Chapter 1
Microscale Technologies for Engineering Complex Tissue Structures

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Abstract Engineered tissue scaffolds aim to reproduce the body's architectural and geometrical intricacies, including vital cell–cell interactions. These scaffolds serve as synthetic extracellular matrices that organize the embedded cells into a three-dimensional (3D) architecture and present them with stimuli for their growth and maturation. Tissue engineering techniques have been applied to many types of tissues; however, numerous challenges regarding their development still remain. These challenges include our inability to generate a functional vasculature that can supply the tissue with nutrients and oxygen and the inability to mimic the complex cell–microenvironmental interactions that regulate the formation of a functional tissue. This chapter focuses on the most recent developments in the field of microfabrication technologies to design vascularized tissue constructs. In particular, we discuss emerging bottom-up approaches to design complex macroscale structures, examine their current limitations, and conclude with future directions in designing more complex tissue architecture.

1 Introduction

Engineering artificial tissues offers great promise for treating patients with organ failures that are associated with disease, injury, and degeneration [1, 2]. Current approaches to engineer three-dimensional (3D) tissue structures are based on encapsulating cells within a porous scaffold and providing structural and molecular cues to facilitate formation of tissue structure [1–5]. These scaffolds serve as synthetic extracellular matrices (ECMs) that assist in cellular organization into a 3D architecture by providing appropriate chemical and physical stimuli to facilitate their growth and maturation [6]. These tissue engineering techniques have been applied to generate a range of tissues including cartilage and skin, as these tissues can survive without the presence of extensive vascularization. However, engineering tissues with complex structures such as the heart, and liver, is not possible until numerous challenges regarding their development are addressed. These challenges include our inability to first generate a functional vasculature that can supply the tissue with nutrients and oxygen and secondly to mimic the complex cell–microenvironmental interactions that regulate the formation of a functional tissue.

The full potential of tissue engineering has not been realized due to the inability to engineer complex tissues that require formation of intrinsic vasculature [1, 7]. One of the major limitations in tissue engineering is diffusion of nutrients, oxygen, and metabolite transport throughout an engineered scaffold [8]. After a cell-seeded scaffold is implanted in vivo, encapsulated cells quickly consume available oxygen and nutrients from the synthetic surroundings to sustain their metabolic activity. The survival of an implanted scaffold initially depends on diffusion of nutrients and later on the formation of neovascularization which leads to full tissue integration. The solubility of oxygen in biological fluids such as culture media or solution containing glucose or amino acid is quite low and is limited. This problem becomes much more severe in the presence of cells (or in vivo) that actively consume oxygen.

The inequality between oxygen supply and consumption at the cellular level may result in hypoxic conditions and consequently change the cellular behavior. Complex tissue formation requires oxygen which is transported by the vasculature. Thus, one of the primary challenges in tissue engineering is to fabricate vascularized networks within the 3D scaffold to facilitate diffusion of oxygen and nutrients that sustain cellular activity of the encapsulated cells [9].

There are two major approaches that have been developed to fabricate vascularized tissue: "top-down" and "bottom-up." The top-down approach involves the use of a porous scaffold to promote the formation of a vascularized structure within a three-dimensional scaffold [10]. Several top-down approaches such as use of angiogenic growth factors, pre-seeding a scaffold with stem cells, and co-culture techniques have been proposed [11–13]. Although most of these approaches have shown promise to facilitate formation of vascular structure, they are ineffective in developing stable and branched vascular structures. This is mainly due to the lack of control over cellular function and organization within a three-dimensional structure. Recently, the bottom-up approach has shown promise in overcoming these challenges by controlling spatial and temporal distribution of cells and directing cell–cell and cell–matrix interactions [7, 14, 15]. The term microfabrication refers to fabrication of miniature structures especially in the micron-size range. The microfabrication techniques have provided new bottom-up approaches such as micropatterning, microprinting, microfluidics, and microassembly (Fig. 1.1) to fabricate complex tissue architectures with pre-vascularized networks [7, 14]. These bottom-up approaches are used to form complex macroscale structures using microfabricated building blocks [16].

This chapter highlights and discusses recent development in bottom-up approaches with a special focus on emerging microscale techniques for engineering complex vascularized tissues. First, we discuss the physiology of the vascularized network and highlight conventional techniques (top-down approaches) to engineer complex tissues. We then focus on recent development in microscale technologies that are currently used to design vascularized networks within a 3D scaffold. We also discuss some of the prevailing technologies that indirectly control cellular microenvironment to promote formation of complex tissues. The use of these emerging technologies in creating and mimicking native tissue architecture is reviewed. Finally, we conclude by listing future direction and outlook on engineering complex vascularized tissues.

