular dimensions they most effectively reproduce. Macroscale techniques (>1 mm) include (a) (EBB, which enables the fabrication of mechanically robust, multilayered vascular grafts suitable for surgical handling (Reprinted from ^[112]), and (b) moulding approaches (Reprinted with permission from Bertassoni et al ^[158] Copyright © 2015 Royal Society of Chemistry), which generate large perfusable conduits with well-defined lumen geometry. Intermediate macro-/microscale (50-1000 μm) techniques comprise (c) DLP (Reprinted from ^[149]), (d) DBB (Reprinted from ^[132]), (e) SLA (Reprinted

from ^[144]), (f) sacrificial bioprinting (Reprinted with permission from Highley et al. ^[159] Copyright © 2015 Wiley), (g) VBB (Reprinted from ^[156]), (h) coaxial bioprinting (Reprinted from ^[160]), and (i) embedded (FRESH) bioprinting (Reprinted with permission from Song et al. ^[161] Copyright © 2018 John Wiley and Sons), which allow the fabrication of perfusable vessels ranging from hundreds of micrometres to approximately 1 mm, while offering improved geometric complexity and spatial resolution compared with conventional extrusion. Microscale approaches include (j) microfluidics (Reprinted from ^[162]), (k) LAB (LIFT) (Reprinted from ^[138]), and (l) acoustic patterning (Reprinted from ^[50]), which are primarily employed to generate capillary-sized structures, endothelial patterning, or microvascular networks. Representative images correspond to tubular or vascularized constructs reported in the cited studies and illustrate the typical vascular scale achievable with each technology rather than direct performance comparisons.

Different techniques are often combined to construct multiscale engineered vasculature, overcoming a technique scale limitation with other technique. Table 2 shows the advantages and limitations between different bioprinting approaches for vascular bioprinting.

At the microvascular scale, fabrication of vessel diameters that approach those of native capillaries prioritize resolution and biological function. These microchannels can be obtained from light-based bioprinting methods or via ECs self-assembling within hydrogels, forming sprouts from pre-existing channels. These processes lead to dense microvascular networks which support mass transport and early inosculation. But construct thickness remains limited and mechanical stability is restricted. Indirect bioprinting plays a key role at the intermediate, or mesoscale, relying on sacrificial templating, moulding, or microfluidic-assisted strategies to create hollow and perfusable channels. Indirect methods enable controlled channel diameters and allow subsequent endothelialisation. They are well suited for distributing flow within engineered tissues and for linking larger conduits to microvascular networks. However, their mechanical strength is generally insufficient for direct surgical implantation. For artery-scale vessels, mechanical integrity becomes essential. Surgical handling is also critical. Extrusion and coaxial bioprinting are commonly used at this scale, producing millimetre-scale channels able to tolerate physiological pressures and which allow suturing to host vessels, achieving stable perfusion conduits.

Functional vascular trees are therefore constructed using hybrid strategies. Different fabrication techniques are combined across scales: large, printed vessels act as perfusion backbones, indirectly printed mesoscale channels distribute flow and microvascular networks form through

high-resolution printing or endothelial self-organization, obtaining a hierarchical organization that reflects native vascular architecture. It provides a practical way to bridge vascular scales in engineered tissues. Only by integrating fabrication strategies across scales can functional vascular hierarchies be achieved.

However, multiscale vascular bioprinting is not only related to the fabrication strategies but is strongly influenced by the properties of the commonly used biomaterials and bioinks. The fabrication of vessels across different length scales depends on material characteristics such as printability, mechanical stability, degradation behaviour, and interactions with cells. These properties directly affect whether capillary networks or surgically relevant conduits can be formed. Therefore, biomaterial selection is a scale-dependent design requirement rather than a separate consideration. For this reason, the following section focuses on natural and synthetic biomaterials for vascular bioprinting.

Table 2. Comparative of the advantages and limitations between different bioprinting approaches for vascular bioprinting.

BIOPRINTING APPROACHES			
	Advantages	Limitations	Ref.
DIRECT PRINTING			
EBB	Simple bioprinting process Flexibility in nozzle selection Printability of high-viscosity bioinks Printability of high cell densities Applicability of multimaterial bioprinting Good post-printing structural integrity Supports complex structures (vertical printability) Wide-range scalability Cost-effective and easy handling Widely available	Low printing resolution compared to other methods ($\sim 100 \mu\text{m}$ - <i>TRACE/FRESH-based bioprinting can reach 10-20 μm</i>). Moderate cell viability ($\sim 80\%$) Limited printing speed Nozzle clogging issues Post-processing requirements Difficulties in printing extremely soft or fragile bioinks Limited cell patterning and organization.	[1,3,109,119–123,125,134,163–165]
DBB	Simple bioprinting process High precision and resolution High cell viability ($> 90\%$) Applicability of multimaterial bioprinting Cost-effective and high availability Scalable High speed and efficiency	Limited to low cell density Limited to low viscosity bioinks Droplet size constraints Structural integrity issues of constructs Clogging and nozzle blockage Limited tissue thickness Challenges in multiscale integration Ink drying and evaporation	[3,109,120,122,127,129,133,163]
LAB	High resolution High accuracy in cell placement Printability of high cell densities No-contact method Biomaterial deposition in solid or liquid phase	Low cell viability Limited bioink options Limited to small scale Slow manufacturing process Expensive and comparatively complex setup	[119,124,125,133,134,166]
SLA	High resolution ($<100 - 300 \mu\text{m}$) and precision High cell viability ($\sim 90\%$) High variety of photo-crosslinkable bioinks No-contact method Smooth surface finish	Requirement of photosensitive bioink Comparatively complex system Common photoinitiator cytotoxicity Challenges in multiscale integration Post-processing requirements Mechanical weakness of printed constructs Increased wastage of source materials	[1,59,133,142,143]

