ADVANCED MATERIALS TECHNOLOGIES
www.advmattechnol.de

4D Printing for Vascular Tissue Engineering: Progress and Challenges

Lubna Zeenat, Ali Zolfagharian, Yeleswarapu Sriya, Shyama Sasikumar, Mahdi Bodaghi, and Falguni Pati**

The hierarchical network of blood vessels comprises the larger vessels (veins and arteries), the smaller ones (venules and arterioles), and the thinnest capillaries. The proper functioning of most tissues in the body relies on vascularization, which is meant for the diffusion of gases, nutrients, and harmful waste. However, it is known that cell survival is compromised as the diffusion of oxygen is limited beyond 100–200 µm and damage can occur at any level of the complex system of the vascular network, as is the case in cardiovascular, musculoskeletal, and neurovascular diseases that recur and progress with age. These may prove fatal, hence the need for vascular tissue engineering (VTE) arises. VTE mainly focuses on the fabrication of vascular constructs using natural, synthetic material, or a combination of both using various techniques. The construct is expected to integrate and anastomose with the host vasculature. 4D bioprinting is an emerging field that allows the fabrication of hollow tubes employing different materials that respond to different stimuli. This review is a comprehensive summary of the major techniques employed in VTE and the recent technique of 4D bioprinting foreseen to revolutionize the field.

1. Introduction

Vasculature related diseases such as cardiovascular diseases and related conditions are killing millions of people every year. Despite pharmacotherapy being in place, surgical treatment is imperative in advanced cases of the diseases. Synthetic material large caliber vessels (10–30 mm) could be developed to replace autologous grafts, but small caliber grafts (lower patency rate than autologous ones) remain unapproved for clinical use. Besides animal and 2D models are not sufficient to study vasculature.[1]

Traditional techniques are faced with the challenges of patient compliance and low patency rate. Additive manufacturing has been promising and has been applied to various fields including regenerative medicine and tissue engineering. 3D bioprinting has allowed printing of biocompatible materials with cells to form complex structures. The latter has been used to print vasculature using fugitive inks using indirect extrusion-based bioprinting. Dual extrusion using two bioinks with multiple cell types have also been performed for vascularization of liver. Freeform reversible embedding of suspended hydrogels has been used to form hollow constructs using biomaterial; collagen, gelatin, and cells; myoblasts and fibroblasts (FBs). Droplets (pico-liters in dimensions) using inkjet printing have been used to form micro-vessel construct. Laser-based bioprinting has been used to form branched vasculature and overhangs using biomaterial and cells (human umbilical vein endothelial cells [HUVECs], FBs). Scaffold free bioprinting has also been used to self-assemble cells and use them for vascular models.[2]

Even though 3D printing is the popular technique for printing vasculature, most of the printing techniques are limited by the type of material to be used and the conditioning required post printing, besides these constructs possess no dynamic nature. Other issues include poor or lack of tissue maturation and simulation. With the advent of 4D bioprinting, the use of advanced smart biomaterials is progressing into a new era to create complex tissues with nearly human tissue resolution, with post printing maturation to address flaws and limitations of 3D bioprinting.[3]

This review provides a background on the different techniques used to fabricate blood vessels (BVs) and discusses the technique of 4D bioprinting that is foreseen to revolutionize the field of tissue engineering.

L. Zeenat, A. Zolfagharian
School of Engineering
Deakin University
Geelong 3216, Australia
E-mail: a.zolfagharian@deakin.edu.au
L. Zeenat, Y. Sriya, S. Sasikumar, F. Pati
Department of Biomedical Engineering
IIT Hyderabad
Kandi, Sangareddy, Telangana 502285, India
E-mail: falguni@bme.iith.ac.in
M. Bodaghi
Department of Engineering
School of Science and Technology
Nottingham Trent University
Nottingham NG11 8NS, UK

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/admt.202300200

DOI: 10.1002/admt.202300200

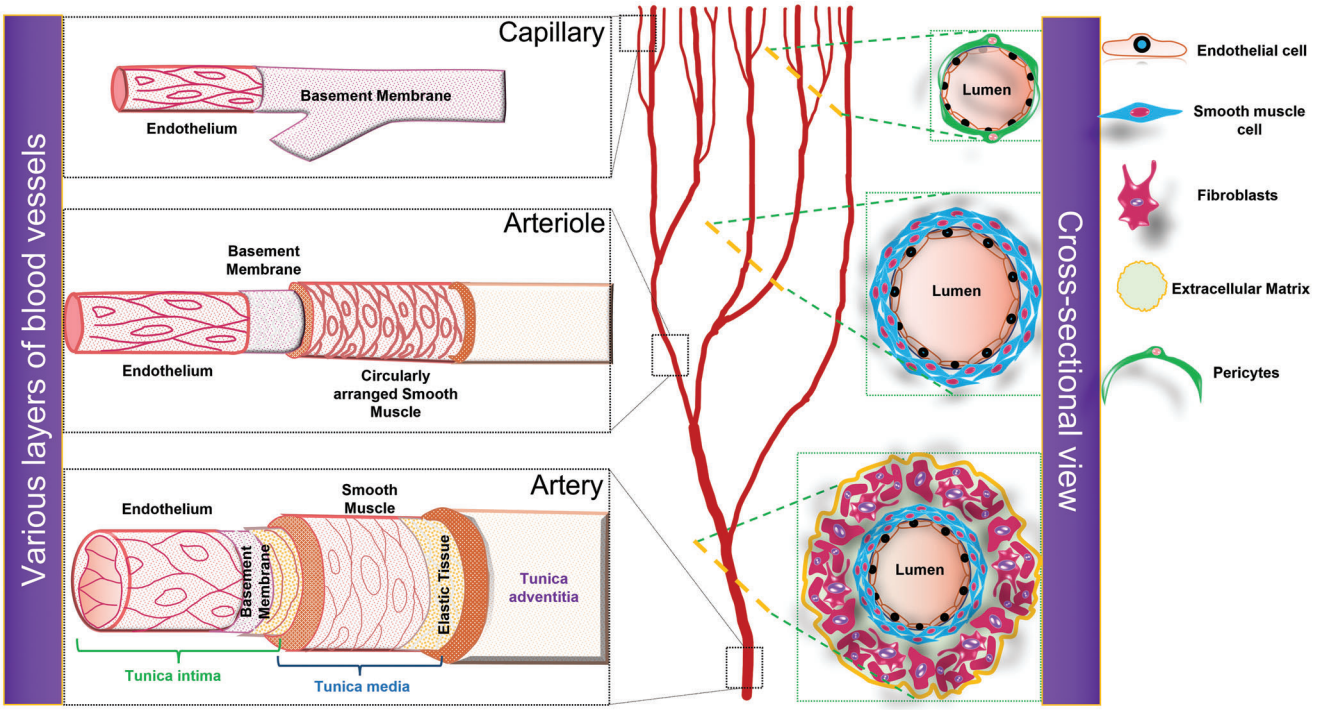

Figure 1. A detailed structural physiology of the blood vessel at various levels of size; artery, arteriole, and the thinnest capillaries.

2. Anatomy of the Blood Vessel

The structure of a BV is comprised of concentric layers that have cellular and non-cellular structures. Each layer is well distinct from the other layers. The innermost layer: tunica intima, consists of a monolayer of endothelial cells (ECs), smooth muscle cells (SMCs), and infrequently of monocytes and lymphocytes. The ECs are closely packed to form a solid barrier between the lumen and the wall of the vessel. This layer not just prevents infections but also thrombosis from occurring. The role of this layer is still complex in producing agents that help maintain the integrity of tunica intima. The ECs produce platelet-derived growth factor (PDGF) and platelet-activating factor and play a role in maintaining homeostasis.[4] Away from the lumen, toward the wall, the intima is composed of type IV collagen and laminin, which form its basement membrane. Surrounding the basement membrane is an elastin layercalled the internal elastic lamina.[5] The middle layer: tunica media, is composed of type I and III collagen with SMCs, arranged spirally, and involved in the contractions and dilations of the vessel.[6] This layer is thickened in large arteries and may receive the nerve supply. The external elastic lamina circumvents the middle layer (also called medial layer). The outermost layer: tunica adventitia, is composed of loose collagen, elastin, and FBs. The interaction of these layers allows for the perfusion of the blood.[7]

The vasculature is mainly divided into macrovasculature and microvasculature; the former is itself composed of separate entities, while the latter forms the network plexus, namely, arterioles, capillaries, and venules. The arterioles are the branched arteries, with an average lumen diameter of 30 µm. The thickness of all the macrovessel layers is reduced in the arterioles. Intima in case of arterioles is as intact but the middle and adventitia are thinned down. Tunica media has only one to two layers of SMCs, and tunica externa is also reduced in thickness. Arterioles are supplied with sympathetic nerves and control blood pressure. The connecting arterioles are called meta-arterioles, these act as sphincters for the arterioles continuing into capillaries, with the latter having a diameter of 5–10 µm. The capillaries are supported and stabilized by pericytes but lack the smooth muscle layers. This allows for easy diffusion of solutes between the capillaries and cells in the tissue. The capillaries are further classified as continuous, fenestrated, or sinusoid capillaries. The continuous capillary has a continuous basement membrane and the clefts between the ECs have tight junctions. These types of capillaries are found in the skin, lungs, and central nervous system. The fenestrated capillaries have intercellular gaps and thus are porous. They are found in renal glomeruli, intestinal mucosa, and exocrine glands. The sinusoidal capillaries have incomplete basement membrane with large gaps and are found in spleen, liver, and bone marrow. Capillaries reunite to form venules, which consist of the endothelium in the inner layer, elastic fibers, and muscle cells in the middle layer, while the external layer has connective tissue fibers.[8] **Figure 1** shows a detailed structural physiology of the BV at various levels of size (artery, arteriole, and the thinnest capillaries), the presence of various types of cells, and supporting cells.

Vascular network formation in both human embryos and adult cells occurs through vasculogenesis and angiogenesis. Vasculogenesis is the formation of vessels de novo from vascular progenitor cells, or the network plexus forms from circulating embryonic progenitor cells (EPCs). Angiogenesis is the formation of new BVs from pre-existing ones[9] (lumen of vessels bifurcates to form new ones), elongation, inosculation (recruitment of EPCs ex vivo

using growth factors such as stromal-derived factor-1 and granulocyte colony stimulating factor which are then transplanted into the host and allowed to form connections with the microvasculature), and sprouting. The response to pro-angiogenic signals causes sprouting, wherein ECs leave their dormant behavior and proliferate. If the basement membrane resorbs and ECs infiltrate and grow, such that there is lumen organization or tubular morphogenesis caused by extracellular matrix (ECM) and tubulogenesis-stimulating molecules secreted by FBs, along with the addition of pericytes adaptation to the tissue needs, it is said to have taken place. There is either regression or stabilization of the vessel. Regression is marked by loss of pro-angiogenic growth factors and blood, whereas stabilization is pronounced by the flow of angiogenic factors such as fibroblast growth factor (FGF), PDGF, vascular endothelial growth factor (VEGF), Ang-1, and Ang-2. The final stage is stabilization, where ECs return to their quiescent state and the adjacent cells adhere to each other and to the ECM as well. PDGF, transforming growth factor, Ang-1, and blood flow through the new sprouts. Thus, this process involves a considerable number of cell types, growth factors, ECM components, and cytokines.[10]

Spontaneous vascularization occurs for thin or avascular tissue upon in vivo transplantation of cell-laden constructs. This is not true for thicker tissues. Thus, the need to develop techniques that help to generate vessels to support the survival of large-sized tissues arises.[11] Large vessel diameters (>8 mm) such as aorta-like, and medium sized ones (6–8 mm, such as carotid femoral artery) have satisfactory results from synthetic vascular grafting. However, this does not work for vessels smaller than 6 mm (like coronary, infrainguinal, or infrageniculate arteries).[1]

3. Biomaterials for Vasculature

The earliest research in the fabrication of vasculature used non-biodegradable synthetic materials like Dacron, expanded polytetrafluoroethylene (ePTFE), which were non-biocompatible and had poor permeabilities across membranes. The surface properties (hydrophobicity, functional groups, charges, and topography) of a biomaterial affect the vasculature (microvasculature), though the exact mechanisms are not known. For instance, metallic surfaces exhibiting RGD and YIGSR peptides promote HUVECs proliferation[12] whereas fibronectin coating on a surface allows EC signaling via integrins.[13] Nanopatterned fibronectin is also known to influence EC behavior. Substrates formed as ridge-groove gratings have been known to assist organization of EPCs and tube formation, thus surface topography such as fibers, grooves, tubes, and crevices play a major role in angiogenesis.[14] It has been suggested that a larger pore size allows infiltration of cells and hence the secretion of angiogenic factors. Moreover, the stiffness of biomaterials is also found to affect vascular network formation. For example, ECs cultured on stiffer collagen (type 1) produced extra stress fibers of actin as compared to those cultured on softer collagen.[15] The physio-chemical properties have a role to play in the interaction between biomaterials and microvasculature. Cells interact with the ECM proteins via integrins and other receptors, while adsorption of proteins, for example, fibronectin occurs on the synthetic materials. Thus, the surface properties (and not the chemical composition of the material), for example, hydrophobicity, are responsible for protein adsorption and cell adhesion. It is also suggested that proliferation of cells is dependent largely on cell-spreading than cell–biomaterial contact.[16] Ultimately, the success of a biomaterial relies on its ability to support cell migration, adhesion, proliferation, and ECM production on the surface of the polymer. Hydrogels with their polymer chains resemble natural ECM, and both natural and synthetic polymers have been used for fabricating vasculature. To build complex vascular network, sacrificial materials have been used including gelatin, pluronics, alginate, polyvinyl alcohol (PVA), agarose, and sugar glass.[17–26] Collagen, which is a natural polymer, is known for not just cell adhesion, cell growth, and differentiation but also for enabling angiogenesis. Fibrin formed during clotting also fosters angiogenesis. Hyaluronic acid (HA) oligomers are known to promote EC sprouting, migration, and proliferation, and further in combination with angiogenic factors, promote angiogenesis. However, the fast degradation of HA fails to exhibit prolonged neovascularization; therefore, a spongy hydrogel of HA and gellan gum (HA-GG) was produced and exposed to macrovascular and microvascular ECs. It was known that while microvascular cells showed no effect, macrovascular cells exhibited higher proliferation.[27] Another study used polyethylene glycol (PEG) hydrogels loaded with fibrin to demonstrate angiogenesis in them as opposed to control PEG hydrogels. Arteriovenous loops consisting of collagen-elastin and collagen-glycosaminoglycan scaffolds could exhibit a homogenous vessel formation. Silk fibroin, alginate (alginate-gelatin capsules), chitosan (chitosan-fibrin scaffold), electrospun microfibers of collagen, and HA-based poly(L-lactide-*co*-ϵ-caprolactone) (PLC/COL/HA) are examples of biomaterials affecting EC behavior and promoting tubular vessel formation.[28]

The advantages of synthetic materials come from their mechanical properties that can be tailored with ease. In 1986, the first BV was fabricated using tissue engineering techniques that employed the synthetic polymer; Dacron. A Dacron mesh was used to support collagen gel that was cultured with ECs, SMCs, and FBs. This attempt proved the feasibility of using human cells on a tissue-engineered graft.[29] ePTFE scaffold variants with large fibrillar sizes are known to cause luminal endothelialization, polyurethanes (PUs) scaffold such as poly(carbonate-urea) urethane are known to be compliant with human arteries.[30]

The degradation of biomaterials is yet another important parameter that governs vessel formation. An example is from the study in which superior neovessels were formed with the release of VEGF by a degrading cell matrix.[31] Biodegradable polymers have therefore found use in the tissue engineering of vascular grafts (TEVG). TEVG obtained by seeding SMCs and ECs on polyglycolic acid (PGA) scaffolds, showcased high burst pressure and contractile responses to various chemical messengers.[32] A copolymer of poly(caprolactone) (PCL)-polylactic acid (PLA) used with woven PGA and seeded with SMCs and ECs exhibited good patency. Biodegradable polymers can also allow for the release of growth factors, for example, VEGF from polylactic glycolic acid (PLGA), which is known to promote angiogenesis in situ.[30] Among the biopolymers, collagen fibers and gels have been used for developing artificial vessels. Collagen fibers can limit deformation when subjected to high strain and thus prevent rupture of the vessel; their integrin binding sequences allow for cell adhesion. Use of collagen also minimizes the inflammatory or

antigenic responses. Fibrin is another biopolymer used for preparing vascular grafts, which along with embedded SMCs have allowed for better collagen and elastin deposition, greater elasticity, and patency up to 15 weeks. GelMA-based bioink[33] has been used along with sacrificial ink (thermosensitive); pluronic F127 to print two varying cell types, the entire structure was encased in a gelatin block. After the sacrificial layer was removed and HUVECs were perfused, there is lumen formation. Another sacrificial material called carbohydrate glass has also been fabricated with cell and lumen formation, along with sprouting was observed.[34]

A variety of strategies have been devised to fabricate the complex network of vasculature. These mainly fall into the following categories: traditional techniques, scaffold-based techniques, cell-based techniques, engineering-based techniques, and rapid prototyping and biofabrication techniques.