2 Physiology and Structure of Complex Vascularized Tissues

The vascularized network present in vivo is composed of complex and highly branched networks of blood vessels. This complex network consists of arteries, capillaries, and veins. The capillaries are mainly responsible for exchange of nutrients, metabolite transport, and oxygen between the tissues and blood. The maximum distance between these capillaries is governed by the oxygen requirement of the tissues. For example, cells present in heart, liver, and muscles consume oxygen and

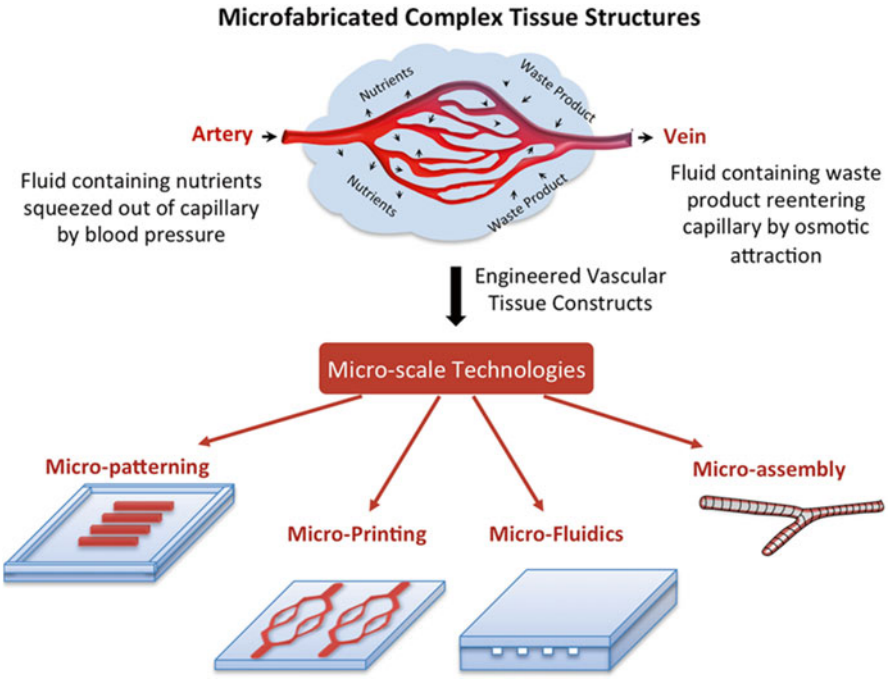

Fig. 1.1 Microscale technologies to fabricate complex vascularized tissue constructs. These technologies include micropatterning, microprinting, microfluidics, and microassembly. The design of mechanically and biologically similar tissue constructs depends on the ability to mimic native microarchitecture of specific tissues. These bottom-up approaches offer distinct advantages in terms of spatial and temporal control over cellular organization

nutrients very rapidly to maintain their function. The maximum allowable distance between capillaries that are present in these tissues is in the range of 100–200 μm; [17] this is well within the oxygen diffusion limit. In the case of cells like pancreatic islets, increasing diffusion distance beyond 100 μm has shown to cause severe necrosis [18]. Cells present in tissues like skin, cartilage, or cornea can sustain their metabolic function even at larger distances (200–1000 μm) [19, 20].

The blood vessels consist of three sub-layers (also known as tunica) that control diffusion of oxygen and nutrients between the blood and the tissues. The innermost layer (tunica intima) is composed of a monolayer of endothelial cells (also known as endothelium). These endothelial cells prevent platelet activation and thrombogenesis by secreting nitric oxide. The middle layer (tunica media) consists of densely populated and well-organized smooth muscle cells that are separated from the endothelial cells by an elastic lamina. The outermost layer (tunica adventitia) consists of ECM and fibroblast cells. Other extracellular proteins such as proteoglycans and glycoproteins are also found around the vascular cells.

Blood vessels can be formed in two ways: vasculogenesis [21] and angiogenesis [22, 23] (Fig. 1.2) [24–27]. Formation of new blood vessels is known as vasculogenesis

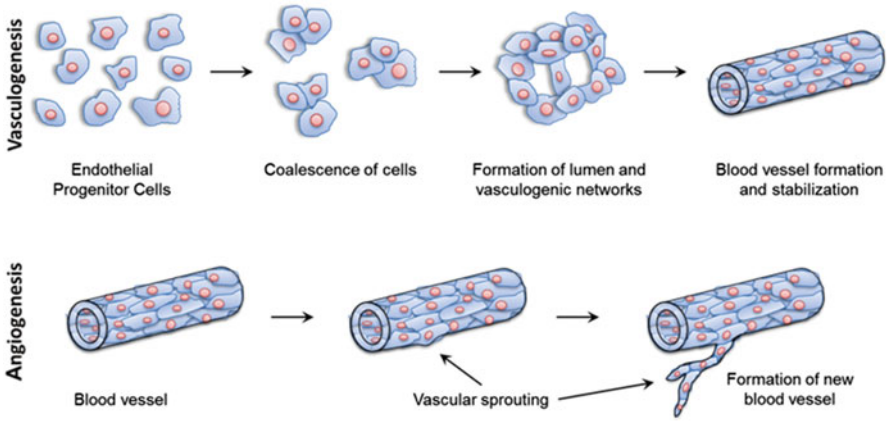

Fig. 1.2 New blood vessel formation occurs via vasculogenesis and angiogenesis. Vasculogenesis is a process of spontaneous blood vessel formation from endothelial progenitor cells. The progenitor cells proliferate and form lumen that ultimately leads to the formation of new blood vessels. Angiogenesis is formation of new blood vessels from pre-existent ones through vascular sprouting