	Wide scalability Potential for perfusable structures		
DLP	High cell viability (85 – 95%) High resolution (50 – >1000 µm) and precision No-contact method Rapid manufacturing process Controlled crosslinking and structural stability Customization and design flexibility Wide scalability Applicability of multi-material bioprinting	Requirement of photocurable, low viscosity bioink Possible to UV or photoinitiators exposure Challenges in generating perfusable capillary networks (< 10 µm) Print volume limitation Possible cell setting/sedimentation Moderate cost Limited access to commercial printers	[74,92,137,163,167]
VBB	Rapid fabrication capability Scalable process (mm to cm) Single-stem fabrication of 3D complex structures Minimum cell exposure to damaging conditions Uniform cell embedding inside intricate geometries	Requirement of photocurable ink Advanced imaging techniques for real-time monitoring Challenges in generating complex vascular networks	[3,118,153–155,168,169]
IN-DIRECT PRINTING			
Sacrificial materials	Enhances structural support Enables complex geometries Facilitates hollow channel formation Compatible with various bioprinting techniques Biocompatible and non-toxic removal methods	Post-processing requirements Potential residue contamination Limited mechanical construct stability after removal Potential for structural deformation during removal. Difficulty in achieving precise vascular diameters Limited material choices Compatibility issue with cell-laden constructs Scalability and reproducibility challenges	[3,98,122,125,170,171]
Moulding	Reproducible results Compatible with various bioprinting techniques	Difficult to create highly complex vascular structures Limited control over vascular diameter and branching Restricted scalability for larger tissue constructs	[58,75,99,100]
Microfluidics	Precise control of fluid dynamics and nutrient perfusion Real-time monitoring and perfusion of nutrients Mimics physiological conditions High resolution vascular models with tuneable properties	Challenging scale-up from microscale constructs Difficulties in maintaining long-term tissue viability and perfusion	[72,103,169,172–176]
COMPLEMENTARY BIOFABRICATION APPROACHES			
Acoustic patterning	Non-contact method High cell viability (90 – 95%) Cell phenotype maintenance Rapid and precise cellular organization (micro scale) Scaffold-free or minimally scaffolded constructs Multicellular and hierarchical patterning Integration with other bioprinting modalities	Low-viscosity hydrogels requirement Limited structural complexity and scale Limited mechanical integrity post-patterning Complexity of system design and control Attenuation and depth limitations Thermal and bioeffects at high power Not inherently perfusable Poor macro-scale complexity	[107,177–180]

4. Bioink selection for bioprinting vasculature

Beyond the capabilities and limitations of individual bioprinting techniques for generating vascular structures across different length scales, the successful fabrication of functional vascular networks critically depends on the properties of the bioinks employed. The design of

bioinks for vascular tissue engineering therefore requires careful optimization of their physicochemical characteristics, as these directly, cell viability and the mechanical integrity of resulting constructs. Key parameters include mechanical and rheological behaviours, viscosity and gelation kinetics, whose optimal ranges vary depending on the specific bioprinting technique used [92,181,182].

An ideal biomaterial in terms of immunological and inflammatory reaction in host, defines in the terms of degradation rate, toxicity and mechanical compatibility with native tissue which has yet to be found. Natural polymers such as collagen, gelatin, fibrin, and tissue-derived decellularized ECM (dECM) generally show good immune tolerance because they resemble components of the native ECM, but immune responses can still occur. These responses are often related to residual biological impurities, batch variability, or incomplete decellularization, especially in dECM-based materials. Modified natural materials such as gelatin methacryloyl (GelMA) are usually well tolerated. However, immune activity of biomaterials may relay on the degree of chemical modification and the presence of residual crosslinking agents. Synthetic hydrogels such as polyethylene glycol (PEG) and its derivatives are largely bioinert. They typically induce minimal immune activation. Their limited bioactivity, however, may reduce immune-mediated tissue remodelling. Sacrificial materials such as Pluronic F127 have minimal immune relevance. This is due to their transient presence during fabrication [183–191].

Bioinks used for vascular bioprinting mainly consist of biomaterials and cell-containing hydrogels, with high-water content beneficial for cell survivability and shielding the cell from fabrication induced forces. They need to form 3D structures with high resolution, suitable strength, elasticity and torsional resistance to be able to withstand physiological blood pressures, while also ensuring blood compatibility [1,7].

4.1. Natural biomaterials

Natural biomaterials are frequently employed in vascular biofabrication due to their inherent biocompatibility, biodegradability, and capacity to mimic the native extracellular matrix [118,181]. They offer an ideal microenvironment for cell adhesion, growth and proliferation, generally without eliciting chronic inflammation or toxicity to the host [7,109,192]. Additionally, their inherent biological cues facilitate cellular interactions critical for vascularization and tissue integration, making them particularly suitable for regenerative medicine applications [193]. However, these natural polymers often exhibit limited mechanical strength and rapid degeneration rates, compromising the long-term structural integrity and functionality of engineered vascular constructs [118,147]. To mitigate these drawbacks, they

are often functionalized or blended with synthetic counterparts, improving their rheological properties and degradation kinetics for enhanced structural support [148,194].