4. Techniques Used for Fabricating Vasculature

Traditionally, vascular grafts were prepared by harvesting an autologous BV segment from the patient's vein or artery. However, it was limited by the existing pathological condition of the patient and, therefore, the high failure rate caused by morbidity at the donor site.[35] The failure rate of the saphenous vein, the commonly used autologous graft, is 50% within 15 years of being implanted.[36] Synthetic materials used in place of autologous grafts, including ePTFE and polyethylene terephthalate (PET), have achieved significant results for long-term BVs in larger (greater than 8 mm) and medium-diameter vessels (6–8 mm). Patency increases up to 80% in 10 years for large-sized vessels and almost the same or higher value as autologous grafts for medium-sized vessels.[37]

Weinberg and Bell[29] constructed the first biosynthetic BV in 1986, which was designed to mimic a native BV. The structural layer resembled the adventitia, which was made of Dacron and contained the FBs, and it held ECs for intima (that were added once the construct was removed from the mandrel) and SMCs for media, grown in an annular collagen gel mold. The construct, however, did not exhibit the required burst pressure. This study, however, paved the way for other research on the use of synthetic biocompatible material for fabricating vasculature. L'Heureux[38] in 1988 constructed a sheet of SMCs wrapped around a mandrel. The SMC layer was then covered with a FB layer, also grown as a sheet. Later, the inner wall of the tubular structure was seeded with ECs. This multilayer approach took around 8–12 weeks to complete, this time frame was a limiting factor in this study, and it was even longer if the cells were harvested from the patient directly.

Traditional methods for vasculature fabrication include solvent-casting, gas-foaming, particulate leaching, and phase-separation. These techniques, however, do not offer much control over pore-size and shape and lack precision in the design of the main construct. Solvent casting is a traditional method of generating scaffolds, in which polymers are dissolved in a solvent containing salt as a porogen, to generate a porous structure by leaching out the salt and seeding cells. A tubular, bi-layered architecture has been made using a hydrogel of chitosan-gelatin by solvent casting. The microporous structure on the inner side allowed FB attachment and proliferation, while the outer side provided mechanical support.[39] Gas foaming is used to generate porous scaffolds, which are formed by the expansion of gases upon releasing high pressure. Carbon dioxide has been used to form a PLA/PCL blend that may be used to form small diameter BVs.[40] Thermal-induced phase separation has been used to generate micro-tubular-oriented scaffolds such as those of BVs.[41]

4.1. Scaffold-Based Techniques

4.1.1. Scaffold Prevascularization

A tissue construct containing cells requires nutrient and gaseous exchanges apart from biomolecules to maintain the viability, proliferation, and metabolism of the cells. Microfluidic networks can be used in vitro that allow perfused culture media to supply the requisites. But the challenge during implantation in vivo is that the time taken for the angiogenesis to occur is large, and hence the tissue construct undergoes ischemia in the embedded cell population. This process further triggers apoptosis and necrosis, thus reducing cell viability. One of the ways researchers have approached this issue is by incorporating a microfluidic network in the construct, followed by seeding a monolayer of ECs and suturing them to the host's arteries and veins. However, blockage of the microfluidic network hinders the entire process, hence the prevascularization of the construct becomes pivotal with subsequent seeding of multiple cell types.[42] There are various techniques to prevascularize the tissue, for instance the arterio-venous loop, in which the vascular cells (ECs, SMCs), angiogenic factors, and fragments of micro-vessels are cultured in vitro and in vivo and later seeded with tissue-specific cells.[43]

The prevascularized graft can model and support tissue growth and integration with the host vasculature. For instance, a prevascularized collagen scaffold seeded with ECs, FBs, and keratinocytes took only 4 days to integrate with the host vasculature as opposed to 14 days for a non-vascularized scaffold.[44] A multiculture is also known to support capillary formation better than a coculture system.[45] The in vitro prevascularization also majorly depends on the physiological conditions applied and maintained during the culture including the dynamic pressure and flow rate, and pulsation to form the vascular network. Stress and strain applied in a cyclical mechanical manner for 8 weeks caused the SMCs seeded on poly-L-lactic acid (PLLA) to proliferate, align, and produce collagen[46] and a 25-day biomechanical stimulation on seeded SMCs and ECs on PGA scaffold promoted ECM deposition and capillary formation.[47] A hypoxic environment caused the cells to produce VEGF, in turn promoting vascularization in vitro.[48]

Prevascularization of scaffolds in vitro requires microfluidic networks delivering culture media in bioreactors to porous scaffolds. The fate of the capillary bed was still uncertain; hence, researchers switched to in vivo prevascularization of the tissue construct.

4.1.2. In Vivo Scaffold Culture

In this technique, angiogenic and cell-loaded constructs are implanted in vascularized live tissue for the capillaries to form as a

result of angiogenesis. The scaffolds embedded with angiogenic factors undergo sprouting with the controlled release of VEGF. The scaffold also contains the autologous cells to produce VEGF since the half-lives of angiogenic factors are short. Thus, the scaffold is first implanted in a vascularized tissue close to arteriovenous loop to promote vasculature formation.[49] When the scaffold forms a capillary bed, it is explanted, seeded with multiple cell types, and then implanted at a specific location in the host body. For example, in one study, Matrigel enriched with FGF-2 was implanted in the diabetic mouse first around the epigastric region. In 21 days of culture, the Matrigel was replaced with vascularized adipose tissue. Later, the seeding of pancreatic islets in these chambers helped to reduce diabetic sugar, referring to better viability and proliferation of islets than in non-vascularized chambers.[50]

4.2. Cell-Based Techniques

4.2.1. Coculture of Cells

Cocultures of ECs and growth factor-producing cells form the major cell-based techniques that have been utilized to form the vasculature. Multicellular 3D spheroids with encapsulated ECs or simple mixing of cultures have been used to fabricate vasculature for skin,[51] bone, skeletal muscle,[45] and adipose tissue. ECs are cocultured with SMCs, MSCs, and FBs. These promote capillary formation, especially with VEGF and basic fibroblast growth factor (bFGF) or coculture with FBs. FBs have been cocultured with HUVECs to generate a stable capillary bed within a span of 14 days. A coculture of MSCs and EPCs not only generated capillary tubes but also the MSCs differentiated into pericytes, further supporting the structure.[52] Although capillary formation has been successfully observed in spheroids, poor anastomosis with the host tissue remains an issue.[53]

Growth factor-producing cells are those that are transfected to overexpress angiogenic growth factors. They tune the migration, proliferation, and maturation of vascular cells into tubular structures by the release of cytokines while being seeded into a biomaterial scaffold. It has also been seen that VEGF transfected cells produce a higher rate of vascularization than scaffolds coated with VEGF plasmids.[54]

4.2.2. Cell Aggregates

The characteristic features of cells such as self-organization and self-assembly help them to develop into tissues and organs, in vivo.[55] Single-cell or multiple-cell aggregates are bioprinted into filaments, spheroids, or cell pellets. Cell pellets, in contrast to spheroids, do not cause nozzle clogging and maintain high cell density, but the organization and fusion of cell pellets require a scaffold. In one example, cell pellets were organized into tissue strands inside permeable alginate capsules, these tissue strands were then made into a 3D structure with layer-by-layer deposition using a bioplotter.[56] Thus, they may be applied to form vascular tissue network.[57] Another study used 3D spheroids or cylinders comprised of many different cells to cause self-adhesion and generate tubular structures.[58]

4.3. Cell Sheet Engineering

A cell-ECM is formed by culturing tissue-specific cells on a temperature-responsive material. The vascularized tissue is formed by stacking a monolayer of ECs with layers of tissue-specific cells in a sandwich-like fashion or coculturing to create multiple cell-sheet layers around a mandrel.[59] In a major study, HUVECs and FBs were cultured in an ascorbic acid-rich medium to stimulate the production of type I collagen. The cell sheets were manually peeled from cell flasks in 30 days' time and wrapped around a mandrel to fabricate tunica media and adventitia. After a maturation period of more than 8 weeks, the inner lumen was endothelized, and a membrane was placed between the intima and media to prevent SMCs from infiltrating into the lumen, much like a native vessel. Cell sheet-engineered vascular tissue is found to produce ECM components much like the native vessel (collagen I, III, fibronectin, chondroitin sulfate, laminin). The burst pressure and mechanical and suture strength were also found to be adequate. The manual peeling of sheets may cause tearing and the sites may act as bursting sites. This problem can be overcome by preventing the use of external forces and using temperature responsive polymers. Agarose-based self-assembled patches have been fabricated as cardiac patches, where SMCs were seeded either on the agarose surface or ring-like structures to form cohesive vascular rings. The drawback of cell sheet engineering is the longer period required for fabrication and maturation.[60]

Temperature-responsive materials such as poly(*N*-isopropylacrylamide) (PNIPAM) coated on a polystyrene tissue culture plate can be used to form tubular structures from cell sheets by attachment and detachment. PNIPAM is hydrophobic at human body temperature (37 °C) and thus allows cells to remain attached and proliferate. On lowering the temperature, PNIPAM turns hydrophilic and absorbs water, thus releasing the confluent layer of cells. Cardiac tissue with two triple-layers and single six-layers has been made to overlay on a vascular bed in a perfusion culture. While the cell density in the former case was observed to be higher, it was attributed to increased cell viability and vascularization.[61] Multiple aligned sheets of MSCs wrapped around a mandrel and allowed to mature before being perfused with EPC's were developed. They resembled the native vessel in form and structure.[62]

4.4. Decellularization

Decellularization is the process of obtaining the ECM while removing the physical, chemical, and enzymatic agents that are otherwise responsible for producing an immune response. The ECM maintains the structure, composition, biological, and mechanical cues that advance the processes of cell adhesion, migration, proliferation, differentiation, organization, and remodeling.[63] Thus, ECM under ideal conditions does not elicit any immune response.[64]

BVs from various sources, such as canine, bovine, ovine, and porcine can be decellularized to obtain decellularized vessel grafts (DVGs). DVGs from the porcine carotid artery have much greater compliance and similar burst pressure to native vessels. Thus, adequate mechanical strength and porosity is maintained,

and infiltration of SMCs is observed on implantation in a canine model.[65] It has also been seen that the patency rate is increased whereas the thrombosis rate is lowered in DVGs recellularized with ECs or cells derived from bone marrow.[66]

The grafts prepared from decellularization are made by culturing cells (FBs, ECs, endothelial progenitor cells) on natural or synthetic scaffolds. Once ECM is produced by the cells in sufficient amounts, the entire structure is decellularized into a DVG. The latter can be integrated into the host BV for further ECM deposition and infiltration of cells. For instance, FB and SMC infiltration was observed with significant deposition of collagen and elastine in DVG produced by decellularization of ECM produced by culturing FBs in a fibrin tube and implantation in an ovine model.[67]

BV decellularization uses several protocols employing physical, chemical (surfactants), and biological (enzymatic) methods. Sodium dodecyl sulfate (SDS) has been used for most vascular decellularization protocols; the latter removes cells by first solubilizing the membrane, followed by dissociating the DNA. Other chemicals used include Triton X100, and tributyl phosphate (TNBP). The enzymes widely used include DNAses and trypsin, the latter causes proteolysis by acting on native collagen and elastin,[68] while DNAses remove the nuclear material. Thus, the detergents remove cells, and the enzymes cause proteolysis.[69] A study combined Triton X100, TNBP, and DNAse in three physical methods of static state, agitation, and perfusion and found that the latter two conditions were able to decellularize better without leaving any debris as in the static condition.[70] Further, it has also been demonstrated by various experiments that SDS works better than Triton in decellularizing vascular tissue, however, a combination of both has been known to cause effective decellularization of carotid arteries.[69]

4.5. Lyophilization

Lyophilization is the process of causing the loss of water at low temperatures, changing an aqueous state into a solid state. In vascular tissue engineering (VTE), lyophilization helps to produce stable, dry grafts, the properties of which remain preserved, but the process of calcification is reduced, helping in the long-term storage of the graft.[71] A study has used lyophilized grafts made of PGA and poly caprolactone-*co*-lactide (PCLA) for use in VTE with a diameter of the order of millimeters (0.91 mm) and a wall thickness of 300 µm.[72] Dual phase separation and lyophilization were used to develop vascular scaffolds using PLLA, poly(L-lactide-*co*-caprolactone) (PLCL), and poly(lactic-*co*-glycolic acid) (PLGA), with an outer microporous layer allowing SMC infiltration and a microporous inner layer supporting endothelialization. These scaffolds had good mechanical strengths but poor suture strengths in comparison to human mammary artery and saphenous vein.[73] Lyophilization has also been combined with electrospinning to fabricate large pore-sized scaffolds of bilayered PCL with heparin for easy infiltration of SMCs.[74]

4.6. Microfluidics and Micropatterning

300 and 500 µm diameter micropipettes have been used to generate pellets that contain SMCs and FBs, the pellets are extruded using 3D bioprinting to form spheroids and cylinders, which in turn fuse to form tubular vessels.[58] Micropatterning techniques like microcontact printing, micromoulding, and photolithography foster cell interaction with their topography and biological factors. Photo-reactive materials are used in photolithography, these are irradiated with light of a specific wavelength through a mask to generate patterns. GelMA hydrogels with different methacrylation degrees have been used to develop vascular tubes of varying diameters. Micromoulding on the other hand, uses a master mold with the required topography and geometry, the polymer is cast into it and later cured to form the required scaffold. Polydimethylsiloxane (PDMS) mold has been used to produce aligned cords of collagen gel encapsulating ECs, the capillaries so developed could become perfused once implanted.[75]

Micropatterned substrates have been used to form a capillary-bed-like structure, resembling a native vessel. Capillary formation is affected by several factors, such as ECM stiffness, shear, and interstitial flow. Researchers have used various techniques such as laser ablation, plasma itching, soft lithography, and replica molding to produce microfluidic patterns on substrates (biocompatible). In one study, a patterned single layer of a substrate was seeded with ECs, and mural cells (pericytes, SMCs) using the microfluidic approach to result in capillary formation after a precise culture period.[76] The use of synthetic substrates (such as polystyrene and PLGA) to form micropatterned EC monolayers show poor degradability and poor membrane barrier function. Thus, natural materials such as silk fibroin, fibrin, collagen (type II), and Matrigel have been used to print micropatterns to form endothelial tubes.[77] Khademhosseini et al.[78] constructed microfibrous scaffolds using a bioink of GelMA and alginate employing coaxial bioprinting. The encapsulated HUVECs form an endothelium monolayer while migrating toward the periphery of the fiber. Neonatal rat cardiomyocytes formed a layer of myocardium that was later combined with a microbioreactor to imitate heart-on-chip model. Thus, microfluidic-based vascular models can be fabricated using the approaches of microfabrication and 3D microfluidic printing, these, however, pose challenges of ease, expense, and convenience.