and it occurs early in the developmental stage within avascular tissue. In vasculogenesis, mesodermal cells first differentiate into angioblasts or hemangioblasts, and then further differentiate into endothelial progenitor cells [21, 26, 27]. These progenitor cells rearrange themselves to form lumen and ultimately result in formation of new capillary blood vessels. Angiogenesis is the term used for the formation of new blood vessels from the pre-existing ones through vascular sprouting [22, 23]. In the normal state, endothelial cells have low turnover rate, but during activated states such as inflammation or wound healing, endothelial cells change their phenotype and release chemotactic factors [28–30]. These chemotactic factors result in vascular sprouting from existing capillaries and formation of new blood vessels [25, 26].

Understanding of the vasculature is paramount in the design and fabrication of complex tissues. As noted, the capillary network varies depending on the tissue; therefore, techniques must be able to mimic these attributes. Angiogenesis contributes to an implant's long-term viability [31]; current methodology will be discussed for the co-creation of vasculature with complex tissues.

3 Current Approaches for Engineering Complex Vascularized Tissues

Traditional tissue engineering strategies (also known as "top-down" approaches) include the use of porous scaffolds often seeded with cells. In this approach, cells are expected to proliferate, secrete ECM, and form vascularized networks

within the already synthesized complex matrix [32, 33]. Several top-down strategies are currently under investigation to create vascularized scaffolds [10, 11]. These include using angiogenesis-inductive materials, incorporation of growth factors (either by conjugation, encapsulation, or supplementing), utilizing co-culture techniques, and using decellularized organs and blood vessels for creating ECM.

The scaffold (made from vascular inductive materials) provides physical, chemical, and biological cues to control cell–matrix interactions and cellular processes such as angiogenesis [34]. Strategy to fabricate vascularized structures includes culturing cell-seeded porous polymeric scaffold in a bioreactor and implanting it in vivo to facilitate remodeling and integration with the host vascular network. In the last two decades, numerous biomaterials composed of natural polymers (such as collagen, hyaluronan, alginate, Matrigel, fibrin, peptide, and decellularized matrix) or synthetic polymers (such as poly(ε-caprolactone) (PCL), poly(ethylene glycol) (PEG), and poly(2-hydroxyethyl methacrylate-co-methacrylic acid) (pHEMA-co-PMA)) have been seeded with endothelial cells to obtain vascularized structure [35]. Natural polymers facilitate cell adhesion and support cell-based remodeling but they have poor processing ability due to weak mechanical properties. Although synthetic materials have poor cell-adhesion capacity, they can be fabricated in complex structures or shapes due to excellent mechanical stability. Most of the studies to evaluate biomaterials for engineering vascularized tissues are limited to endothelial cell adhesion and formation of cord-like structures [36]. Future studies should focus on evaluating cell–matrix interactions, activation status of endothelial cells, integration of monolayer, and stability of the layer under hemodynamic conditions.

Another strategy to develop a vascularized network within a 3D scaffold is to utilize topographical features in directing and guiding cells to promote microvascularization. For example, Sukmana et al. used poly(ethylene terephthalate) (PET) microfibers as contact guidance to orient and facilitate formation of microvessels using human umbilical vein endothelial cells (HUVECs) [37]. Addition of fibrin to PET microfibers promoted adhesion of HUVECs. They observed that fiber-to-fiber distance played a major role in lumen formation and development of microvessels. It was shown that formation of vascular structure can be controlled only by controlling the scaffold architecture (such as fiber diameter and fiber-to-fiber distance). Other groups have also used electrospun scaffold for engineering vascular tissue structures [38, 39].

In most cases, biomaterials alone are not capable of inducing angiogenesis. Thus to further assist formation of microvascular structure within a scaffold, growth factors are conjugated, encapsulated, or supplemented during in vitro and in vivo studies [40]. Most common growth factors used to induce vascularization are vascular endothelial growth factor (VEGF) [41–45], fibroblast growth factor (FGF) [46–49], transforming growth factor (TGF-b) [50], platelet-derived growth factor (PDGF) [51–53], and angiopoietins (Ang) [43, 54].

In more complex in vivo conditions, apart from growth factors, the surrounding microenvironment (such as cell–cell interactions, cell–ECM interactions) plays a

critical role in directing cell fate [8]. The presence of different cell types (co-culture) can lead to unique responses that cannot be obtained by using growth factors alone. For example, Melero-Martin et al. showed that in vivo vasculogenesis can be obtained by co-implantation of endothelial progenitor cells and mesenchymal stem cells suspended in Matrigel (act as a support structure) [55]. In another effort, Levenberg et al. engineered vascularized skeletal muscle tissue by culturing myoblasts, embryonic fibroblasts, and endothelial cells within a highly porous 3D scaffold made from poly-(L-lactic acid) (PLLA) and poly(lactic-co-glycolic acid) (PLGA) [56]. This study showed that the addition of embryonic fibroblasts enhances VEGF expression and results in the formation of stable endothelial vessels.