Collagen, a major component of the native ECM, is widely utilized due to its excellent biocompatibility and cell-interactive motifs, though its low mechanical stiffness often requires cross-linking or blending with other materials to achieve adequate structural support for vascular applications [181,185,195–205]. Gelatin, a denatured form of collagen, shares many of its biological advantages but possess enhanced processability and thermos-responsive gelation.[68,167,184,206–213] Functionalized gelatin, such as GelMA, further enhances printability and mechanical tunability through photocrosslinking, enabling precise control over mechanical properties and degradation rates, essential for neovessel formation [132,176,207,214–218]. Gelatin-norbornene is a another promising derivative that combines the biocompatibility of gelatin with enhanced photocrosslinking capabilities and improved mechanical stability, offering a refined platform for vascular biofabrication [149,193,212]. Despite this modifications, gelatin-based bioinks often encapsulate cells, limiting cell-to-cell interactions required for robust vascular network formation [7]. This can be overcome with micropatterning or co-culturing with supporting cells, facilitating the formation of perfusable vascular structures. Similarly, while dECM offer excellent biomimicry and support cell viability and function, their cost and complex preparation limit widespread applications [20,42,118,170,191,219–223].

Fibrin, which is a crucial protein in the blood clotting cascade, forms natural hydrogels that supports cell adhesion and proliferation, highly desirable for vascular tissue engineering due to its role in wound healing and angiogenesis [43,224–227]. Fibrin's rapid polymerization kinetics, initiated by thrombin, allow quick gelation, advantageous for structural integrity during printing, but also requires precise control to prevent premature clogging of nozzles [223,228]. Despite its biological activity, its mechanical properties, such as their Young's modulus, often are insufficient for high pressure vascular applications without reinforcements. Alginate is widely favoured in biofabrication due to its biocompatibility, low cost and hydrogel forming capability. With a porous structure that allows biochemicals diffusion, its mild gelation conditions make it suitable for encapsulating cells without compromising their viability, but to slow down its biodegradability and to improve their mechanical properties, functionalization are often made [15,26,211,229–233].

To further enhance control and bioactivity, researchers have also turned to hybrid bioinks, which leverage the strengths of both natural and synthetic materials, offering a range of advantages for vascular network formation or targeted support to cell function. However, a key challenge

lies in balancing biocompatibility with the potential for reduced cell responsiveness in highly synthetic environments.

4.2. Synthetic biomaterials

While natural polymers offer inherent biocompatibility, synthetic biomaterials provide superior control over mechanical properties, degradation rates, and chemical functionalization, making them highly attractive for engineering robust and functional vascular networks [234]. This level of control allows for precise tuning of properties such as stiffness, porosity and degradation kinetics to match specific physiological requirements of different vascular beds. Conversely, while synthetic materials often enhance mechanical tunability, they frequently lack inherent biological cues required for optimal cellular integration and function, requiring surface modifications or composite formulations to improve biocompatibility.

PEG and its derivatives is probably the most explored synthetic biomaterial for vasculature bioprinting [73]. Bioinert, easy to conjugate and with strong and tuneable mechanical properties, it improves bioprinting, shape retention and mechanical strength. However, it often requires surface modifications or functionalizations with bioactive molecules to promote cell adhesion and integration within the host tissue [148,235,236]. PEG is often modified into PEGDA (polyethylene glycol diacrylate) or PEGTA (polyethylene glycol triacrylate), enabling UV photocrosslinking and efficient cell encapsulation with adjustable mechanical properties and ability to form intricate microarchitectures via light-based bioprinting techniques, facilitating high resolution printing of complex vascular networks while maintaining cell viability and controlling biomaterial degradation [7,125,188,217,237–240].

PLGA (polylactic-co-glycolic acid), PLA (polylactic acid) and PCL (polycaprolactone) are widely recognized polymers for their versatility in tissue engineering applications, due to their tuneable mechanical characteristics, controlled degradation rates and ease of processing into intricate architectures. These polymers are particularly advantageous for their ability to modulate scaffold stiffness and degradation kinetics, crucial parameters for successful vascular regeneration. They provide high strength and stiffness, along with excellent processability, making them a viable option for fabrication mechanically demanding vascular structures [31,58,113,241–250].

Pluronic F127 is an example of a thermoresponsive polymer commonly used in vasculature bioprinting, with ability to self-assemble into a gel at physiological temperatures while remaining a solution at lower temperatures, which greatly enhances printability and cell viability during bioprinting processes. This behaviour allows for precise deposition of cell-laden bioinks without harsh crosslinking agents, facilitating removal of sacrificial components

in multi-material printing and creating perfusable lumens, and enabling intricate vascular networks with high spatial resolution and structural integrity. Because of its amphiphilic nature, it allows the formation of self-assembled micelles in aqueous solutions, with a potential for localized drug delivery that can promote angiogenesis. Despite its good printability, Pluronic F127 has inherent weak mechanical properties and limited cell binding, so it is mainly used as sacrificial material [220,251–259].

To provide a clearer and more comprehensive overview of the relationship between biomaterials, their vascular applications, relevant vessel scales, and fabrication strategies, Table 3 summarizes the key associations discussed in this section.

Table 3. Overview of biomaterials commonly used in vascular bioprinting, highlighting their typical vascular applications, relevant vessel scales, and associated fabrication strategies.