4.7. Engineering-Based Techniques

4.7.1. Electrospinning

Electrospinning is the process of producing polymer fibers in nano- and micro-scales in the presence of an electric field. It consists of a high-power voltage, a syringe pump, a syringe needle, and a metal collector. The high-power voltage generates the electric field, and the syringe pump fits the syringe and controls the flow rate. While the material extrudes from the syringe, it is collected on the metal collector. The polymer solution is held at the tip of the needle by the surface tension, and a Taylor's cone is formed and elongates under an increasing voltage. Continuous, twisted, and intertwined fibers are obtained with high viscous polymer solutions, while with low viscous polymers, particles are formed much like electrospraying.[79] **Figure 2** illustrates the process of electrospinning. Fiber alignment is the pivotal concern in electrospinning, for instance, with blends of silk with polydioxanone (PDO)/PCL, it was found that the diameters of

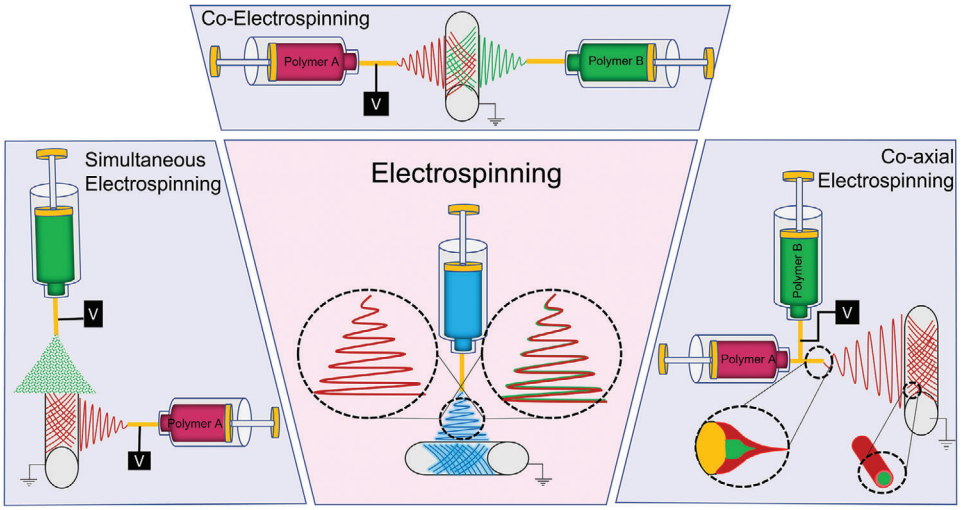

Figure 2. Illustration of the different types of electrospinning performed for the construction of vasculature.

fibers around an 80 mm mandrel were not affected by the rotational speed. However, for a smaller diameter of 4 mm at 500–8000 RPM, a considerable difference in diameter was observed. Thus, it was known that high packing density could be achieved for large diameter mandrels rotating at higher RPM.[80]

Electrospun vascular grafts (ESVGs) have evolved from single-layer, single-component to multilayer, multi-component. The fibers can be spun on a rotating mandrel or formed into a sheet that eventually rolls up. Natural or synthetic polymers have been used either as a single material or in combination to form (ESVGs).[81] On one hand, natural components like tropoelastin and silk fibroin[82] support endothelialization and SMC infiltration.[83] On the other hand, they have poor mechanical properties in comparison to autologous grafts.[84] A blend of PGA and poly(lactide-*co*-ϵ-caprolactone) (PLCL)[85] and that of collagen and elastin improves burst pressure and cell-scaffold interaction, respectively.[86]

In order to tune the degradation rate and mechanical properties, co-electrospinning is performed. For instance, PCL and PDO, where PDO allows fast degradation, assists in ECM deposition and cell infiltration, while PCL degrades slowly and thus maintains the tubular structure.[87] PVA and PCL/gelatin are co-spun where gelatin acts as a sacrificial material and results in increased porosity and cell migration.[88] Electrospinning can be combined with electrospraying in which fibers and particles are simultaneously deposited on a mandrel. In one study PCL/collagen nanofibers are electrospun with nanoparticles of heprasil (thiol-modified HA)-gelatin. Heprasil is responsible for cell infiltration by creating pockets upon enzymatic degradation.[89] **Table 1** summarizes the techniques and biomaterials used in the fabrication of tubular constructs.

To mimic the tunica layers of the BVs, multilayer sequential electrospinning is performed, in which the different layers are made of different polymers. For instance, an inner layer of PVA/poly glycerol sebacate (PGS) was fabricated, over which a PGA layer was electrospun over PGS, (PVA being the sacrificial material) forming a tube-like structure. Where PGS provides cell infiltration, PCL maintains structural integrity.[97] **Table 2** summarizes the various biomaterials employed in the fabrication of tubular constructs using electrospinning.

Two concentric syringe needles can be used to form a core–shell structure mimicking an ESVG in a process called coaxial electrospinning. The core layer can consist of a synthetic material, meant for mechanical strength while the biocompatibility is provided by the shell. Synthetic materials such as PLLA, PU, and PCL provide tissue-like mechanical properties with gelatin as the shell material to promote ECs and SMCs proliferation in a sandwich like coculture system.[107] A recent study that involves the parameter of time for the electrospun biopolymers and can be classified as a 4D biofabrication technique, made use of PCL/polyhydroxybutyrate (PHB). The polymers were electrospun into a bilayer, with one layer of soft thermoplastics (PCL) and other of HA-MA; or one layer of hard thermoplastics (PHB) and other of HA-MA. PHB-based layer folded transversely while the PCL-based folded longitudinally, further, PHB-based bilayer was mechanically strong and resisted any collapse during manipulation.[108]

4.7.2. Molding

Molding is the process of casting a polymer solution into a customized shape of the desired structure, the mold is removed upon solidification of the polymer. Thus, this technique can be used to obtain the prevascularization of tubular and branching vascular structures, called molded vascular grafts (MVGs).[109]

MVGs are fabricated by casting into an annular ring separated on the inside by an internal rod to set the inner and outer diameter and the wall thickness. This technique can be combined with any pore-generating technique to increase the porosity of the construct (**Figure 3**). For example, an MVG of PGS was fabricated and salt particles with diameters ranging from 5 to 150 µm were allowed to fuse before the addition of the polymer solution. Once the solvent was evaporated, a highly porous scaffold was obtained that facilitated cell infiltration. Larger salt particles (>75 µm) led

Table 1. Summarizes the various techniques and biomaterials used for the fabrication of vasculature.

Technique	Materials for VTE	Pros	Cons	Reference
Scaffold culture (in vivo)	Matrigel	Allows seeding of multiple cell types. Microfluidic networks can be integrated in it.	It is time consuming and the in vivo culture results in loss of donor tissue.	[90]
Cell sheet rolling	SMC sheet	Mimics the native ECM, and high resistance, non-immunogenic vessels are produced.	Obtaining cells directly from patients is a long process. Long culture periods are needed.	[91]
Electrospinning	PCL, PLCL, PVA, collagen, elastin, chitosan, Lecithin, silk fibroin, PLLA, PCU, PEUU.	Bioactive substances can be used, multiple layers can be generated with tunable mechanical properties.	Complex setup, use of high voltage and toxic solvents. The complex constructs are difficult to be fabricated.	[92]
Molding	PGS, collagen, fibrin, silk, hyaluronic acid, PLCL.	Micropatterned surfaces can be formed, employing pore generating techniques with simple device setup.	Complex channels cannot be formed. Toxic solvents are used, mechanical reinforcement is needed.	[93]
Decellularization	PCLA, PGA.	Preserves the ECM, mechanical and biochemical composition, in addition to 3D microarchitecture.	Recellularization of multiple cell types at precise locations is a challenge.	[94]
Laser degradation	Collagen, PEGDA.	Patterned hydrogels can be formed.	Increased time with decreased thickness of the construct.	[95]
Coculture of cells	ECs, FBs.	Multicellular spheroids are used. Overexpressed angiogenic factors.	It is time taking. Different cells cultured at the same time do not show capillary formation.	[96]
Bioprinting	Thermoplastics, resins, hydrogels.	Automatic, assists in the formation of complex constructs.	Limited availability of materials.	[2]

to cell infiltration up to 90%, while smaller ones (25–32 μm) facilitated up to 50% of the scaffold thickness.[110]

Knitted and non-woven meshes have also been used to improve mechanical strength and enhance the structural integrity of the structure. In one study, a Dacron mesh was placed between two layers of collagen, the inner encapsulated SMC, and the outer encapsulated FB, thus preventing any tearing or delamination.[29] Electrospun coating over molded structures is also known to provide additional strength. For instance, a cast poly(esterurethane urea) (PEUU) mold was coated with a thin electrospun fiber layer, causing the rupture rate to decrease by 50%. The electrospun fiber layer can also be of another synthetic polymer, just as in the case of coating electrospun PCL fiber on cast PGS for the tubular graft to withstand pulsatile pressure in rat aortas.[110]

The adhesion, proliferation, and recruitment, as well as the alignment of ECs and SMCs, respectively, have been found to be affected by the surface topography; thus, micropatterning of the inner and outer walls needed to be developed.[111] One method uses a thin layer of PDMS on the inner or outer surface before casting the polymer solution in an annular mold. Yet another example uses a grating (2 μm) into a molded PVA construct resulting in an increase in the graft luminal area.[110]

4.7.3. Laser Degradation

A cross-linked hydrogel can be formed into a patterned construct by selective degradation at predetermined regions. The mechanism works on the two-photon absorption profile of the hydrogel, where a low absorption profile generates high-intensity radiation, causing free plasma to form free electrons created in the process. The plasma causes a shock wave to be generated by compressing the surrounding liquid. The shock wave forms vapor bubbles upon propagation that rupture the hydrogen bonds, causing physical degradation of the hydrogel.[112] For those hydrogels that possess high two-photon absorption, the degradation occurs by the heat generated with a few seconds exposure to laser light. Both the chemical denaturation of proteins and heat dissipation assist in the degradation of the hydrogel. A short laser-pulse of high intensity causes degradation to take place at high-resolution.[113] This technique can therefore be utilized to generate vasculature. Table 1 summarizes the various techniques, their advantages and disadvantages, and the biomaterials used for the fabrication of vasculature.

4.8. Rapid Prototyping or Biofabrication

Biofabrication, or bioprinting, has transformed the area of fabrication for designing tissue constructs. It has exploited the ability to design complex, heterocellular structures with precise positioning of the materials and cells. Biofabrication holds a great scope to develop perfusable and transportable vascular structures. A great leap of progress has already been made toward forming perfusable and branched vascular structures.[114]

4.8.1. 3D Bioprinting

The introduction of 3D bioprinting in the field of regenerative medicine has generated great possibilities for patient-specific

Table 2. Summarizes the various biomaterials employed in the fabrication of tubular constructs using electrospinning.

Biomaterial	Features	Pros	Cons	Reference
Poly(glycolic) acid (PGA)	One of the first biodegradable polymers used for biomedical applications.	In vivo electrospun PGA can support SMCs up to 5 days.	Its mechanical properties decrease with its rapid bulk degradation.	[98]
Poly(lactic) acid (PLA)	It exits in a semicrystalline (Poly(L-lactide) (PLLA)) and an amorphous form (poly (DL-lactide) (PDLLA)).	PLLA undergoes slow deformation, has high mechanical strength, and is resistant to hydrolysis. Electrospun PLA allows more SMC infiltration and lesser fibrotic effect than many other polymers like PGA.	PDLLA has lower mechanical strength.	[99]
Poly(lactic-*co*-glycolic) acid (PLGA)	It is FDA approved biopolymer for human use.	It has excellent biocompatible, mechanical, and degradation properties. Electrospun tubular nanofibers degraded very slowly compared to PGA.	PLGA degrades rather quickly in enzymatic environmental.	[100]
Poly(ϵ-caprolactone) (PCL)	It can be well blended with other polymers and has a good formability at low temperature.	It can be used for long-term application. Its slow degradation makes it a good candidate for vascular application. The suture strength and burst pressure for electrospun PCL is closer to native vessel.	Its use may cause local hypoxia to develop.	[101]
Poly(L-lactide-*co*-ϵ-caprolactone), P(LLA-CL)	Non-toxic with a suitable degradation period.	Electrospun nanofibers of P(LLA-CL) support long term viability of cells.	The structural integrity and mechanical strength are less.	[102]
Polydioxanone (PDS)	Its biocompatible, bioabsorbable, and crystalline.	Electrospun PDS has a mechanical strength, burst pressure closer to collagen and elastin.	—	[80]
Polyurethanes (PUs)	They have superior mechanical and elastic properties.	(PUs)	Less biostable, susceptible to enzymatic, oxidative, hydrolytic degradation.	[103]
Collagen and elastin	They are the principal proteins in a blood vessel.	Small diameter vessels can be made by electrospinning soluble collagen and elastin.	The source of the material causes varied risk of infections, degradation, and physiochemical properties.	[84]
Silk fibroin	It is hydrophobic, crystalline, and environmentally stable.	Electrospun chemically modified silk fibroin mitigates thrombogenicity.	Its degradation rate is not suitable for long-term use.	[104]
Gelatin	It is denatured collagen.	Electrospun gelatin has higher tensile strength and a collagen comparable diameter.	It is temperature sensitive. Liquid at 37 °C.	[105]
PCL and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)	PHBV blended with PCL shows better results than PHBV used alone.	The electrospun blend can be made into small-diameter vessels with good mechanical strength.	Pure PCL has better cytocompatibility.	[80]
Collagen, elastin and PCL, PLCL, PLGA, and PLLA	The electrospun nanofibers can be made into vascular grafts.	The scaffolds have suitable mechanical and dimensional stability.	—	[106]
Elastin and PDO	Fiber diameter is inversely proportional to polymer ratio.	Nearly same compliance as the native femoral artery.	Pure PDO has greater values for all tested parameters of the blend.	[80]
Silk fibroin and PLCL	The blend allows endothelialization better than individual polymers.	The properties of wettability, endothelialization, and mechanical strength are enhanced.	—	[80]