In the past few years, researchers have been trying to use combinatorial methods to achieve rapid neovascularization within an engineered scaffold. For example, Phelps et al. designed bioartificial matrices from PEG-based hydrogels to obtain stable vascularized network [57]. They incorporated protease-degradable sites for controlled degradation, adhesion motifs to facilitate cell adhesion and migration, and growth factor (VEGF) to induce new tissue vascularization. Due to the presence of degradable moieties within the PEG hydrogels, the covalently conjugated VEGF showed a sustained release for 2 weeks. A significantly higher degree of vessel density was observed after the matrix containing VEGF was subcutaneously implanted in a mouse model. Researchers attributed the significantly enhanced rate of neovascularization to the simultaneous control of matrix degradation and growth factor release. The main goal of all these "top-down" strategies is to incorporate vascularized structure within an engineered scaffold. However, most of the approaches might take a few days to several weeks to develop vascularized structure after implantation. The sustainability of implanted construct might rely on surrounding vessels and angiogenesis of existing vessels. During this time frame, encapsulated cells might extract nutrients and oxygen, and tissue necrosis might be observed. Even if these strategies are able to form complex vascularized networks in vitro, most of them fail to integrate with the host tissues and result in undesirable side effects such as inflammation and poor resorption.

4 Microscale Technologies for Engineering Complex Vascularized Tissues

The ultimate goal of the bottom-up approach is to control cell–cell and cell–matrix interactions to fabricate complex vascularized tissue. Microfabrication techniques have been extensively used to pattern cell-laden hydrogels for studying fundamental cell biology. Several techniques have been developed for microfabrication including photolithography, microcontact printing, microfluidics, micropatterning, and microassembly (Fig. 1.3).

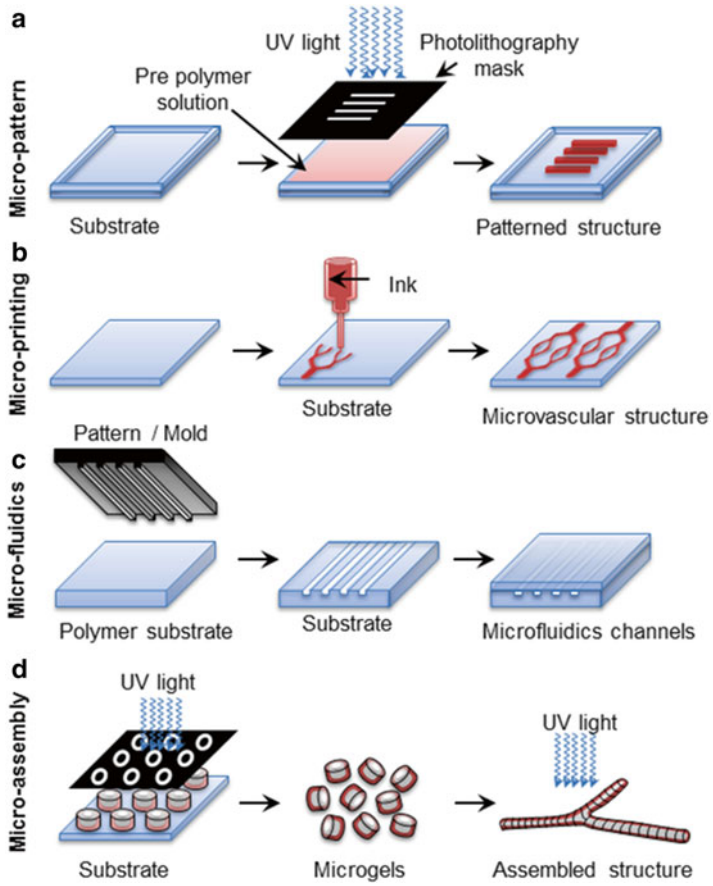

Fig. 1.3 Bottom-up approaches in vascularized tissue engineering. (**a**) Micropatterning utilizes soft lithographic techniques to fabricate microscale structure. In this process, photocrosslinkable polymer precursor solution along with initiator is exposed to ultraviolet radiation through a mask to fabricate micropatterns. (**b**) Microprinting utilizes conformational contact to form a pattern of ink on the surface. (**c**) Microfluidic channels can be fabricated by using micromolds. These channels can then be used to form microfibers of a sacrificial substance that is coated to form hollow fibers. These microfluidic channels can be utilized to form hollow vascularized conduit. (**d**) Complex vascularized structures can also be fabricated by assembly of microgels

4.1 Micropatterning Techniques for Engineering Complex Vascularized Tissues

Photolithography is widely used in the microelectronics device industry to fabricate micropatterned surfaces. The recent development of various photocrosslinked polymers has provided the impetus to engineer micropatterned hydrogels for various biomedical applications. In this process, precursor solution consisting of

photocrosslinkable polymer along with initiator is exposed to ultraviolet radiation through a mask. The selective exposure of the precursor solution to the radiation results in photoreaction and cross-linked polymer patterns. The pre-polymer solution can be easily washed away after the removal of photomask.