Biomaterial	Typical vascular application	Relevant vessel scale	Representative fabrication strategies	Ref
Collagen	Angiogenic matrices, capillary formation	Microvascular	EBB, embedded printing, self-assembly	[197,200,203,260]
Gelatin	Sacrificial templates, viscosity modifier	Micro–mesoscale	Sacrificial printing, embedded printing	[209,210,261–263]
GelMA	Perfusable microchannels, endothelialised networks	Micro–mesoscale	SLA, DLP, EBB, coaxial printing	[199,264–267]
Alginate	Perfusable tubes, coaxial conduits	Micro–mesoscale	Coaxial, sacrificial printing	[268–271]
Fibrin	Pro-angiogenic capillary beds	Microvascular	Coaxial printing, cell-driven assembly	[226,227,272,273]
dECM	Tissue-specific vascular niches	Microvascular	EBB, hybrid bioinks	[101,222,274,275]
PEG-based hydrogels	Structurally stable microchannels	Micro–mesoscale	SLA, DLP, EBB	[236,240,276–278]
Pluronic F127	Fugitive ink for hollow channel formation	Micro–mesoscale	Sacrificial and embedded printing	[257,258,279–283]
PLGA / PLA	Rigid vascular scaffolds	Mesoscale–macrovascular	Indirect printing, moulding	[247,284–286]
PCL	Implantable vascular grafts	Macrovascular	EBB, hybrid scaffolds	[248,249,287,288]

4.3. Structure proprieties of biomaterials.

Mechanical properties, permeability, and burst pressure are critical parameters for evaluating the functionality of bioprinted vascular constructs. However, the relative importance of these properties depends strongly on the target vascular scale. While burst pressure is a key

performance indicator for medium and large diameter vascular grafts, permeability and mass transport properties become increasingly important in microvascular networks. Therefore, successful vascular biofabrication requires balancing structural integrity with the transport characteristics necessary to support cellular viability and tissue function.

Natural hydrogels such as collagen, gelatin (or GelMA), and fibrin are characterized by low elastic moduli, typically in the range of 0.1 to 50 kPa, closely matching the mechanical properties of many native soft tissues. Collagen hydrogels exhibit highly-tuneable mechanical properties through crosslinking, conditions, concentration, pH and temperature. For example, increasing polymer concentration from 5 to 40 mg/mL, and adjusting pH and temperature, the elastic modulus can increase from 0.13 to 900 kPa [205]. Collagen also provides an inherently porous fibrillar architecture that supports molecular diffusion, cell migration and vascular remodelling. However, its rapid enzymatic degradation remains a significant limitation, with degradation rates of $72.8 \pm 0.2\%$ after 3 hours reported for native collagen. Functionalization to methacrylated collagen (ColMA) reduces degradability to $55 \pm 2.8\%$, while subsequent photocrosslinking further decreases degradation to $35.9 \pm 4\%$ [258]. In microvascular application, collagen-based constructs have demonstrated sufficient structural stability to maintain perfusion under physiologically relevant pressure gradients. For example, one study successfully mimicked postcapillary pressure differences of 5 – 10 mmHg between source and sink channels and maintained vessel integrity while allowing transport of 40 kDa dextran across the engineered microvascular network [289].

Similarly, gelatin and its derivative GelMA offer adjustable mechanical properties that depend strongly on their concentration and degree of methacrylation. Young's modulus can increase from 2.0 ± 0.3 kPa in a 5% GelMA solution to 22.0 ± 3.2 kPa for formulations with threefold higher concentration. The degree of methacrylation also regulates degradation behaviour by controlling crosslink density. Low methacrylation levels produce loosely crosslinked networks that degrade within days, whereas highly methacrylated formulation generates denser polymer structures with fewer accessible cleavage sites, resulting in prolonged stability. Beyond mechanical tunability, GelMA has become one of the most widely used bioinks for vascular bioprinting because its porous network supports nutrient diffusion while enabling endothelial cell adhesion and vascular maturation. Mechanical strength increases with concentration and crosslinking density; for example, Zeenat et al. estimated a theoretical burst pressure of 72.62 kPa (≈ 544.5 mmHg) for a 20% GelMA construct [290], whereas Siminska-Stanny et al. Reported experimentally measured failure pressures of 283.3 ± 39.97 mbar in printed vessels [291]. Although these values demonstrate adequate mechanical robustness for perfusion and

handling, their physiological relevance depends on the intended application, as microvascular constructs typically operate under substantially lower pressure conditions than arterial grafts. Fibrin also exhibits soft mechanical properties ranging from 0.5–5 kPa depending on its concentration. Its properties can be enhanced by combining it with other materials. For example, combining it with spider silk can generate structures with burst pressures of 362 mmHg [43]. This material possesses an intrinsic fibrillar structure that creates a porous network, allowing for high permeability and facilitating the transport of growth factors and cell migration. However, its degradation by plasmin is very rapid, necessitating combination strategies with other polymers to extend its functional stability [224–227].

Alginate offers a stiffness range of 10 to 100 kPa when crosslinked with Ca^{2+} ions at concentrations between 1% and 3% w/v [15,211], with burst pressures of 100 to 400 mmHg depending on the structure design [26]. Its permeability depends largely on the pore size generated during the gelation process, and its degradation can be controlled by crosslinking.