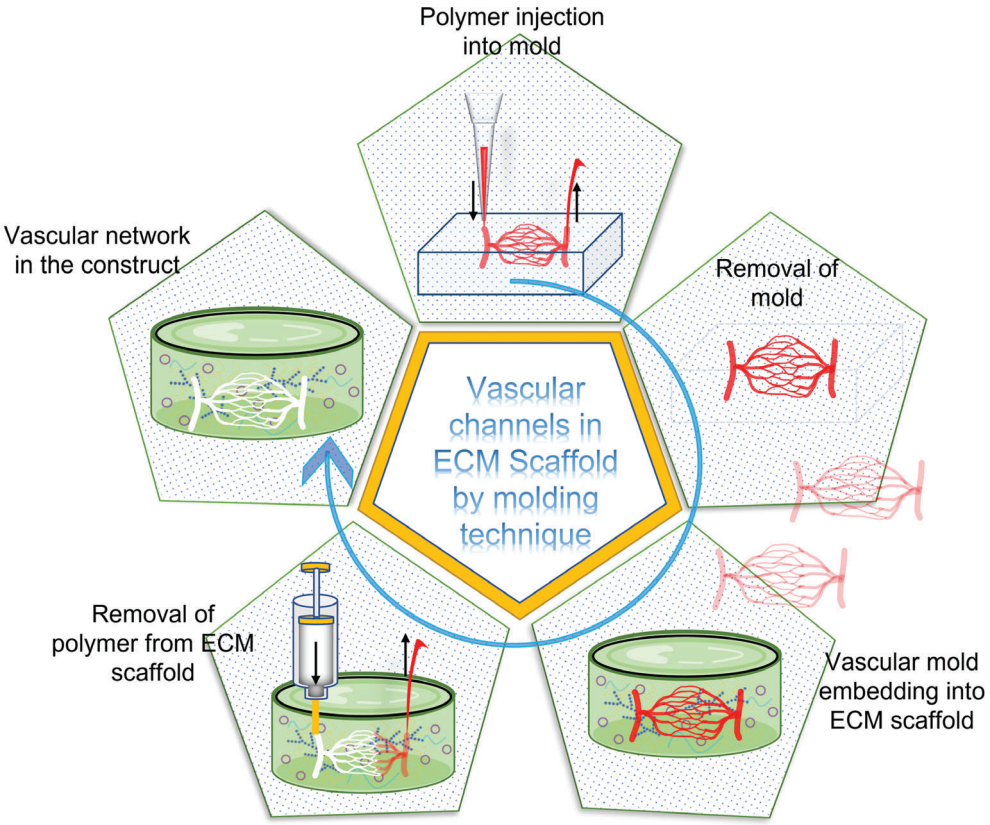

Figure 3. The process of molding in building the vascular network in a construct.

tissue regenerative therapy. 3D bioprinting is an additive manufacturing technique for constructing complex models of cells and tissues, with a layer-by-layer deposition using a 3D printer and guided by a computer-aided design model. It is marked by higher reproducibility, repeatability, precision and control over the external and internal geometry, cellular orientation, and spatial distribution to mimic the structure and function of their tissue (native) counterparts.[115]

For the 3D printed constructs, currently, there are two main strategies for the formation of the vasculature, one of which is the release of angiogenic factors to allow the growth of vasculature in the 3D printed construct.[116] The other is the direct printing of the vascular network around which the target tissue cells[114] are printed to form an organoid construct.

Extrusion is the most common technique for vasculature fabrication. Fused deposition modeling (FDM) uses molten filaments (thermoplastics) of a polymer extruded through the nozzle. Since this process involves heating at higher temperatures, bioinks containing cells and bioactive components are deposited using screw or piston-driven, pneumatic extrusion or solenoid forces (at or below physiological forces).[117] FDM allows the fabrication of printed vascular grafts (PVG), ranging from single tubular to bifurcated ones (single and multiple bifurcated grafts with diameters from 2 to 4 mm).

The nozzle diameter and the speed of extrusion decide the resolution of the final product. The properties of shear thinning behavior and thixotropy are well-suited for extrusion and interconnectivity in the scaffold and the pore geometry depends on the printing parameters.[118] Extrusion bioprinting has been used to fabricate a graft, 50 mm long, where FB aggregates were extruded between a supporting agarose gel made into two concentric circles. The aggregates finally fused to generate an aortic ring-like construct.[110]

Many materials have been used as sacrificial biopolymers in order to create constructs with channels. For instance, PDO hydrogels fabricated with channels made from sacrificial filaments of PVA exhibited higher suture retention strength (142 N mm^2) and high elastic modulus (9.5 MPa) and promoted attachment and proliferation of HUVECs. But the trapezoidal shape of the channel altered the blood flow dynamics.[119] **Figure 4** illustrates many examples of the use of sacrificial materials in 3D bioprinting.

Coaxial extrusion bioprinting uses a concentric extrusion of core and shell material, in which the core material causes the cross-linking of the shell. For example, a sodium alginate shell is cross-linked by a $CaCl_2$ solution diffusing from the core. The washing steps result in the removal of the core material, thus forming the mesochannels. The channels may be seeded with ECs.[126] The limitation of this study is that alginate requires modification (e.g., coating with Matrigel) in order to interact with HUVECs, and the polymer away from the core remains ungelled and needs to be washed causing a poor mechanical construct to form. In a recent study, a tri-layer coaxial printing was performed using pluronics as the sacrificial layer, sodium alginate and collagen to

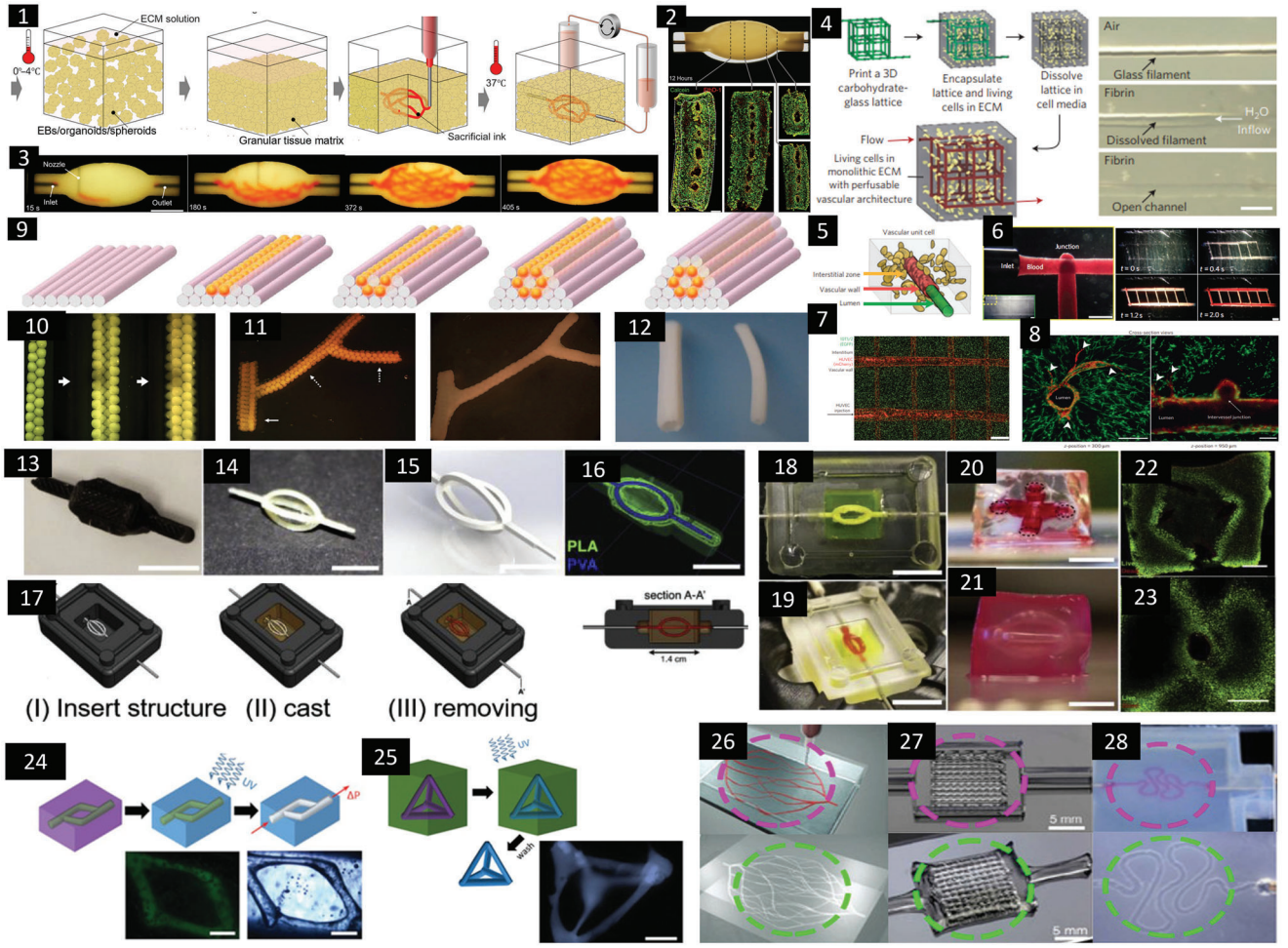

Figure 4. 4D printing of vasculature with sacrificial materials and cellular self-assembly. (1) shows the various steps of sacrificial printing/writing onto a functional tissue. (2) shows a perfusable tissue construct after 12 h of perfusion and the live/dead stain images at various sections of the construct. (3) shows an image of a sequence of a perfusable matrix in which a 3D bioprinted vascular network resides. 1–3) Reproduced with permission.[120] Copyright 2019, American Association for the Advancement of Science. (4) shows a carbohydrate self-supporting glass lattice used as a sacrificial material for vascular network. (5) shows the compartments of a vascular unit cell. (6) shows patterned channels that support pressure and flow. (7) shows the endothelium lining formed by encapsulating HUVECs in interstitial space. (8) shows endothelialization of intersecting channels. 4–8) Reproduced with permission.[121] Copyright 2021, Springer Nature. (9) illustrates the small diameter vascular channels made of agarose (pink) and multicellular spheroids (orange). (10) shows the assembly of spheroids. (11) shows the branched structure formed of the spheroids. (12) shows SMC tube formed by fusion post printing. 9–11) Reproduced with permission.[58] Copyright 2009, Elsevier. (13) depicts a branched construct with four curved arms made using PVA as a sacrificial material and PLA as support material. (14) shows the structure after PLA is dissolved. (15) CAD model of 3D vascular branch. (16) shows a visualized motion diagram where green is dispensing path of PLA and blue is dispensing path of PVA. (17) shows stepwise use of printed PVA as a sacrificial material in a fluidic mold, followed by casting of ECM over the template and removal of PVA and perfusion by a peristaltic pump. (18) shows a branched structure in a hydrogel before removal of PVA. (19) is a vascularized 3D construct. (20) shows volumetric distribution and stability of the channel formed. (21) shows the stiffer construct after perfusion. (22) shows the cross section of a perfused inlet of the channel. (23) shows the spheroid viability after perfusion for 15 days in the center and the channels. 13–23) Reproduced with permission.[122] Copyright 2018, Elsevier. (24) shows the printing of a channel by writing an ink into a support followed by UV cross-linking of the support gel and removal of the ink to form channels. (25) shows the writing of an ink into a support structure followed by cross-linking the ink and dissolution of support. 24,25) Reproduced with permission.[123] Copyright 2015, Wiley-VCH. (26) shows the printed microvasculature in a hydrogel that is omnidirectional. Reproduced with permission.[124] Copyright 2011, Wiley-VCH. (27) and (28) shows the fabrication of the convoluted proximal tubule using fugitive ink on a matrix. Reproduced with permission.[125] Copyright 2016, Springer Nature.

form a triple layer. The inner layer was loaded with HUVECs, while the outer layer was seeded with human aortic SMCs. A BV mimicking the natural one was formed.[127]

The other most common bioprinting methods are laser-assisted, inkjet bioprinting, stereolithography (SLA), and acoustic bioprinting. The first two are based on the deposition of droplets and the self-assembling of the latter to form a construct. Inkjet printing can either be continuous or drop-on-demand and can be achieved by different methods such as piezoelectric, thermal, acoustic, solenoid forces, electrostatic, and electrohydrodynamic.[128] The earliest use of inkjet printing in the fabrication of PVGs was to extrude ink droplets onto a

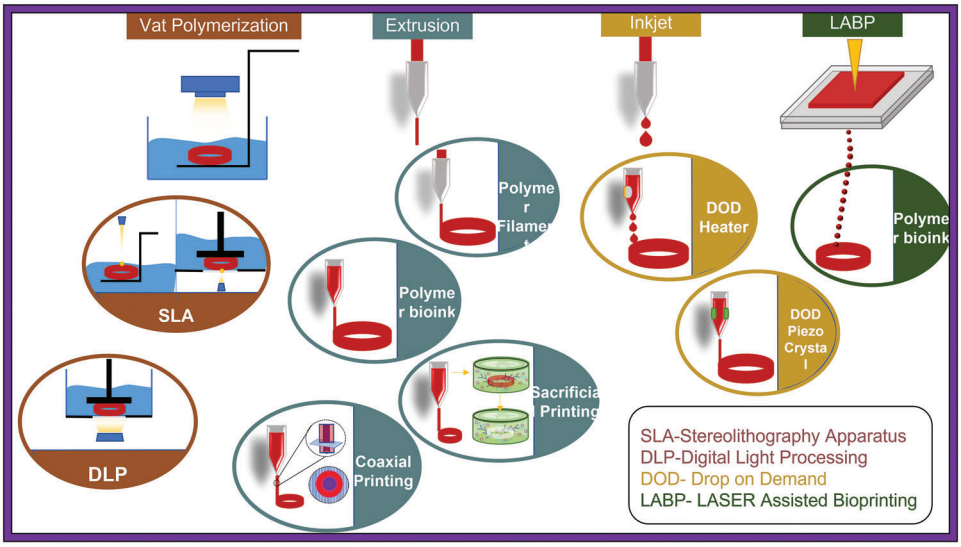

Figure 5. Demonstration of the various types of 3D bioprinting techniques employed for the generation of vasculature.

substrate and allow them to self-assemble into a vessel-like conduit. However, the fusion of the cell aggregates is a time-consuming process, hence the need for support structures for long, tubular, and overhung structures, which was overcome with the use of $CaCl_2$[129] and fluorocarbon.[130] The buoyancy of these chemicals provides time for the integration of droplets to form an integral structure and supports both horizontal and vertical bifurcation. Inkjet printing has also been used to print water droplets at a low temperature (−30 °C) to allow easy solidification into ice. The sacrificial ice is coated with natural (tropoelastin, silk) or synthetic material (PCL, PDMS) to form large, small, tubular, and bifurcated grafts.[131]

Laser-induced forward transfer (LIFT)[132] focuses laser light onto a gold or titanium energy-absorbing layer that forms a vapor and exerts pressure on the donor layer that releases a bioink droplet on a collector. LIFT has the advantage of printing with higher cell densities in a range of viscosities, whereas inkjet printing works with lower viscosity (the mechanical strength of the constructs is lower), and the resolution and speed are higher compared to extrusion. LIFT was also used to print fine capillaries (size tens of microns), ECs printed on a scaffold of Matrigel were able to form a capillary lumen.[133] Both laser-induced and droplet bioprinting help in constructing mesovasculature models in which supporting materials such as agarose are, printed along cell droplets, and upon the fusion of the droplets, the support is removed and mesovasculature is formed.