Initial development to construct complex vascularized networks using photolithography utilized non-degradable synthetic polymers such as polydimethylsiloxane (PDMS). Although PDMS has good mechanical properties, biocompatibility, and high optical transparency, it is not degradable, limiting its utility as an implantable material. Recently, numerous biodegradable polymers have been explored for scaffolding purposes in micropatterning such as PLGA [58, 59], PCL [60, 61], poly(glycerol sebacate) (PGS) [62–65], and hyperbranched polyesters [66]. Currently, micropatterning techniques utilize scaffolds made from natural polymers that closely mimic native ECM; these polymers include gelatin [67, 68], alginate, chitosan [69], and carrageenan [70].

Photolithography has been utilized to obtain desired microscale features to control formation of vascularized structure in 2D and 3D microenvironments. For example, gelatin methacrylate (GelMA) can be patterned as 2D microchannels of width from 50 to 200 μm can be fabricated using photolithography [67, 68]. By tuning material properties and channel width, the adhesion, spreading, and elongation of HUVECs seeded on micropatterns were controlled. However, one of the drawbacks of 2D microenvironment is lack of control over cellular orientation and vascular network. Nikkhah et al. showed that endothelial cells entrapped within these microchannels align and organize within 1 week and facilitate formation of cord-like tubular structures [67]. These results indicate that micropatterned structures can provide confined geometries that can result in endothelial cord formation and thus can be used to design complex vascularized tissue constructs.

The photolithography technique produces microvascular channels with high precision at the micron scale. However, to create a 3D network, several patterned layers must be aligned and stacked together. The physical alignment of several microscale patterned layer precision might limit the practical application of fabricating thick tissue-engineered construct. To overcome the difficulty of spatially controlling the microfeatures within a three-dimensional scaffold, Chiu et al. developed a technique to create microchannels within a 3D PEG hydrogel by selective degradation of micropatterned structures [71]. They used non-contact photolithography to fabricate poly (ethylene glycol)-co(L-lactide) (PEG-PLLA) microchannels within multilayer PEG hydrogels. After exposing the hydrogel construct to high pH, PEG-PLLA micropatterns degraded rapidly and uniform-sized microchannels were obtained. Researchers were able to fabricate interconnected microchannels within a 3D hydrogel structure by introducing a second patterned layer.

Another micropatterning technique to fabricate microscale features with desired architecture and topography is micromolding [72, 73]. In this process, a master mold is used to fabricate a micropattern by casting polymer solution on prefabricated silicon wafers. Then the cast pattern is used as a negative mold to fabricate microscale architecture. Fidkowski et al. used PGS to microfabricate a biodegradable and elastomeric capillary network. In this study, they used microelectromechanical

systems to etch capillary patterns onto a silicon wafer [74]. The pattern was then transferred to PGS film and was later bound by a flat PGS film to obtain capillary networks. After seeding the channels with endothelial cells and subjecting to continuous flow, a confluent cell layer was formed along the channels within 2 weeks.

Zheng et al. fabricated microstructured tissue templates with embedded micropores and a microfluidic network from alginate and collagen hydrogels [75]. They showed that by controlling physical properties and material chemistry, biophysical mechanism and in vivo host responses could be tailored. The scaffold with micropatterns showed higher tissue in growth and invasion of blood vessels compared to the unpatterned scaffold murine wound model. Their study indicates that pro-angiogenic signals secreted by tissue present within the pores primarily drives rapid blood vessel invasion and tissue vascularization.

In another study, Diez et al. microfabricated elastomeric patterned hydrogels by combining soft lithography and micromolding techniques known as fill-molding in capillaries (FIMIC) [76]. In this method, a PEG-based hydrogel is molded on microfabricated silicon master using UV radiation. The grooves created on the surface of the hydrogel are filled with a second hydrogel by utilizing capillary action. One of the primary advantages of this technique is that two different polymeric hydrogels with different physical, chemical, or biological properties can be obtained. One of the potential advantages of this method is fabricating two different types of materials encapsulated with different cells. For example, the capillaries can be filled with softer hydrogels (may be conjugated with growth factor) to promote formation of vascularized tissue and the outer hydrogels (strong and tough network) promote osteogenic differentiation to obtain vascularized bone tissue. However, this technique is only applicable to fluidic secondary materials that can be filled within the capillaries.