PEGDA and PEGTA offer the widest and most adjustable stiffness range compared to the materials analyzed in this review. Stiffness can be modulated between 10 and 1500 kPa depending on the concentration, molecular weight, and degree of polymer crosslinking. For example, PEGDA hydrogels created by both bulk crosslinking and 3D printing with a similar molecular weight of 700 Da had compression moduli in the range of 20 to 1290 kPa [240]. Their permeability can be precisely controlled by the crosslinking density and mesh size, although their use in vascular configurations with defined burst pressures is infrequent in the literature [237–239]. Degradable synthetic polymers such as PLGA, PLA, and PCL exhibit mechanical properties in the MPa range (around 10^3 – 10^6 kPa), which is substantially higher than the values obtained for the other materials studied [31]. Thanks to their superior mechanical properties, they can also withstand burst pressures exceeding 1000–3000 mmHg and long degradation times ranging from months to years [31,246].

Finally, Pluronic F127 is primarily used as a sacrificial material in fusion printing strategies, taking advantage of its reversible thermal gelation behavior. With storage moduli of around 1–16.5 kPa at 37°C, it is a thermoresponsive gel, so its rapid dissolution upon cooling allows for the creation of perfusable vascular channels within carrier hydrogels [258]. While it does not act as a permanent structural material, its function as a sacrificial template makes it an indispensable tool in the bioprinting of complex vascular networks.

Overall, the selection of biomaterials for vascular bioprinting requires balancing mechanical stability, degradation kinetics and transport properties according to the target vessel size and function. While high burst pressure and mechanical strength are essential for larger vascular

grafts, permeability and mass transport characteristics are often the dominant determinants of performance in capillary-scale and microvascular constructs, where efficient exchange between the vascular lumen and surrounding tissue is fundamental to physiological function.

4.4. Cell types used for bioprinting vasculature

Beyond the ink formulation, successful biofabrication of vascular constructs depends on the selection and incorporation of appropriate cellular components, which enable a more accurate replication of the native vascular microenvironment, essential for the development of fully functional tissue-engineered blood vessels. Beyond material formulation and printing strategy, cellular incorporation represents a functional design consideration rather than a descriptive listing of cell types. A variety of cell types, including ECs, SMCs and mesenchymal stem cells (MSCs) are frequently employed to replicate the intricate cellular architecture and functionality of native blood vessels. Each cell type plays a distinct role in vascular homeostasis and regeneration: ECs form the endothelium and innermost layer, SMCs contribute to structural integrity and vasomotor tone typical of tunica media, and MSCs offer vasculogenic and immunomodulatory properties. The use of fibroblast in an outer layer of the vessel mirrors native macroscale vessels. The integration of the diverse cell populations into biofabricated constructs is essential for achieving functionality, as their synergic interactions are critical for lumen formation, vessel maturation and long-term patency. Co-culture or additional incorporation of bioactive cues can enhance both the structural and functional integration [1,292].

ECs are crucial for forming the tunica intima, essential for maintaining blood compatibility and regulating vascular tone. They maintain blood homeostasis, assist in molecular exchange, interact with immune cells and stimulate contractile perivascular cells maintaining successful perfusion after transplantation. They are crucial for barrier function, nutrient exchange and vascular homeostasis [7,122]. Human umbilical vein endothelial cells (HUVECs) are frequently utilized due to their accessibility and established protocols for isolation and expansion [111,238,293]. Human microvascular endothelial cells (HMVECs), found and extracted from different tissues, are particularly valuable for microvascular applications due to capillary-like structure formation and angiogenesis capabilities [2,46,294]. Endothelial progenitor cells (EPCs) can be differentiated from induced pluripotent stem cells (iPSCs) or bone marrow and offer enhanced angiogenic capabilities, neovascularization and vascular repairing properties [20,122,295,296]. Pericytes are sometimes added as support cells, since they offer physical support to ECs and modulate their paracrine effects, regulating vessel permeability and regulation [11,297–299].

SMCs are a critical cell component for structural integrity and contractile properties, vital for blood pressure regulation and vessel reactivity. Human aortic SMCs (HAoSMCs) and vascular SMCs (VSMCs) have been used to mimic natural structure of blood vessels [165,239], and cardiomyocytes, less common, play a supportive role in vascularized cardiac patches, promoting paracrine signalling relevant to vascular health [300].

Fibroblasts are frequently integrated into co-culture as support cells, due to their capacity to produce ECM components, providing structural support and biomechanical cues [14,39,79,132,301].

MSCs are also frequently incorporated because of its multipotent differentiation capacity and paracrine effects, promoting vascular maturation, angiogenesis and modulating immune responses [136,232,302]. iPSCs also hold considerable promise for vascular tissue engineering due to their multipotent differentiation capability and immunomodulation [2,122,238].

These cell types together constitute the core cellular toolkit for most current vascular tissue engineering strategies, whether the goal is a small-diameter graft, a perfusable capillary network, or a large-scale blood vessel construct.

But cell-selection alone is often insufficient to obtain stable and functional vascular constructs immediately after bioprinting. In many cases, a post-print culture phase is mandatory, since dynamic cues gradually influence endothelial organization and matrix remodelling [303,304]. Under static conditions, long-term vascular stability is difficult to maintain, whereas flow-related stimuli support endothelial alignment and junction development. Exposure to physiological shear stress has been associated with improved endothelial integrity, reduced thrombogenic behaviour, and progressive deposition of vascular extracellular matrix [305–307]. Perfusion-based bioreactors introduce cell cultures to controlled shear stress in a pressure-driven micro-physiological systems, improving cell morphology and expression of endothelial marker in comparison to static culture conditions [12]. These perfusion bioreactors mimic the dynamic physiological environments of native blood vessels, providing mechanical stimulation that ultimately leads to the development of robust vascular-like constructs. These bioreactors are designed to generate negative pressure for enhanced cell seeding and dynamic fluid flow for uniform cell distribution and matrix deposition, creating conditions that closely approximate the *in vivo* microenvironment. The precise control over parameters such as fluid flow rate and pressure waveforms enables the preconditioning of vascular grafts, increasing luminal ECs density, reducing thrombus formation and enhancing mechanical properties such as burst pressure and resistance to shear stress through an increased production of essential ECM proteins [308–310]. Overall, these observations indicate that flow preconditioning and

perfusion bioreactors are important elements towards having a bioprinted vasculature with functional and physiological outcomes.