SLA[134] makes use of photocurable polymers (uses UV or visible light). It is a high-resolution photopolymerization process that works with detailed surface topography and may or may not incorporate cells.[135] The irradiated part undergoes a chemical reaction and solidifies into a layer-by-layer structure. Vascular grafts with controllable geometries (including parameters of curvature, wall thickness, and diameter) have been fabricated by this technique.[136] It mainly uses methacrylate polymers and others such as poly(ethylene oxide) for the process. Non-cytotoxic photosensitive polymers such as vinyl carbonate and PLGA-acrylate blend have been developed to be used in SLA to support cell attachment and proliferation. SLA can further be used to fabricate mesovasculature, in which an unmasked hydrogel surrounds a masked one. The unmasked region is photopolymerized, while the masked one is washed away to create a mesochannel. Proteins, peptides, and other biomaterials can also be incorporated into SLA on patterned hydrogels such as poly(ethylene glycol) diacrylate (PEGDA). Arg–Gly–Asp–Ser motif, for instance, allows the attachment of FBs, while Arg–Glu–Asp–Val allows HUVECs. The micropatterning on the hydrogel allowed the HUVECs to form the mesovasculature. Its disadvantages mainly include poor cell viability and inefficiency to print multiple cell types. Moreover, SLA needs a washing step in order to remove the unreacted polymer. A recent study used a multi-material SLA printer and a photoink that was enzymatically degradable to fabricate an EC channel that was stable in culture for 28 days. **Figure 5** shows the various types of bioprinting approaches used to generate vascular conduits.

Acoustic bioprinting stands apart from the traditional techniques and allows contactless motion and aggregation of cells to achieve high ordered arrangements. This technique was used to create pressure fields from standing surface acoustic waves to pattern cells in a modified HA hydrogel (catechol-conjugated) to form an aligned and perfusable vessels, demonstrating angiogenesis in mouse model.[127]

One of the major limitations in developing vascular grafts using the 3D bioprinting technique is the resolution of the lumen diameter that can be achieved. The minimum lumen size attained using inkjet, extrusion, and light bioprinting are ≈200, 100, and 1 µm, respectively.[137] Gong et al. developed a strategy of complexation-induced resolution enhancement which can enhance the resolution of 3D bioprinted vascular grafts. They generated constructs with a diameter of 355 ± 21 µm using HAMA hydrogel with pluronics as the sacrificial material. These constructs were then immersed in a solution of chitosan in acetic acid for 24 h to achieve shrinkage and to obtain a diameter of 174 ± 12 µm, which is 49.0 ± 3.4% of the original size. The distortions in the construct pre- and post-shrinkage were found to

Table 3. Represents the various parameters achieved using different techniques of 3D bioprinting.[137]

Parameters	Extrusion	Inkjet	Light
Cell viability	Low (less than 80%)	Medium (greater than 80%)	High (greater than 90%)
Minimum lumen size achieved	≈200 μm	≈100 μm	≈1 μm
Speed of fabrication	Medium	Low	High
Resolution	Medium (100–300 μm)	Low (100–1000 μm)	High (1–100 μm)

Table 4. Summarizes the comparisons of the biomaterials and techniques used for the fabrication of vasculature.

Biomaterial	Technique	Cross-linking technique	Pros	Cons	Reference
Collagen (type I)	Inkjet (drop on demand). Extrusion. Laser.	Thermally cross-linked or nebulized using sodium hydrogen carbonate.	Supports in vivo and in situ bioprinting with long-term stability. Allows high functionality of cells.	Post printing processing (gelation). Increase in cell density increases chances of cell death. Support material is required.	[139–141]
Alginate	Inkjet (drop on demand). Freeform reversible embedding of suspended hydrogel (FRESH).	Chemically cross-linked with $CaCl_2$.	The tube constructs made of alginate are stable with good fidelity.	Complex geometries aren't possible with inkjetting of alginate. Alginate lacks any peptides for cell adhesion.	[142–144]
Fibrin	Inkjet. Laser.	Fibrinogen and thrombin react to form fibrin.	Stable tubular structures can be formed that resemble the native vessel.	The process is long and time consuming. In inkjet thrombin and fibrinogen must be deposited exactly at the same location.	[145,146]
Hyaluronic acid	Extrusion	Photopolymerization	Cell viability and mechanical properties are suitable for vessel fabrication.	UV exposure time is long, and HA-MA is difficult to be removed.	[147]
Gelatin	Extrusion	Thermal. Blended with cross-linkers (thrombin, $CaCl_2$).	Supports cells and release of growth factors (VEGF). Supports angiogenesis.	Temperature sensitive. Angiogenesis is supported only in the periphery in vivo	[148,149]
GelMA	Extrusion	Photopolymerization	Interconnected stable capillary network can be formed	Initial cell death caused by UV exposure. Perfusion is a challenge in small diameter vessels.	
Agarose	Extrusion	Thermal	Flexible branched structures can be formed. Supports spheroids fusion.	Spheroid fusion is time consuming besides large number of spheroids are needed to form a continuous structure.	[144]
PEG	Extrusion	Photopolymerization	Provides good mechanical strength and supports proliferation.	PEGTA has higher viscosity.	[147]
Pluronic	Extrusion	Photopolymerization and thermal.	Higher stability and fidelity.	Requires humid environment.	[124]

be negligible.[138] **Table 3** summarizes the various parameters of the most common methods of bioprinting and **Table 4** summarizes the comparisons of the biomaterials and techniques used for the fabrication of vasculature.

4.8.2. 4D Bioprinting

4D printing can be seen as an advanced fabrication technique that includes 3D printing and a fourth dimension: time. The desired change in shape, size, or functionality occurs due to the application of an external stimulus. It was introduced for the first time by Tibett from the Massachusetts Institute of Technology (MIT), other groups such as Qi's from the Georgia Institute of Technology have performed some exciting work using 4D printing. It is a dynamic technique that can be carried out using biomaterials with or without cells to form complex constructs for tissue engineering.[150] It is the material and the type of stimuli used that set 4D bioprinting apart from 3D. The application of the stimuli exerts a certain control over the process, and the material responsive to one or more stimuli is termed stimuli-responsive material. Hydrogel is the most common water-responsive material. For example, a composite of agarose, PEG, and PCAD (an

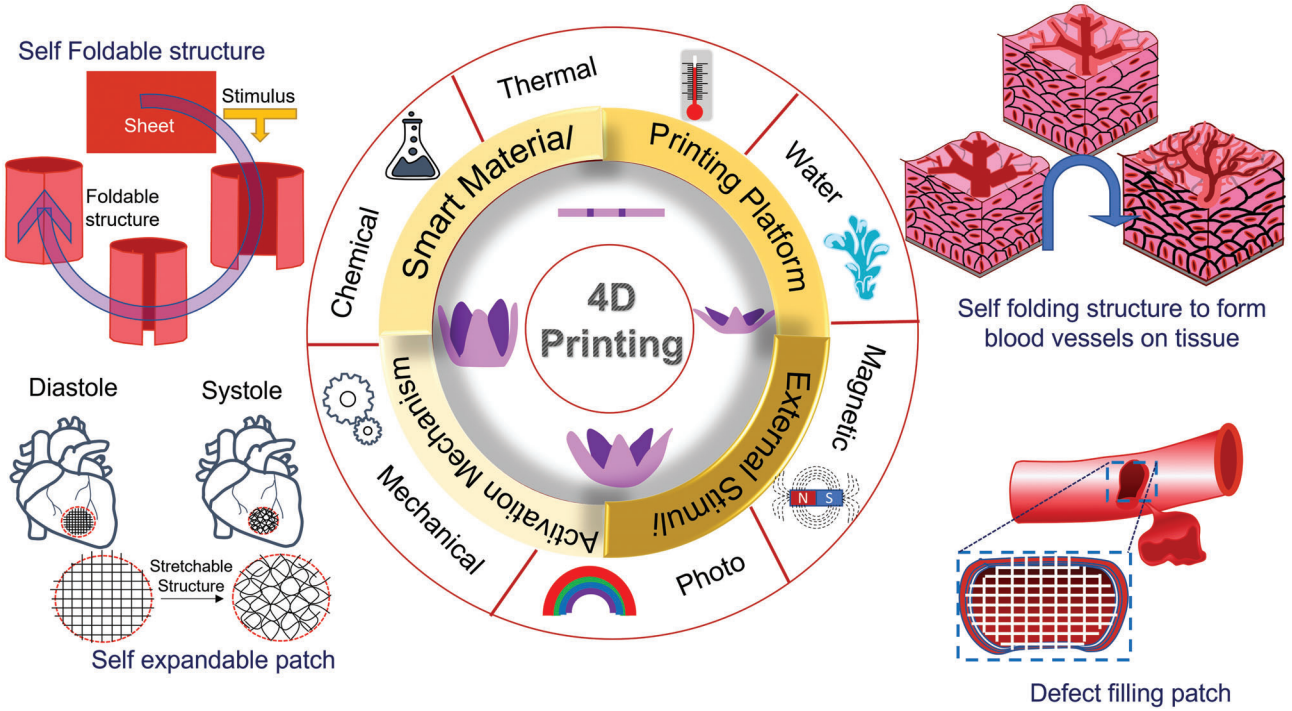

Figure 6. Demonstration of the various aspects of 4D printing and the application of 4D bioprinting in VTE.

azobenzene derivative) can undergo quick deformation in the presence of moisture.[151] Other stimuli, like electric and magnetic fields, light, pH, and sound are also used. Polyelectrolytes are responsive to the electric field; magnetic nanoparticles are responsive to the magnetic field. For example, magnetic platelets are used in printable ink to guide the orientation of particles in response to the magnetic field.[152] Polymers such as collagen,[153] and keratin[154] are responsive to pH such that there is a change in the polymer chain arrangement from globule to coil form, which in turn causes shape change. **Figure 6** demonstrates the various aspects of 4D printing and the application of 4D bioprinting in VTE.

4D bioprinting has been used to develop patches to treat myocardial infarction using hydrogels consisting of gelatin and PEG.[155] A shape-morphing construct was fabricated using near-infrared consisting of a composite cell-laden bioink of alginate and polydopamine.[156] The maturation of cells over time has also been regarded as an instance of 4D bioprinting. A bioprinting strategy involves a bilayer imparting shape morphing to a 3D construct consisting of an actuation layer that drives the shape morphing and a cell-supporting microgel layer, that is, both biodegradable and photocurable. The microgel layer allows the printing of a cell-only bioink that forms a condensate and, later, upon degradation, results in the formation of mature tissue.[157]

The fabrication of BVs is challenging with 3D bioprinting, it is limited in terms of the material, size, and strength of the conduit formed. 4D bioprinting overcomes these challenges by printing a structure that is flat in shape and forms a conduit upon being stimulated externally.

A lot of research carried out in 3D bioprinting can be classified as 4D because the change of shape or functionality or maturation of cells takes place over a period. A common example of is the use of cell-traction force that has been used previously to pull together a sheet into a tube-like structure. It is an example of 4D bioprinting as the shape and functionality of the given construct change over time. A study utilizing cell tension and surface tension forces is the culturing of NIH 3T3 cells on parylene C microplates that were initially flat. Upon confluency, the cell-traction force drove the folding of the sheet into a tube-like structure. Various shapes can be formed using this technique by changing or customizing the initial geometry.[158] Cell condensation is a scaffold-free approach and in comparison, to cell-laden hydrogel, it does not need any synchronization between hydrogel degradation and new tissue formation. The deformation of the cell condensation construct can be programmed through shape transformation and maturation of tissues. For example, a bilayer structure was constructed using a hydrogel layer and a microgel layer that allowed cell bioink to be printed. Upon degradation of the microgel layer, the 4D transformation into a configured and matured tissue was obtained.[159]

An approach has been reported about the temperature-triggered self-folding tubes that are biocompatible and biodegradable as well, with an active layer of gelatin (used either in native or photocross-linked form). The cross-linked and non-cross-linked gelatin layers differ in properties and as a result, trigger either folding at room temperature and unfolding at 37 °C or non-deformation at room temperature and folding at 37 °C. The study has also demonstrated the encapsulation of neural cells in the folded tubes (at the time of formation) and their viability for 7 days.[160]

Another study also reports the formation of self-folding tubular structures from a bilayer structure, in which the lower layer is made up of PCL and the upper layer consists of sodium

alginate. There are various parameters that govern the self-folding behavior of the bilayer including aspect ratio, layer thickness, and pattern. The folding is caused by the difference in the swelling ratio and heat-responsiveness of PCL and sodium alginate to the external stimulus of calcium ions and heat. The bilayer tubular structures can be tailored by altering the parameters and the external stimuli. The constructs can be used in VTE.[161]

Tubular structures have been fabricated from soft swellable PNIPAM segments and stiff non-swellable polyacrylamide segments such that the latter is the passive layer while the former is the active layer. The two inks were extruded from two separate nozzles to form alternate vertical segments in the tubular structure. The constructs were immersed in deionized water (DI water) kept at 50 °C, preceding which they were illuminated by UV light. The tubes maintained their structure at 50 °C but deformed by decreasing the temperature to 25 °C.[162]

Another study optimized alginate and methylcellulose with excellent properties of printability, shape-fidelity, and rheological properties. The properties of anisotropic stiffness and swelling were programmed by controlling the density gradients of networks perpendicular to the orientation of the patterned strips. By changing the spacing between the strips and the angle, the hydrogel could be made into tubes, helices, double-helices, and flowers.[163]

A 4D biofabrication approach has also been reported by Kirillova et al.[164] Therein, two different shape morphing hydrogels were employed to form self-folding tubes with high resolution and controllable architectures and diameters. The biopolymers used were alginate and HA, these were modified with methacrylate groups and made into photocross-linkable hydrogels (AA-MA and HA-MA). Greenlight was used instead of UV to help maintain cell viability and demonstrate the differentiation of mouse bone marrow stromal cells into a variety of other cell types. The self-folded hollow hydrogel tubes can be used in VTE. Figure 6 shows the various ways in which 4D bioprinting has been performed to generate vascular structures. In another study, employing GelMA as the base material, micro hollow tubular structures were fabricated utilizing the self-folding property of the material. GelMA was UV cross-linked, and rhodomine was added to the solution of GelMA and Irgacure. This resulted in a stable, uniform, and integrally walled structure that formed when placed in water. Some tubular structures remained open with unclosed walls; these could be closed by increasing the extent of folding. The cracks, however, assist in the migration of cells in the initial phases, facilitating the formation of neovessels, and help in integration in the later phases. Different branching patterns in the flakes allow branched structures to form with varied types of anastomosis. The resulting leaking tubes and an absence of programmed bifurcation were among the drawbacks of their work.[165] Yet another study reported the fabrication of a bifurcated tubular structure using shape-changing layers of a 3D construct using a mathematical model. They described bifurcation, forming T or Y-shaped junctions, as an important element of network formation. They elucidated that these bifurcations can't be formed by simple rolling of separate sheets. Therefore, they constructed a T-junction bifurcated tubular structure from a bioink consisting of alginate, dialdehyde, and gelatin (ADA gel). They found that UV exposure strengthened the construct, but longer exposure decreased the tube diameter; besides, increasing gelatin concentration increased the thickness of the films.[166]

Tiny tubular structures used to treat constricted vessels, for example, BVs are called stents.[167] Nearly half of the stents used are shape-memory alloys, nitinol being an example. The disadvantage of the latter is the high rate of restenosis, failure of cell regeneration, and thrombus formation.[168] Shape-memory polymers (SMPs) reduce this risk due to their biocompatibility and biodegradability and modulation in their shape.[169] High-resolution SLA[170] and SMP fibers[171] have been used to develop 4D printed stents. 4D printed PLA stents have been studied for their degradation using finite element analysis to develop insightful relations between vascular injury, stent and its deployment temperature.[172]

4D bioprinting can thus be used as a strategy to control the shape and size of constructs over time and has various applications in the field of tissue engineering. However, it is still a technique that is in its infancy with major challenges that need to be addressed to improve its potential as a tool for tissue engineering. One of the challenges is to optimize the stimuli-responsive biomaterials such that they are bioprintable.[173] Moreover, the existing 4D bioprinted shape transformations such as folding and assembling cannot currently meet the complex needs of tissue engineering. The stimuli-responsive biomaterials should be able to maintain long-term applications without losing their properties as only a few of the structures recovered their original shape in limited situations. In addition, the mechanical properties of the biomaterial are not enough to withstand the high pressure associated with constant changes in response to the stimuli (**Figure 7**).[174]

5. Challenges and Future Directions

The composition of the BV at the biomolecular level is varied; hence, structural and functional components must be recapitulated. Besides, the reproduction of the layer in direct contact with the blood is a major challenge. Thus, well-designed biomaterials and cells remain a bottleneck in constructing functional tissue. The fabrication techniques used for assembling the materials and the cells must allow for proper distribution, maximum cell survival, and ideal mimicking of the native BVs.