4.2 Microprinting to Create Interconnected 3D Microvascular Structure

Microcontact printing is another method to fabricate well-defined and controlled architectures using biopolymers. Bianchi et al. combined microprinting techniques with growth factors and co-culture [77]. They fabricated fractal-like structures to mimic the capillary network using a pressure-assisted microfabrication method [77, 78]. The scaffold was then seeded with endothelial cells along with fibroblasts. The endothelial cells were transfected with an adenoviral vector carrying human tissue kallikrein (angiogenic promoter). The study showed that the metabolic activity of cells was enhanced due to synergistic contribution from co-culture and viral transfection. This study indicates that dynamic reciprocity between microstructural features and biochemical signals is important for controlling cellular activity.

Direct ink writing offers an alternate fabrication method for designing 3D microvascular structures with high fidelity [79, 80]. For example, Therriault et al. used a direct ink writing method to fabricate 3D microvascular networks by using a fugitive organic ink [81, 82]. In this work, they first deposited a fugitive ink in a layer-by-layer

fashion to generate 3D periodic square-spiral architecture. The deposited ink was self-sustained and was able to span large distances without curving or bending. The second step consisted of infiltrating the 3D periodic structure with an epoxy resin and curing it to form structural matrix. Finally, the fugitive ink was removed to create an interconnected 3D microvascular structure. Although this technique provides a simple and high-fidelity method to create vascular structure, it is limited to generate periodic and interconnected geometries.

In a recent report, Wu et al. proposed to use omnidirectional printing (a slight variation of direct ink writing) to fabricate biomimetic microvascular structure (Fig. 1.4) [83]. In this process, viscoelastic ink consisting of sacrificial material is directly patterned to create microvascular structures into a 3D scaffold. The patterned structure is encapsulated within a photocrosslinked matrix and subsequently, the sacrificial pattern is removed to yield microvascular structure. This approach has potential to develop complex vascular architecture within a 3D hydrogel network.

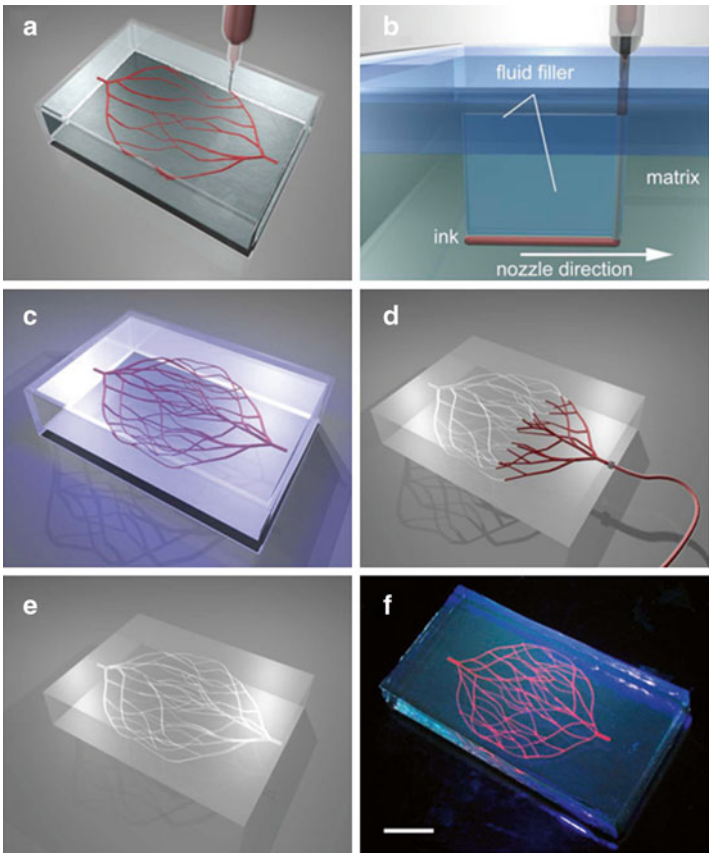

Fig. 1.4 Fabrication of microvascular structure within a photopolymerizable hydrogel matrix using omnidirectional printing of a fugitive organic ink as demonstrated by Wu et al. [83]

However, further optimization of materials in terms of biocompatibility and degradability needs to be performed to utilize this technique for complex tissue engineering applications.

Recently, Xavier et al. investigated precursor solutions containing gelatin methacrylate (GelMA) and silicate nanoparticles for 3D printing [84]. In using this approach cells can be incorporated in specific geometries, thereby more recapitulating complex tissues structures. Specifically, they demonstrated that hydrogel precursor ink is a viable alternative to traditional 3D printer inks by exploiting the shear-thinning properties of nanocomposite-hydrogel solution. Through this work, there is a possible shift in the paradigm for 3D printing from conventional polymeric inks to nanocomposite inks.

4.3 Microfluidics Technologies for Enhanced Perfusion of Complex Vascularized Constructs

One of the major approaches in fabricating complex vascularized structures within a 3D scaffold is to take advantages of microfluidic technologies. Microfluidics deals with the precise control and manipulation of fluids in confined space and volumes [85]. A range of biological phenomena such as cell–cell interactions, cell–biomaterial interactions, and mass transport can be manipulated by spatiotemporal control of fluid flow within a 3D scaffold [86]. Recently, microfluidic technology has shown potential to fabricate vascularized 3D scaffolds. In this approach, microfluidic channels provide a structural framework for cells to form vascular-like structure and the fluid flow provides soluble factors to control tissue regeneration.