5. Bridging scales: multiscale bioprinting of vascular vessels

Significant progress has been made in vascular biofabrication over the past decade, with successful demonstrations of vascular structures across a broad range of length scales. At the macroscale, bioprinting technologies can now generate hollow conduits with dimensions, geometries and mechanical properties approaching those of native arteries and veins. At smaller scales, several techniques have produced endothelialised microvessels and perfusable channels with diameters below 100 μm , enabling controlled transport and barrier functions *in vitro*. However, it is important to distinguish these achievements from the generation of a true microcirculatory system. Despite substantial advances in microvascular fabrication, no current bioprinting technology has demonstrated the reproducible fabrication of a fully functional microcirculation that recapitulates the hierarchical organization, vessel density, perfusion dynamics and exchange functions of native capillary networks. Consequently, the fabrication of an integrated vascular tree spanning arteries, arterioles, capillaries, venules and veins remains an unresolved challenge (Figure 4).

A major reason for this limitation is that current manufacturing strategies remain inherently scale specific. Technologies capable of reproducing the dimensions and mechanical requirements of large vessels generally lack the resolution necessary to generate capillary-scale architectures. Conversely, approaches capable of producing microvessels often suffer from slow fabrication rates, or dependence on biologically driven self-organization processes [5,6,73]. As a result, no single bioprinting method currently provides simultaneous control over vascular architecture, mechanical performance, and physiological function across all vascular scales.

To address this limitation, most multiscale vascularization strategies combine complementary fabrication approaches. Typically, larger perfusable vessels are generated through direct bioprinting, while the microvascular compartment is established through endothelial self-assembly, vasculogenic, or angiogenic sprouting within porous biomaterials. These systems frequently incorporate biodegradable scaffolds, ECs, supporting stromal cells, and proangiogenic matrices to promote the formation of capillary-like networks. While such approaches can generate vascular structures spanning multiple length scales, the smallest vessels are generally formed through biological remodelling rather than direct fabrication.

Consequently, network architecture, connectivity, and functionality remain difficult to standardize and reproduce between constructs.

From a translational perspective, this distinction is crucial. Structures intended for clinical implantation require mechanical integrity and surgical handling properties, while *in vitro* models prioritize rapid fabrication, perfusion, and reproducibility. Therefore, multiscale vascularization should not be approached as a single optimization problem, but rather as the integration of complementary functionalities at different scales.

To overcome the limitations of individual fabrication methods, recent efforts have increasingly focused on hybrid manufacturing strategies. The integration of multiple biofabrication technologies within a unified workflow provides a precise route toward generating hierarchical vascular networks and complex tissue gradients. For example, a hybrid strategy combining the top-down microfabrication of tubular scaffolds via electrospinning with the bottom-up 3D bioprinting of cell-laden bioinks has successfully enabled the engineering of interconnected triple-scale vascular networks, spanning mechanically robust macrovessels, mesovessels, and organized capillaries [311]. Similarly, the integration of extrusion-based bioprinting with custom digital light processing (DLP)-printed static mixers allows for the creation of continuous cellular and stiffness gradients that mimic native tissue hierarchy while preserving high cell viability [312]. Such approaches aim to seamlessly integrate macro- and microvascular compartments along with multi-material transitions, significantly reducing the structural and biological trade-offs that currently constrain individual technologies.

Advances in biomaterials are also contributing to this transition. Four-dimensional (4D) bioprinting extends conventional 3D bioprinting by introducing constructs capable of undergoing controlled changes in structure, function, or biological behaviour in response to internal or external stimuli [313]. Smart bioinks with adaptive and stimuli-responsive properties provide new opportunities for generating dynamic vascular systems that better mimic developmental and regenerative processes observed in native tissues [60]. The combination of these materials with advanced bioprinting technologies may facilitate the generation of vascular constructs that evolve after fabrication, potentially improving network maturation, remodelling, and functional integration [314].

Alongside advances in manufacturing and biomaterials, increasing attention is being directed toward the incorporation of vascular cells during fabrication and the use of computationally guided vascular design. Integrating endothelial and supporting mural cells directly into printed constructs can promote earlier vascular stabilization and reduce the need for post-printing seeding procedures. In parallel, computational modelling and data-driven design approaches

are increasingly used to optimize vascular topology, perfusion behaviour, transport characteristics, and mechanical performance across multiple scales. These tools provide a more systematic framework for designing vascular networks that satisfy physiological constraints while improving reproducibility and scalability [315,316].

Overall, the field is moving away from single-scale approaches toward more integrated methods for constructing vascular networks. Faster printing, improved control over cell placement, and computational guidance help address various practical challenges, working in conjunction with existing bioprinting methods rather than replacing them.