All the techniques employed till date have served some purpose, but no vascular model has been able to perform all the natural processes of complex vasculature. The most desirable characteristic of the vasculature is integration with the host tissue.

The introduction of synthetic and tissue engineering materials revolutionized the field with biodegradable, biocompatible, non-thrombogenic materials with controlled degradation, mechanical properties, and functionalization, though the use of responsive materials indeed changed the course of the use of biomaterials in tissue engineering. However, the use of a single biomaterial is not sufficient for constructing a BV; hence, formulations of materials along with cells emerged. Hydrogels, being unique in their characteristics and variety have focused the research on developing multifunctional scaffolds with an appropriate ratio of degrading biomaterials and ECM production by cells.

For cells, proliferative potential and source are major considerations, and the technique of fabricating vascular conduits was initially based on ECs and SMCs as cell components. However,

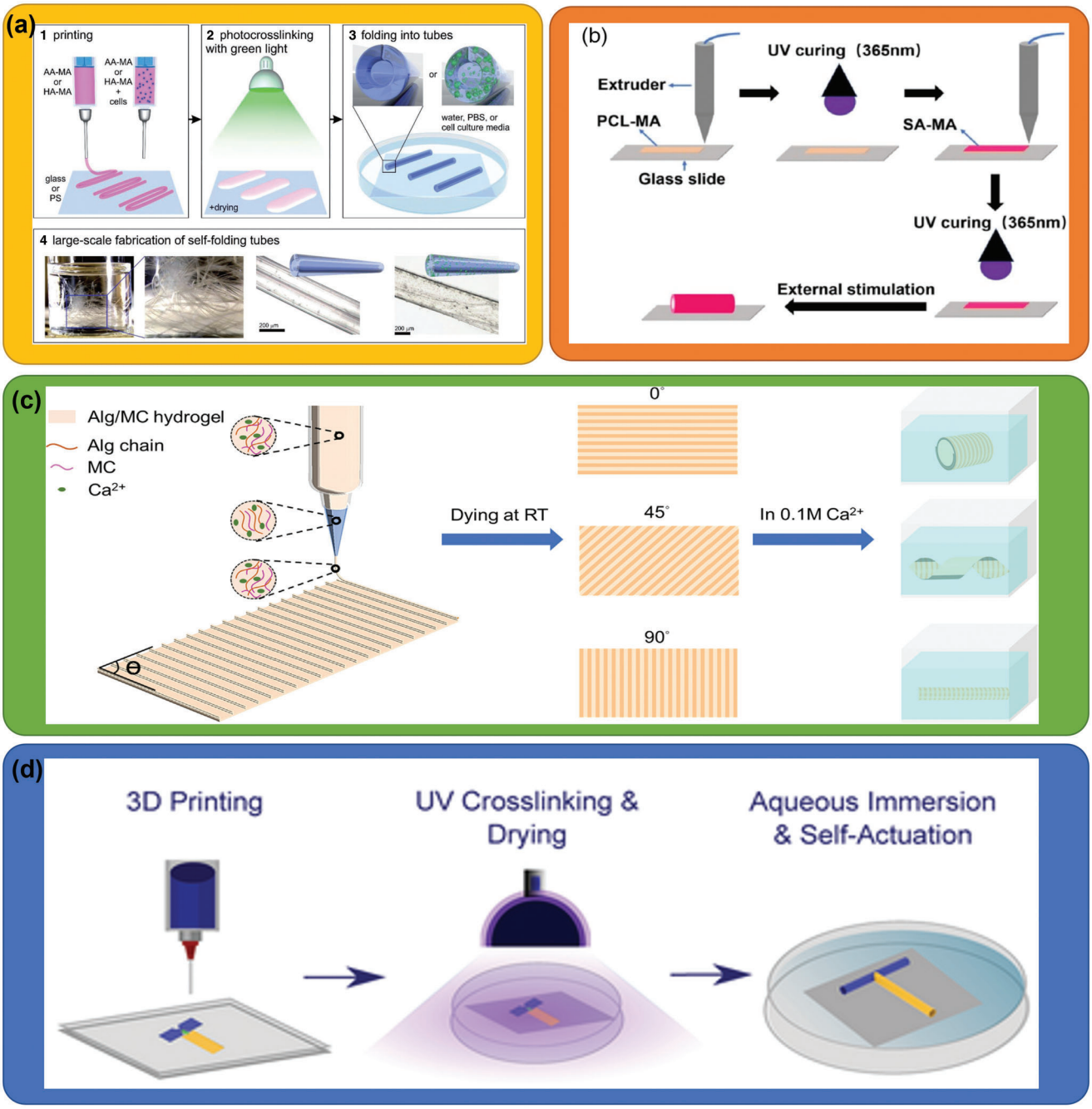

Figure 7. a) The methacrylated alginate and hyaluronic acid forming tubular structures on being illuminated by green light. Reproduced with permission.[164] Copyright 2017, Wiley-VCH. b) The PCL and sodium alginate layer on being cured with UV light and stimulated forms tube-like structures. Reproduced with permission.[161] Copyright 2023, Wiley-VCH. c) Different angles at which alginate and methylcellulose can be printed to morph into different shapes. Reproduced with permission.[163] Copyright 2021, Elsevier. d) Shows the formation of a T-shaped perfusable structure formed upon UV cross-linking and stimulated by aqueous immersion. Reproduced with permission.[166] Copyright 2023, Wiley-VHC.

recent studies show that they aren't enough. Besides, seeding cells via static methods falls short of infiltration of SMCs in the middle layer and homogenous distribution of ECs in the intimal layer (despite the attachment of biomolecules and cell adhesion peptides). Even though the development of stem cells, induced-pluripotent stem cells, and embryonic stem cells has emerged as alternatives for ECs and SMCs, they still pose a concern for tumorogenesis.

Furthermore, the maturation of cells is a longer, pivotal, and complex phenomenon where cells establish connections between themselves and lay an ECM. Studies show that maturation is increased in the presence of mechanical stresses exerted

by fluid flow. The role of bioreactors comes into play for maturation and endothelialization of the intimal layer and is perhaps a major challenge. Moreover, the clinical applications of the vascular grafts are limited, and no technology has progressed to meet patient demands.

Bioprinting is a promising tool that allows the precise and reproducible fabrication of multi-material to mimic native BV, and the construction of perfusable vessels in matrix hydrogel has been made possible but mimicking the distinct layers for a fully functional multi-scale vascular network remains as a gap. Besides, patient compliance is also a challenge.

In future, a robust and highly organized vascular network needs to be developed that supports and assists in the supply of both the cells and the nutrients resembling the native structure of arterioles, capillaries, and venules. Besides, substantial knowledge regarding the biological functioning and structure needs to be built to develop materials and tune strategies to allow multiple cells to be spatially combined with responsive materials to achieve controlled differentiation and new tissue formation. 4D bioprinting satisfies all the characteristics of 3D bioprinting along with use of stimuli-responsive materials to help customize scaffolds and cell behavior.

However, even 4D bioprinting is limited by the number of stimuli-responsive materials available and those that are responsive to multiple stimuli are still fewer. The types of 3D printers that can work with smart materials are also limited. Moreover, the responses of most materials are limited to simple bending or folding only at the macro level. Thus, design control needs to shift from the macroscale to the microscale. The precise control of external stimuli, the response, and the resolution can allow control of the deformation and therefore are the future directions for 4D bioprinting.

More 4D biomaterials are being investigated to cater to patient specific therapeutics. There is a need to develop advanced printing tools and materials that are both biocompatible and biodegradable.

6. Conclusion

This review summarizes the various fabrication techniques for VTE. While the traditional techniques have their own advantages, the advent of additive manufacturing completely transformed the area of tissue engineering by catering to patient-specific needs. 3D bioprinting improved the reproducibility, scalability, and resolution of the tissue constructs formed, but it still considered the initial state of the printed construct. A more specialized fabrication technique is 4D biofabrication, which includes time as its fourth dimension to consider the change in shape, size, functionality, and cell maturation over time. The printing of hollow tubes has been a challenge for 3D bioprinting, and even though it has been addressed via various techniques of 3D bioprinting, the limitation of size and resolution is still a bottleneck. 4D bioprinting is widely accepted and considered to resolve issues with static polymer constructs. It is gaining pace gradually and is known to help in the fabrication of dynamic constructs with tunable properties and reconfigurable architectures. The technique is dependent on the choice of materials and stimulus to inculcate a desired change in the final printed construct. 4D bioprinting would play a promising role in future for fabricating vascular networks built using materials that either help in the assembly of heterologous droplets of cells or create various types of gradients for materials to form complex structures such as that of vasculature.

Acknowledgements

Open access publishing facilitated by Deakin University, as part of the Wiley - Deakin University agreement via the Council of Australian University Librarians.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

4D printing, bioprinting, cardiovascular, neurovascular, vascular tissue engineering

Received: February 10, 2023
Revised: April 11, 2023
Published online:

[1] B. B. J. Leal, N. Wakabayashi, K. Oyama, H. Kamiya, D. I. Braghirolli, P. Pranke, *Front. Cardiovasc. Med.* **2021**, *7*, 376.
[2] P. Sasmal, P. Datta, Y. Wu, I. T. Ozbolat, *Microphysiol. Syst.* **2018**, *2*, 1.
[3] G. Kumari, K. Abhishek, S. Singh, A. Hussain, M. A. Altamimi, H. Madhyastha, T. J. Webster, A. Dev, *Nanomedicine* **2022**, *17*, 255.
[4] M. Tennant, J. K. Mcgeachie, *Aust. N. Z. J. Surg.* **1990**, *60*, 747.
[5] J. P. Stegemann, S. N. Kaszuba, S. L. Rowe, *Tissue Eng.* **2007**, *13*, 2601.
[6] P. Berillis, *Open Circ. Vasc. J.* **2013**, *6*, https://doi.org/10.2174/1877382601306010001.
[7] H. Ardalani, A. H. Assadi, W. L. Murphy, in *Engineering in Translational Medicine*, Springer, New York **2014**, pp. 155–182.
[8] S. Y. Yuan, R. R. Rigor, *Colloq. Ser. Integr. Syst. Physiol.* **2010**, *3*, https://doi.org/10.4199/C00025ED1V01Y201101ISP013.
[9] O. C. Velazquez, *J. Vasc. Surg.* **2007**, *45*, A39.
[10] P. Carmeliet, R. K. Jain, *Nature* **2011**, *473*, 298.
[11] C. Tomasina, T. Bodet, C. Mota, L. Moroni, S. Camarero-Espinosa, *Materials* **2019**, *12*, 2701.
[12] M. I. Castellanos, J. Guillem-Marti, C. Mas-Moruno, M. Díaz-Ricart, G. Escolar, M. P. Ginebra, F. J. Gil, M. Pegueroles, J. M. Manero, *J. Biomed. Mater. Res., Part A* **2017**, *105*, 973.
[13] M. H. Lee, P. Ducheyne, L. Lynch, D. Boettiger, R. J. Composto, *Biomaterials* **2006**, *27*, 1907.
[14] C. J. Bettinger, Z. Zhang, S. Gerecht, J. T. Borenstein, R. Langer, *Adv. Mater.* **2008**, *20*, 99.
[15] F. J. Byfield, R. K. Reen, T.-P. Shentu, I. Levitan, K. J. Gooch, *J. Biomech.* **2009**, *42*, 1114.
[16] A. L. Sieminski, K. J. Gooch, *Biomaterials* **2000**, *21*, 2233.
[17] S. Li, K. Wang, X. Jiang, Q. Hu, C. Zhang, B. Wang, *ACS Biomater. Sci. Eng.* **2020**, *6*, 2297.
[18] Q. Zou, X. Tian, S. Luo, D. Yuan, S. Xu, L. Yang, M. Ma, C. Ye, *Carbohydr. Polym.* **2021**, *269*, 118222.
[19] B. Ren, K. Song, A. R. Sanikommu, Y. Chai, M. A. Longmire, W. Chai, W. L. Murfee, Y. Huang, *Appl. Phys. Rev.* **2022**, *9*, 011408.
[20] S. Li, W. Wang, W. Li, M. Xie, C. Deng, X. Sun, C. Wang, Y. Liu, G. Shi, Y. Xu, X. Ma, J. Wang, *Adv. Funct. Mater.* **2021**, *31*, 2102685.