In the past decade, a range of polymers have been investigated to design microfluidic devices to engineer complex vascularized constructs. Initially, elastomeric and biocompatible polymers such as PDMS [87] were extensively used, whereas recent methodologies focus on use of biodegradable polymers such as PLGA [61, 88], PGS [64, 74], and silk fibroin [89].

For example, Fidkowski et al. used biodegradable and biocompatible elastomer (PGS) to microfabricate capillary networks using soft lithography techniques [64, 74]. The tensile properties of the PGS elastomer closely match those of veins and do not elicit any chronic inflammation when implanted in vivo [74, 90]. To microfabricate capillary networks, standard MEMS were first used to etch a pattern on silicon wafers. The pattern was then transferred to the PGS film by spreading pre-polymer solution on the wafer and polymerizing it at 150 °C. The patterned PGS film was bonded to flat film to create enclosed capillary channels that were seeded with endothelial cells (HUVECs). The cells readily attached to the PGS surface compared to the PDMS- and PLGA-based microdevices without the use of adhesion protein. After 14 days of culture, a nearly confluent cell layer was formed within the PGS channels.

Although a PGS-based microfluidic device shows feasibility for developing microvascularized scaffolds, rapid degradation after subcutaneous implantation

(~60 days) is one of the major limitations [90, 91]. To fabricate long-lasting and mechanically strong microfluidic devices, Wang et al. developed poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate) (APS)-based microfluidic scaffolds [91]. The degradation time of this elastomer can be tuned from a few weeks to a year by changing the chemical composition. Although the mechanical strength of APS is much lower compared to PGS and PLGA, the process of microfabricating capillary networks is rapid, cost effective, and easily reproducible. This makes the APS-based microfluidic scaffolds attractive for engineering complex vascularized tissues.

In a recent study, Borenstein et al. proposed to use silk fibroin as a degradable biopolymer to fabricate a microfluidic scaffold that can support formation of microvascular networks [89]. They fabricated the microfluidic channels by first obtaining a pattern on a silicon wafer using photolithographic techniques. The pattern was then transferred to an elastic mold (made from PDMS). A trenched layer was obtained by casting silk fibroin on the elastic mold that was subsequently bound to a flat silk film to obtain microfluidic channels. The microfluidic conduits provide a physical template to cells and help them to reorganize into a microvascular structure. The study showed that the microfluidic device made from the biopolymer sustained fluid flow without leakage and delamination. Human dermal microvascular endothelial cells (HDMVECs) were successfully infused within the microfluidic network.

One of the problems with microchannel formation is controlling channel geometry. Most of the earlier attempts report formation of distorted rectangular-shaped microchannels. Borenstein et al. overcame this problem by combining micromolding and embossing techniques [92]. They were able to obtain nearly perfect cylindrical channels with the inner diameter ranging from 100 μm to 1 mm.

Alternative approach to fabricate microfluidic channels is to use a sacrificial element. Golden et al. used gelatin to fabricate sacrificial micromolded meshes using a PDMS stamp [93]. The mesh structure was encased in a secondary network (collagen type I, fibrinogen, or Matrigel) and later the micromolded meshes were washed away to obtain a 3D scaffold with microchannels. After perfusing the microchannels with endothelial cells, a uniform cell monolayer was formed that lined the microchannels. One of the difficulties in obtaining uniform-size microfeatures is low mechanical properties and the highly hydrophilic characteristic of gelatin limits the formation of rigid micromolded meshes.

In a similar technique Miller et al. printed multiscale vascular network from carbohydrate glass as sacrificial materials by combining thermal extrusion and fiber drawing processes [94]. This 3D sacrificial structure was coated with poly (D-lactide-co-glycolide) (PDLGA) before impregnated with a pre-polymer solution laden with cells. After cross-linking the pre-polymer solution, 3D vascular network was dissolved to generate a cylindrical network. This technique allows independent control over the vessel geometry, surrounding matrix, and endothelialization and can be used to form complex 3D interconnected structures. They showed feasibility of this concept by endothelialization of channel walls and entrapping primary rat hepatocytes within the surrounding matrix. After 1–2 weeks of culture, an endothelial monolayer lining the vessel wall was observed along with the formation of

multicellular sprouts from these patterned vascular structures. This technique has shown potential in designing complex 3D vascularized tissue structures and can be used for a range of tissue engineering applications.

Recently, Bellan et al. also used melt-spun shellac microfibers to design a 3D interconnected network within enzymatically cross-linked gelatin hydrogels [95]. The pH-dependent solubility of shellac microfibers was used to dissolve the fibrous structure after embedding it in enzymatically cross-linked gelatin hydrogels. This sacrificial method results in formation of a 3D interconnect network with enhanced perfusion through the scaffold. The concept reported here is promising; however the effect of pH on cell viability and effect of perfusion on cellular function still need to be investigated.