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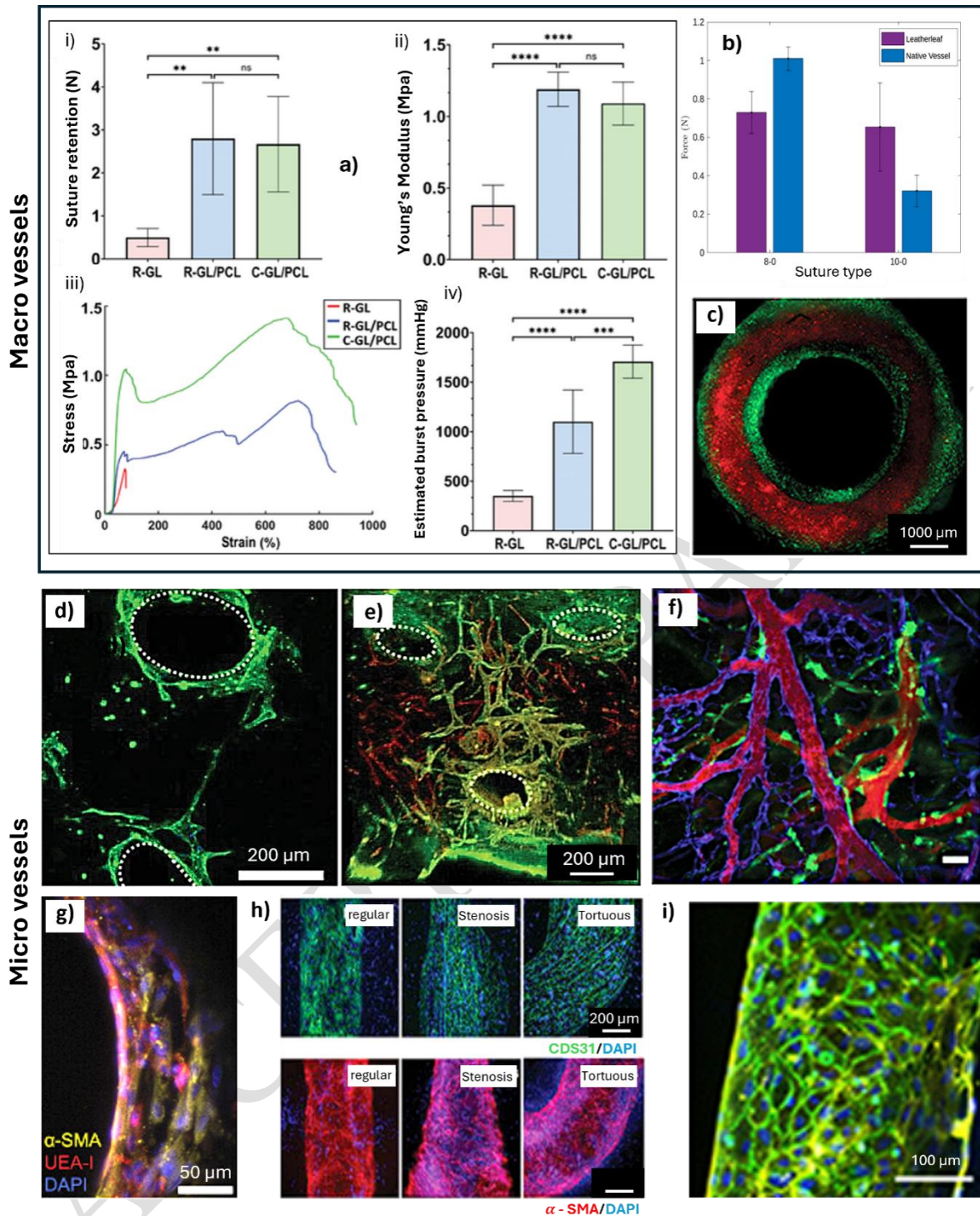


Figure 4. Vascular biofabrication features achieved both for macro and micro vessels. On the macroscale: a) Mechanical characterization of small diameter arteries mimics fabricated with electrospun gelatin (GL) and PCL nanofibers (PCL), with adequate mechanical strength (A) and similar Young's Modulus (B), tensile strength (C) and burst pressures (D) on the range of native vessels. (Reprinted from ^[11]). b) Suture retention comparison under max load of 8 – 10 and 10 – 0 sutures looped through small diameter vascular grafts and native vessels, meeting transplantation standards. (Reprinted from ^[308]). c) Cross-section of a multimaterial artificial

macro vessel populated by fibroblasts (outer layer green), SMCs (red) and HUVECs (inner layer green). (Reprinted from ^[301]). Regarding microvessel biofabrication: d-e) Capillary networks achieved can be achieved via endothelial sprouting after 14 of induction on the peripheral (d) and the centre (e) of the bioprinted tissue. (Reprinted from ^[160]). f) Hierarchical meso-microvascular implants in a subcutaneous mouse mode, with *in vivo* integration and perfusion of the engineered vessels (green) and host vessels (blue) with TRITC (red). (Reprinted from ^[113]). g) A mature endothelium of a printed vessel on day 7 of perfusion. (Reprinted from ^[68]). h) Immune response and atherosclerosis development of engineered vessels with various geometrical shapes. (Reprinted from ^[60]). i) Presence of CD31 marker expression showing full maturation of a confluent monolayer endothelial barrier (Reprinted from ^[317]).