[21] M. Wang, X. Yang, L. Liang, *J. Chem.* **2020**, *2020*, 3148652.
[22] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, C. S. Chen, *Nat. Mater.* **2012**, *11*, 768.
[23] L. E. Bertassoni, M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, G. Barabaschi, D. Demarchi, M. R. Dokmeci, Y. Yang, A. Khademhosseini, *Lab Chip* **2014**, *14*, 2202.
[24] D. Dranseikiene, S. Schrüfer, D. W. Schubert, S. Reakasame, A. R. Boccaccini, *J. Mater. Sci. Mater. Med.* **2020**, *31*, https://doi.org/10.1007/s10856-020-06369-7.
[25] S. Khalighi, M. Saadatmand, *Int. J. Biol. Macromol.* **2021**, *193*, 2153.
[26] S. C. Millik, A. M. Dostie, D. G. Karis, P. T. Smith, M. Mckenna, N. Chan, C. D. Curtis, E. Nance, A. B. Theberge, A. Nelson, *Biofabrication* **2019**, *11*, 045009.
[27] L. P. D. Silva, R. P. Pirraco, T. C. Santos, R. N. Carballal, M. T. Cerqueira, V. M. Correlo, A. P. Marques, *ACS Appl. Mater. Interfaces* **2016**, *8*, 33464.
[28] S. V. Lopes, M. N. Collins, R. L. Reis, J. M. Oliveira, J. Silva-Correia, *ACS Appl. Bio Mater.* **2021**, *4*, 2941.
[29] C. B. Weinberg, E. Bell, *Science* **1986**, *231*, 397.
[30] S. Ravi, E. L. Chaikof, *Regener. Med.* **2010**, *5*, 107.
[31] M. Ehrbar, V. G. Djonov, C. Schnell, S. A. Tschanz, G. Martiny-Baron, U. Schenk, J. Wood, P. H. Burri, J. A. Hubbell, A. H. Zisch, *Circ. Res.* **2004**, *94*, 1124.
[32] L. E. Niklason, J. Gao, W. M. Abbott, K. K. Hirschi, S. Houser, R. Marini, R. Langer, *Science* **1999**, *284*, 489.
[33] D. D. Swartz, J. A. Russell, S. T. Andreadis, *Am. J. Physiol.: Heart Circ. Physiol.* **2005**, *288*, 1451.
[34] N. M. Seale, S. Varghese, *J. Mater. Chem. B* **2016**, *4*, 3454.
[35] C. Arrigoni, D. Camozzi, A. Remuzzi, *Cell Transplant.* **2017**, *15*, 119.
[36] S. Goldman, K. Zadina, T. Moritz, T. Ovitt, G. Sethi, J. G. Copeland, L. Thottapurathu, B. Krasnicka, N. Ellis, R. J. Anderson, W. Henderson, *J. Am. Coll. Cardiol.* **2004**, *44*, 2149.
[37] K. Hu, Y. Li, Z. Ki, H. Yang, C. Liu, Y. Li, Y. Guo, W. Wang, *Biomater. Transl. Med.* **2022**, *3*, 81.
[38] N. L'heureux, S. Pâquet, R. Labbé, L. Germain, F. A. Auger, *FASEB J.* **1998**, *12*, 47.
[39] R. V. Badhe, D. Bijukumar, D. R. Chejara, M. Mabrouk, Y. E. Choonara, P. Kumar, L. C. Du Toit, P. P. D. Kondiah, V. Pillay, *Carbohydr. Polym.* **2017**, *157*, 1215.
[40] D. Wang, Y. Xu, Q. Li, L.-S. Turng, *J. Mater. Chem. B* **2020**, *8*, 1801.
[41] X. Hu, H. Shen, F. Yang, J. Bei, S. Wang, *Biomaterials* **2008**, *29*, 3128.
[42] A. M. Hiscox, A. L. Stone, S. Limesand, J. B. Hoying, S. K. Williams, *Tissue Eng., Part A* **2008**, *14*, 433.
[43] T. Dvir, A. Kedem, E. Ruvinov, O. Levy, I. Freeman, N. Landa, R. Holbova, M. S. Feinberg, S. Dror, Y. Etzion, J. Leor, S. Cohen, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 14990.
[44] P.-L. Tremblay, V. Hudon, F. Berthod, L. Germain, F. A. Auger, *Am. J. Transplant.* **2005**, *5*, 1002.
[45] S. Levenberg, J. Rouwkema, M. Macdonald, E. S. Garfein, D. S. Kohane, D. C. Darland, R. Marini, C. A. Van Blitterswijk, R. C. Mulligan, P. A. D'amore, R. Langer, *Nat. Biotechnol.* **2005**, *23*, 879.
[46] S. I. Jeong, J. H. Kwon, J. I. Lim, S.-W. Cho, Y. Jung, W. J. Sung, S. H. Kim, Y. H. Kim, Y. M. Lee, B.-S. Kim, C. Y. Choi, S.-J. Kim, *Biomaterials* **2005**, *26*, 1405.
[47] C. Williams, T. M. Wick, *Tissue Eng.* **2004**, *10*, 930.
[48] D. Shweiki, A. Itin, D. Soffer, E. Keshet, *Nature* **1992**, *359*, 843.
[49] K. J. Cronin, A. Messina, K. R. Knight, J. J. Cooper-White, G. W. Stevens, A. J. Penington, W. A. Morrison, *Plast. Reconstr. Surg.* **2004**, *113*, 260.
[50] A. J. Hussey, M. Winardi, X.-L. Han, G. P. L. Thomas, A. J. Penington, W. A. Morrison, K. R. Knight, S. J. Feeney, *Tissue Eng., Part A* **2009**, *15*, 3823.
[51] A. F. Black, F. Berthod, N. L'heureux, L. Germain, F. A. Auger, *FASEB J.* **1998**, *12*, 1331.
[52] BORIS, https://boris.unibe.ch/42748/ (accessed: February **2023**).
[53] A. Wenger, A. Stahl, H. Weber, G. Finkenzeller, H. G. Augustin, G. B. Stark, U. Kneser, *Tissue Eng.* **2004**, *10*, 1536.
[54] F. Geiger, H. Lorenz, W. Xu, K. Szalay, P. Kasten, L. Claes, P. Augat, W. Richter, *Bone* **2007**, *41*, 516.
[55] K. Jakab, C. Norotte, B. Damon, F. Marga, A. Neagu, C. L. Besch-Williford, A. Kachurin, K. H. Church, H. Park, V. Mironov, R. Markwald, G. Vunjak-Novakovic, G. Forgacs, *Tissue Eng., Part A* **2008**, *14*, 413.
[56] Y. Yu, K. K. Moncal, J. Li, W. Peng, I. Rivero, J. A. Martin, I. T. Ozbolat, *Sci. Rep.* **2016**, *6*, 28714.
[57] M. D. Sarker, S. Naghieh, N. K. Sharma, X. Chen, *J. Pharm. Anal.* **2018**, *8*, 277.
[58] C. Norotte, F. S. Marga, L. E. Niklason, G. Forgacs, *Biomaterials* **2009**, *30*, 5910.
[59] Y. Jung, H. Ji, Z. Chen, H. F. Chan, L. Atchison, B. Klitzman, G. Truskey, K. W. Leong, *Sci. Rep.* **2015**, *5*, https://doi.org/10.1038/srep15116.
[60] D. G. Seifu, A. Purnama, K. Mequanint, D. Mantovani, *Nat. Rev. Cardiol.* **2013**, *10*, 410.
[61] K. Moschouris, N. Firoozi, Y. Kang, *Regener. Med.* **2016**, *11*, 559.
[62] M. Yamato, T. Okano, *Mater. Today* **2004**, *7*, 42.
[63] S. F. Badylak, D. Taylor, K. Uygun, *Annu. Rev. Biomed. Eng.* **2011**, *13*, 27.
[64] J. J. Song, H. C. Ott, *Trends Mol. Med.* **2011**, *17*, 424.
[65] B. S. Conklin, E. R. Richter, K. L. Kreutziger, D.-S. Zhong, C. Chen, *Med. Eng. Phys.* **2002**, *24*, 173.
[66] B. W. Tillman, S. K. Yazdani, L. P. Neff, M. A. Corriere, G. J. Christ, S. Soker, A. Atala, R. L. Geary, J. J. Yoo, *J. Vasc. Surg.: Venous Lymphat. Disord.* **2012**, *56*, 783.
[67] Z. Syedain, J. Reimer, M. Lahti, J. Berry, S. Johnson, R. Bianco, R. T. Tranquillo, *Nat. Commun.* **2016**, *7*, https://doi.org/10.1038/ncomms12951.
[68] P. M. Crapo, T. W. Gilbert, S. F. Badylak, *Biomaterials* **2011**, *32*, 3233.
[69] J. Li, Z. Cai, J. Cheng, C. Wang, Z. Fang, Y. Xiao, Z.-G. Feng, Y. Gu, *J. Biomater. Sci., Polym. Ed.* **2020**, *31*, 999.
[70] R. Simsa, X. M. Vila, E. Salzer, A. Teuschl, L. Jenndahl, N. Bergh, P. Fogelstrand, *PLoS One* **2019**, *14*, e0220743.
[71] J. R. Olmos-Zúñiga, R. Jasso-Victoria, N. E. Díaz-Martínez, M. O. Gaxiola-Gaxiola, A. Sotres-Vega, Y. Heras-Romero, M. Baltazares-Lipp, M. E. Baltazares-Lipp, P. Santillán-Doherty, C. Hernández-Jiménez, *Braz. J. Med. Biol. Res.* **2016**, *49*, e5001.
[72] J. W. Reinhardt, J. de Dios Ruiz Rosado, J. C. Barker, Y.-U. Lee, C. A. Best, T. Yi, Q. Zeng, S. Partida-Sanchez, T. Shinoka, C. K. Breuer, *Regener. Med.* **2019**, *15*, 389.
[73] W. Wang, W. Nie, D. Liu, H. Du, X. Zhou, L. Chen, H. Wang, X. Mo, L. Li, C. He, *Int. J. Nanomed.* **2018**, *13*, 7003.
[74] S. K. Norouzi, A. Shamloo, *Mater. Sci. Eng., C* **2019**, *94*, 1067.
[75] C. Vyas, R. Pereira, B. Huang, F. Liu, W. Wang, P. Bartolo, *Curr. Opin. Biomed. Eng.* **2017**, *2*, https://doi.org/10.1016/j.cobme.2017.05.008.
[76] D. R. Myers, Y. Sakurai, R. Tran, B. Ahn, E. T. Hardy, R. Mannino, A. Kita, M. Tsai, W. A. Lam, *J. Visualized Exp.* **2012**, e3958, https://doi.org/10.3791/3958.
[77] B. M. Baker, B. Trappmann, S. C. Stapleton, E. Toro, C. S. Chen, *Lab Chip* **2013**, *13*, 3246.
[78] Y. S. Zhang, A. Arneri, S. Bersini, S.-R. Shin, K. Zhu, Z. Goli-Malekabadi, J. Aleman, C. Colosi, F. Busignani, V. Dell'erba, C. Bishop, T. Shupe, D. Demarchi, M. Moretti, M. Rasponi, M. R. Dokmeci, A. Atala, A. Khademhosseini, *Biomaterials* **2016**, *110*, 45.
[79] J. Doshi, D. H. Reneker, *J. Electrost.* **1995**, *35*, 151.

[80] E. Ercolani, C. D. Gaudio, A. Bianco, *J. Tissue Eng. Regener. Med.* **2015**, *9*, 861.
[81] M. Rychter, A. Baranowska-Korczyc, J. Lulek, *RSC Adv.* **2017**, *7*, 32164.
[82] H. J. Park, J. S. Lee, O. J. Lee, F. A. Sheikh, B. M. Moon, H. W. Ju, J. H. Kim, D. K. Kim, C. H. Park, *Macromol. Res.* **2014**, *22*, 592.
[83] E. C. Filipe, M. Santos, J. Hung, B. S. L. Lee, N. Yang, A. H. P. Chan, M. K. C. Ng, J. Rnjak-Kovacina, S. G. Wise, *JACC: Basic Transl. Sci.* **2018**, *3*, 38.
[84] K. A. Mckenna, M. T. Hinds, R. C. Sarao, P.-C. Wu, C. L. Maslen, R. W. Glanville, D. Babcock, K. W. Gregory, *Acta Biomater.* **2012**, *8*, 225.
[85] T. Fukunishi, C. A. Best, T. Sugiura, J. Opfermann, C. S. Ong, T. Shinoka, C. K. Breuer, A. Krieger, J. Johnson, N. Hibino, *J. Thorac. Cardiovasc. Surg.* **2017**, *153*, 924.
[86] E., D. Boland, *Front. Biosci.* **2004**, *9*, 1422.
[87] Y. Pan, X. Zhou, Y. Wei, Q. Zhang, T. Wang, M. Zhu, W. Li, R. Huang, R. Liu, J. Chen, G. Fan, K. Wang, D. Kong, Q. Zhao, *Sci. Rep.* **2017**, *7*, https://doi.org/10.1038/s41598-017-03851-1.
[88] Z. Tan, H. Wang, X. Gao, T. Liu, Y. Tan, *Mater. Sci. Eng., C* **2016**, *67*, 369.
[89] A. K. Ekaputra, G. D. Prestwich, S. M. Cool, D. W. Hutmacher, *Biomacromolecules* **2008**, *9*, 2097.
[90] M. W. Laschke, H. Mussawy, S. Schuler, A. Kazakov, M. Rücker, D. Eglin, M. Alini, M. D. Menger, *Tissue Eng., Part A* **2010**, *17*, 841.
[91] J. Zhao, L. Liu, J. Wei, D. Ma, W. Geng, X. Yan, J. Zhu, H. Du, Y. Liu, L. Li, F. Chen, *Artif. Organs* **2012**, *36*, 93.
[92] Y. Wu, Y. Qin, Z. Wang, J. Wang, C. Zhang, C. Li, D. Kong, *J. Biomed. Mater. Res., Part B* **2018**, *106*, 1618.
[93] L. Gui, A. Muto, S. A. Chan, C. K. Breuer, L. E. Niklason, *Tissue Eng., Part A* **2009**, *15*, 2665.
[94] A. Porzionato, M. Sfriso, A. Pontini, V. Macchi, L. Petrelli, P. Pavan, A. Natali, F. Bassetto, V. Vindigni, R. De Caro, *Int. J. Mol. Sci.* **2015**, *16*, 14808.
[95] K. A. Heintz, M. E. Bregenzer, J. L. Mantle, K. H. Lee, J. L. West, J. H. Slater, *Adv. Healthcare Mater.* **2016**, *5*, 2153.
[96] E. T. Bishop, G. T. Bell, S. Bloor, I. J. Broom, N. F. K. Hendry, D. N. Wheatley, *Angiogenesis* **1999**, *3*, 335.
[97] M. Gultekinoglu, Ş. Öztürk, B. Chen, M. Edirisinghe, K. Ulubayram, *Eur. Polym. J.* **2019**, *121*, 109297.
[98] L. S. Nair, C. T. Laurencin, *Prog. Polym. Sci.* **2007**, *32*, 762.
[99] S. Wang, Y. Zhang, H. Wang, G. Yin, Z. Dong, *Biomacromolecules* **2009**, *10*, 2240.
[100] M. J. Kim, J. H. Kim, G. Yi, S. H. Lim, Y. S. Hong, D. J. Chung, *Macromol. Res.* **2008**, *16*, 345.
[101] M. A. Woodruff, D. W. Hutmacher, *Prog. Polym. Sci.* **2010**, *35*, 1217.
[102] Y. Dong, T. Yong, S. Liao, C. K. Chan, M. M. Stevens, S. Ramakrishna, *Tissue Eng., Part A* **2009**, *16*, 283.
[103] H. Bergmeister, C. Grasl, I. Walter, R. Plasenzotti, M. Stoiber, C. Schreiber, U. Losert, G. Weigel, H. Schima, *Artif. Organs* **2012**, *36*, 54.
[104] S. Wang, Y. Zhang, H. Wang, Z. Dong, *Int. J. Biol. Macromol.* **2011**, *48*, 345.
[105] S. Panzavolta, M. Gioffrè, M. L. Focarete, C. Gualandi, L. Foroni, A. Bigi, *Acta Biomater.* **2011**, *7*, 1702.
[106] S. J. Lee, J. Liu, S. H. Oh, S. Soker, A. Atala, J. J. Yoo, *Biomaterials* **2008**, *29*, 2891.
[107] N. Nagiah, R. Johnson, R. Anderson, W. Elliott, W. Tan, *Langmuir* **2015**, *31*, 12993.
[108] M. Trujillo-Miranda, I. Apsite, J. A. R. Agudo, G. Constante, L. Ionov, *Macromol. Biosci.* **2023**, *23*, 2200320.
[109] R. M. Allaf, in *Functional 3D Tissue Engineering Scaffolds: Materials, Technologies, and Applications*, Woodhead Publishing, **2018**, pp. 75–100, https://doi.org/10.1016/B978-0-08-100979-6.00004-5.
[110] Z. Wang, S. M. Mithieux, A. S. Weiss, *Adv. Healthcare Mater.* **2019**, *8*, 1900742.
[111] T. Gong, K. Zhao, X. Liu, L. Lu, D. Liu, S. Zhou, *Small* **2016**, *12*, 5769.
[112] S. Pradhan, K. A. Keller, J. L. Sperduto, J. H. Slater, *Adv. Healthcare Mater.* **2017**, *6*, 1700681.
[113] O. Sarig-Nadir, N. Livnat, R. Zajdman, S. Shoham, D. Seliktar, *Biophys. J.* **2009**, *96*, 4743.
[114] P. Datta, B. Ayan, I. T. Ozbolat, *Acta Biomater.* **2017**, *51*, https://doi.org/10.1016/j.actbio.2017.01.035.
[115] S. V. Murphy, A. Atala, *Nat. Biotechnol.* **2014**, *32*, 773.
[116] T. Y. Park, Y. J. Yang, D.-H. Ha, D.-W. Cho, H. J. Cha, *Biofabrication* **2019**, *11*, 035001.
[117] I. Zein, D. W. Hutmacher, K. C. Tan, S. H. Teoh, *Biomaterials* **2002**, *23*, 1169.
[118] F. Pati, J. Jang, J. W. Lee, D. W. Cho, in *Essentials of 3D Biofabrication and Translation*, Academic Press, **2015**, pp. 123–152, https://doi.org/10.1016/B978-0-12-800972-7.00007-4.
[119] E. M. Jeffries, S. Nakamura, K.-W. Lee, J. Clampffer, H. Ijima, Y. Wang, *Macromol. Biosci.* **2014**, *14*, 1514.
[120] M. A. Skylar-Scott, S. G. M. Uzel, L. L. Nam, J. H. Ahrens, R. L. Truby, S. Damaraju, J. A. Lewis, *Sci. Adv.* **2019**, *5*, eaaw2459.
[121] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, C. S. Chen, *Nat. Mater.* **2012**, *11*, 768.
[122] R. C. Pimentel, S. K. Ko, C. Caviglia, A. Wolff, J. Emnéus, S. S. Keller, M. Dufva, *Acta Biomater.* **2018**, *65*, 174.
[123] C. B. Highley, C. B. Rodell, J. A. Burdick, *Adv. Mater.* **2015**, *27*, 5075.
[124] W. Wu, A. Deconinck, J. A. Lewis, *Adv. Mater.* **2011**, *23*, H178.
[125] K. A. Homan, D. B. Kolesky, M. A. Skylar-Scott, J. Herrmann, H. Obuobi, A. Moisan, J. A. Lewis, *Sci. Rep.* **2016**, *6*, https://doi.org/10.1038/srep34845.
[126] W. Liu, Z. Zhong, N. Hu, Y. Zhou, L. Maggio, A. K. Miri, A. Fragasso, X. Jin, A. Khademhosseini, Y. S. Zhang, *Biofabrication* **2018**, *10*, 024102.
[127] E. Bosch-Rué, L. M. Delgado, F. J. Gil, R. A. Perez, *Biofabrication* **2020**, *13*, 015003.
[128] B. Derby, *Annu. Rev. Mater. Res.* **2010**, *40*, 395.
[129] C. Xu, W. Chai, Y. Huang, R. R. Markwald, *Biotechnol. Bioeng.* **2012**, *109*, 3152.
[130] A. Blaeser, D. F. D. Campos, M. Weber, S. Neuss, B. Theek, H. Fischer, W. Jahnen-Dechent, *BioRes. Open Access* **2013**, *2*, 374.
[131] R. Wang, J. Ozsvar, B. Aghaei-Ghareh-Bolagh, M. A. Hiob, S. M. Mithieux, A. S. Weiss, *Biomaterials* **2019**, *192*, 334.
[132] M. Duocastella, J. M. Fernández-Pradas, J. Domínguez, P. Serra, J. L. Morenza, *Appl. Phys. A: Mater. Sci. Process.* **2008**, *93*, 941.
[133] L. Koch, A. Deiwick, B. Chichkov, *Micromachines* **2021**, *12*, 1538.
[134] V. L. Tsang, S. N. Bhatia, *Adv. Drug Delivery Rev.* **2004**, *56*, 1635.
[135] R. F. Pereira, P. J. Bártolo, *J. Appl. Polym. Sci.* **2015**, *132*, 42458.
[136] A. J. Melchiorri, N. Hibino, C. A. Best, T. Yi, Y. U. Lee, C. A. Kraynak, L. K. Kimerer, A. Krieger, P. Kim, C. K. Breuer, J. P. Fisher, *Adv. Healthcare Mater.* **2016**, *5*, 319.
[137] A. K. Miri, A. Khalilpour, B. Cecen, S. Maharjan, S. R. Shin, A. Khademhosseini, *Biomaterials* **2019**, *198*, 204.
[138] J. Gong, C. C. L. Schuurmans, A. M. V. Genderen, X. Cao, W. Li, F. Cheng, J. J. He, A. López, V. Huerta, J. Manríquez, R. Li, H. Li, C. Delavaux, S. Sebastian, P. E. Capendale, H. Wang, J. Xie, M. Yu, R. Masereeuw, T. Vermonden, Y. S. Zhang, *Nat. Commun.* **2020**, *11*, https://doi.org/10.1038/s41467-020-14997-4.
[139] V. K. Lee, D. Y. Kim, H. Ngo, Y. Lee, L. Seo, S.-S. Yoo, P. A. Vincent, G. Dai, *Biomaterials* **2014**, *35*, 8092.
[140] O. Kérourédan, J. M. Bourget, M. Remy, S. C. Manciet, J. Kalisky, S. Catros, N. B. Thebaud, R. Devillard, *J. Mater. Sci.: Mater. Med.* **2019**, *30*, https://doi.org/10.1007/S10856-019-6230-1/.

[141] J. W. Lee, Y.-J. Choi, W.-J. Yong, F. Pati, J.-H. Shim, K. S. Kang, I.-H. Kang, J. Park, D.-W. Cho, *Biofabrication* **2016**, *8*, 015007.
[142] Y. Nishiyama, M. Nakamura, C. Henmi, K. Yamaguchi, S. Mochizuki, H. Nakagawa, A. Takiura, *J. Biomech. Eng.* **2009**, *131*, 035001.
[143] G. Gao, J. Y. Park, B. S. Kim, J. Jang, D.-W. Cho, *Adv. Healthcare Mater.* **2018**, *7*, 1801102.
[144] K. Christensen, C. Xu, W. Chai, Z. Zhang, J. Fu, Y. Huang, *Biotechnol. Bioeng.* **2015**, *112*, 1047.
[145] M. Gruene, M. Pflaum, C. Hess, S. Diamantouros, S. Schlie, A. Deiwick, L. Koch, M. Wilhelmi, S. Jockenhoeval, A. Haverish, B. Chichkov, *Tissue Eng., Part C* **2011**, *17*, 973.
[146] X. Cui, T. Boland, *Biomaterials* **2009**, *30*, 6221.
[147] A. Skardal, J. Zhang, L. Mccoard, X. Xu, S. Oottamasathien, G. D. Prestwich, *Tissue Eng., Part A* **2010**, *16*, 2675.
[148] S. Li, Z. Xiong, X. Wang, Y. Yan, H. Liu, R. Zhang, *J. Bioact. Compat. Polym.* **2009**, *24*, 249.
[149] J. Y. Park, J.-H. Shim, S.-A. Choi, J. Jang, M. Kim, S. H. Lee, D.-W. Cho, *J. Mater. Chem. B* **2015**, *3*, 5415.
[150] Q. Yang, B. Gao, F. Xu, *Biotechnol. J.* **2020**, *15*, 1900086.
[151] L. Zhang, H. Liang, J. Jacob, P. Naumov, *Nat. Commun.* **2015**, *6*, 7429.
[152] D. Kokkinis, M. Schaffner, A. R. Studart, *Nat. Commun.* **2015**, *6*, https://doi.org/10.1038/ncomms9643.
[153] S. Anandhakumar, G. Krishnamoorthy, K. M. Ramkumar, A. M. Raichur, *Mater. Sci. Eng., C* **2017**, *70*, 378.
[154] M. L. P. Ramos, J. A. González, L. Fabian, C. J. Pérez, M. E. Villanueva, G. J. Copello, *Mater. Sci. Eng., C* **2017**, *78*, 619.
[155] H. Cui, C. Liu, T. Esworthy, Y. Huang, Z.-X. Yu, X. Zhou, H. San, S.-J. Lee, S. Y. Hann, M. Boehm, M. Mohiuddin, J. P. Fisher, L. G. Zhang, *Sci. Adv.* **2020**, *6*, eabb5067.
[156] Y. Luo, X. Lin, B. Chen, X. Wei, *Biofabrication* **2019**, *11*, 045019.
[157] A. Ding, S. J. Lee, R. Tang, K. L. Gasvoda, F. He, E. Alsberg, *Small* **2022**, *18*, 2202196.
[158] K. Kuribayashi-Shigetomi, H. Onoe, S. Takeuchi, *PLoS One* **2012**, *7*, e51085.
[159] A. Ding, S. J. Lee, R. Tang, K. L. Gasvoda, F. He, E. Alsberg, *Small* **2022**, *18*, 2202196.
[160] V. Stroganov, S. Zakharchenko, E. Sperling, A. K. Meyer, O. G. Schmidt, L. Ionov, *Adv. Funct. Mater.* **2014**, *24*, 4357.
[161] P. Cao, J. Yang, J. Gong, L. Tao, T. Wang, J. Ju, Y. Zhou, Q. Wang, Y. Zhang, *J. Appl. Polym. Sci.* **2023**, *140*, e53241.
[162] J. Liu, W. Liu, A. Pantula, Z. Wang, D. H. Gracias, T. D. Nguyen, *Extreme Mech. Lett.* **2019**, *30*, 100514.
[163] J. Lai, X. Ye, J. Liu, C. Wang, J. Li, X. Wang, M. Ma, M. Wang, *Mater. Des.* **2021**, *205*, 109699.
[164] A. Kirillova, R. Maxson, G. Stoychev, C. T. Gomillion, L. Ionov, *Adv. Mater.* **2017**, *29*, 1703443.
[165] L. Zhang, Y. Xiang, H. Zhang, L. Cheng, X. Mao, N. An, L. Zhang, J. Zhou, L. Deng, Y. Zhang, X. Sun, H. A. Santos, W. Cui, *Adv. Sci.* **2020**, *7*, 1903553.
[166] W. Kitana, I. Apsite, J. Hazur, A. R. Boccaccini, L. Ionov, *Adv. Mater. Technol.* **2023**, *8*, 2200429.
[167] M. Shaikh, G. Kichenadasse, N. R. Choudhury, R. Butler, S. Garg, *J. Controlled Release* **2013**, *172*, 105.
[168] A. Melocchi, M. Uboldi, N. Inverardi, F. Briatico-Vangosa, F. Baldi, S. Pandini, G. Scalet, F. Auricchio, M. Cerea, A. Foppoli, A. Maroni, L. Zema, A. Gazzaniga, *Int. J. Pharm.* **2019**, *571*, 118700.
[169] C. S. Yang, H. C. Wu, J. S. Sun, H. M. Hsiao, T. W. Wang, *ACS Appl. Mater. Interfaces* **2013**, *5*, 10985.
[170] Q. Ge, A. H. Sakhaei, H. Lee, C. K. Dunn, N. X. Fang, M. L. Dunn, *Sci. Rep.* **2016**, *6*, 31110.
[171] M. Bodaghi, A. R. Damanpack, W. H. Liao, *Smart Mater. Struct.* **2018**, *27*, 065010.
[172] X. Wang, Y. Zhang, P. Shen, Z. Cheng, C. Chu, F. Xue, J. Bai, *Biomater. Sci.* **2022**, *10*, 2302.
[173] Y.-C. Li, Y. S. Zhang, A. Akpek, S. R. Shin, A. Khademhosseini, *Biofabrication* **2016**, *9*, 012001.
[174] L. Ionov, *Adv. Healthcare Mater.* **2018**, *7*, 1800412.

Lubna Zeenat is pursuing her Ph.D., jointly from IIT Hyderabad and Deakin University, Australia. Her research project pertains to the fabrication of functional tissue construct using 4D bioprinting. She holds a master's degree in biotechnology and her research interests include biofabrication, 4D bioprinting, and tissue engineering.

Ali Zolfagharian is the Alfred Deakin medalist for best doctoral thesis, titled "3D-printed Soft Actuators," and the Alfred Deakin Postdoctoral Fellowship awardee. He is co-founder of the 4D Printing Society and co-editor of the Smart Materials in Additive Manufacturing book series published by Elsevier, while also being a senior lecturer in the School of Engineering at Deakin University, Australia, where he established 4D Printing and Robotic Materials Lab. He has thus far had outputs in the field of 3D and 4D printing of smart robotic materials, including 120 journal articles, 15 special issues, 7 book chapters, and 5 books.

Mahdi Bodaghi is senior lecturer in the Department of Engineering at Nottingham Trent University. He is the founder and leader of the 4D Materials & Printing Lab that hosts a broad portfolio of projects focusing on electro-thermo-mechanical behaviors of smart materials, meta-materials, soft robotics, biomedical devices, and 3D/4D printing technologies. His vast experience and research on smart materials and additive manufacturing has led him to co-found the 4D Printing Society and to co-edit the book series—Smart Materials in Additive Manufacturing. His research has also resulted in the publication of over 200 scientific papers in prestigious journals (h-index: 38, citations: 4500).

Falguni Pati is an associate professor in the Department of Biomedical Engineering at IIT Hyderabad (IITH), India. He completed his Ph.D. from IIT Kharagpur and had postdoctoral positions at POSTECH (South Korea) and KTH (Sweden). His research interests include developing novel bioinks, bioprinting methods, and technologies to produce tissue analogs-on-demand and in vitro tissue/organ models. His lab at IITH, Biofabrication and Tissue Engineering Lab, is at the forefront of developing various tissue engineering solutions based on 3D and 4D bioprinting technologies. His team developed a novel biomimetic hydrogel for treatment of blinding corneal diseases and progressing toward clinical translation.