5.1. Desing framework for multiscale vascular biofabrication

Successful multiscale vascularization should be regarded as a sequential design process rather than the selection of a single printing technology. The biological function required at each vascular scale determines the corresponding mechanical properties, bioink characteristics, fabrication strategy and post-printing maturation approach. Large-calibre vessels primarily require structural integrity, burst pressure resistance and surgical handling, whereas capillary-scale networks depend on endothelial self-organization, matrix remodelling and perfusion-induced maturation [311]. Consequently, the most effective multiscale strategies integrate complementary fabrication techniques rather than attempting to reproduce the entire vascular hierarchy using a single platform.

One of the principal challenges of multiscale vascular biofabrication is that the design requirements of macrovascular and microvascular compartments are often incompatible. Large conduits require mechanically robust biomaterials capable of withstanding arterial pressure, surgical suturing and long-term cyclic loading [26]. In contrast, capillary formation depends on soft, remodelable ECMs that permit EC migration, proteolytic degradation and cell-driven morphogenesis [311]. Consequently, material formulations and fabrication conditions that optimize macrovascular performance frequently compromise microvascular development, whereas matrices that favour capillary maturation generally lack sufficient mechanical strength for surgical implantation. Hybrid biofabrication strategies therefore arise from the need to reconcile these conflicting biological and mechanical requirements rather than simply combining different printing technologies.

From this perspective, fabrication technologies should be considered complementary rather than competing approaches. EBB provides structural support from macrovascular conduits

[71,92], SLA techniques offer improved geometric precision [109,125,134], sacrificial printing facilitates perfusable networks [3,98], while cell self-assembly and microfluidic approaches enable capillary formation [103,171,173,225,226]. Hybrid strategies therefore emerge naturally from the functional requirements of each vascular level rather than representing merely a combination of different printing methods.

Current evidence suggests that the most promising multiscale strategies combine complementary fabrication technologies rather than relying on a single printing modality. Representative strategies include EBB to fabricate mechanically stable macrovascular conduits integrated with sacrificial printing or microfluidic patterning to generate perfusable microchannels, while high-resolution photopolymerization techniques may be incorporated to reproduce capillary-scale architectures. Such hybrid approaches allow each technology to operate within the vascular scale for which it is best suited, thereby overcoming the intrinsic limitations of individual fabrication methods.

Successful vascular biofabrication should be evaluated not by the performance of individual printing technologies, but by their collective ability to generate mechanically stable, biologically functional and clinically translatable multiscale vascular systems.

5.2. Considerations for clinical translation

Although the combination of complementary biofabrication strategies provides a rational route toward multiscale vascular integration, successful clinical translation depends on factors that extend beyond fabrication itself. The generation of perfusable channels or endothelialized conduits does not necessarily ensure long-term functionality after implantation. Instead, engineered vascular constructs must rapidly establish stable anastomosis with the host circulation [113], maintain long-term patency, resist thrombosis [40], and undergo physiological vascular remodelling while preserving tissue perfusion [40].

Likewise, the biological integration of multiscale vascular networks requires coordinated interactions with the host microenvironment. Besides endothelial maturation, successful vascular regeneration depends on appropriate immune modulation [52,53], ECM remodelling [79,80] and the coordinated development of supporting systems such as lymphatic vessels and perivascular innervation, all of which contribute to long-term tissue homeostasis [79,80]. Consequently, macro-to-micro vascular integration should be regarded not only as a printing challenge but also as a dynamic biological process that continues after implantation.

Finally, future clinical translation will require manufacturing processes that are reproducible, scalable and compatible with Good Manufacturing Practice (GMP), together with standardized quality control, sterilization protocols and regulatory approval pathways. Therefore, future

developments should aim not only to improve printing resolution or bioink performance but also to ensure that engineered vascular constructs can be manufactured consistently and maintain long-term biological functionality following implantation.

6. Conclusion

Vascular bioprinting has progressed beyond early demonstrations to focus on developing integrated, multiscale vascular systems. While many existing methods perform well at specific scales, none alone can meet all the requirements for large, clinically relevant structures, including manufacturing speed, spatial control, biological performance, and mechanical stability.

Future developments will likely rely on combining different manufacturing approaches rather than further optimizing individual techniques. Extrusion-based methods remain the most viable for generating large-diameter, mechanically stable vessels, while light- and droplet-based techniques offer superior spatial resolution for microvascular features. Conversely, indirect and sacrificial strategies are particularly well-suited for the rapid fabrication of perfusable networks in *in vitro* models, where reproducibility and throughput are often prioritized over complete physiological fidelity. A key challenge for emerging technologies is overcoming current limitations by integrating rapid manufacturing, precise cell positioning, and computer-guided design. Taken together, these strategies represent a potential shift toward the next generation of vascular biofabrication and deserve consideration, either within the main text or as future lines of research.

Biomaterial design remains a limiting factor. Insufficient mechanical strength and degradation kinetics, among other factors, limit and compromise the quest to mimic the vasculature. New bioink developments must go further, focusing on dynamic and instructive biomaterials capable of evolving over time, providing both structural support and biochemical signals that guide endothelial organization and vascular stabilization.

The success of bioprinting advancements will depend on the proper integration of manufacturing methods, material design, and post-printing conditioning into a unified process. This approach should prioritize functional integration over isolated technical improvements and could thus help vascular bioprinting move closer to producing stable and perfusable tissues suitable for advanced models and future therapeutic applications.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

No primary research results have been included, and no new data were generated or analysed as part of this review. All information discussed in this review is based on previously published literature, which is cited throughout the text.

Further disclosure

During the preparation of this work, the authors used AI tools to improve the grammatical structure in some paragraphs. The authors reviewed all the references cited to ensure the accurate representation of the original research findings. After using ChatGPT, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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