In a recent study, a microfluidic approach was combined with cell sheet technology to fabricate a perfusion bioreactor to obtain in vitro-vascularized tissue surrogates [96]. This study utilized an approach of stacking a multilayered cultured cardiac cell sheet, along with endothelial cells, on collagen microchannels. Then these collagen microchannels were perfused with culture media containing fibroblast growth factor (bFGF) and VEGF to facilitate cell migration and to promote formation of tubular structures. The proposed approach indicates rapid recruitment of endothelial cells for the formation of pre-integrated and vessel-populated architectures within multilayered tissue-like surrogates. This method has shown promise in fabricating organs with complex vascularized networks and high metabolic capacity.

4.4 Microassembly of Microgels to Fabricate Complex Architecture

Microassembly is another approach to fabricate complex tissue architecture using direct assembly of microscale hydrogels [97, 98]. These microgels can be tailored to mimic the microarchitectures and functions of micron-size subunits obtained in natural tissue. By mimicking microarchitecture of natural tissues, microenvironmental interactions (such as cell–cell, cell–matrix, and cell–soluble factors) can be controlled.

In a recent study, assembly of micropatterned structure in well-defined shapes with multiple functionalities was demonstrated [99]. Du et al. demonstrated feasibility of sequential assembly of cell-laden concentric microgels to form tubular constructs (Fig. 1.5) [100]. Each microgel unit consisted of two concentric hydrogel rings loaded with two different types of cells. The fabrication process involved sequential photolithography using two overlaying masks. First, the endothelial cell-laden inner ring was fabricated and then the smooth muscle cell-laden outer ring was fabricated. These concentric microgels were assembled into a tubular structure and were further stabilized by applying a second UV cross-linking. Although this modular approach shows promising results, stability of the assembled structure under long-term perfusion needs to be evaluated.

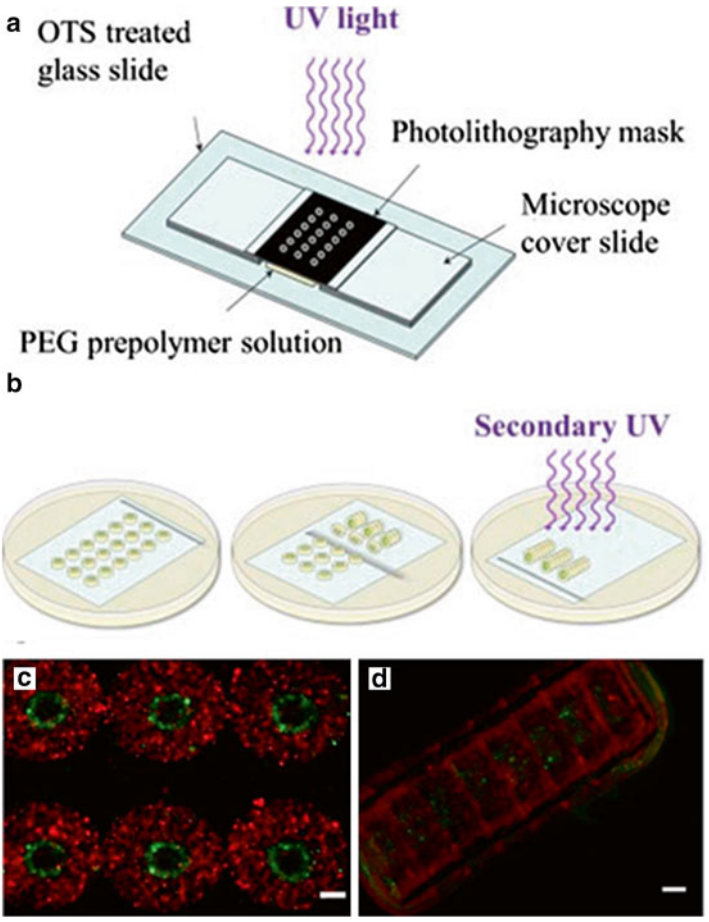

Fig. 1.5 Formation of tubular constructs via sequential assembly of cell-laden concentric microgels. (**a**, **b**) Fabrication and assembly of 3D concentric microgels. (**c**, **d**) Cell-laden microgels and their assembly. The inner rings contain endothelial cell (*green*) and the outer ring contains smooth muscle cells (*red*). Scale bar: 100 μm [100]

Jakab et al. fabricated prescribed constructs and geometries by utilizing the self-organization potential of cells and tissues [101, 102]. They used 3D bioprinter to fabricate multicellular spheroids and then placed them in a biocompatible environment (Fig. 1.6). They showed that this technique was able to recapitulate early morphogenesis events through controlling various developmental and genetic patterns. For example, by assembling a cardiac construct consisting of embryonic cardiac and endothelial cells, synchronously beating tissue consisting of vascularized structure can be obtained. Assembly of endothelial cells into vessel-like conduits resulted in formation of neovascularization. This 3D printing technique can be used to self-assemble complex cellular structure with various shapes.

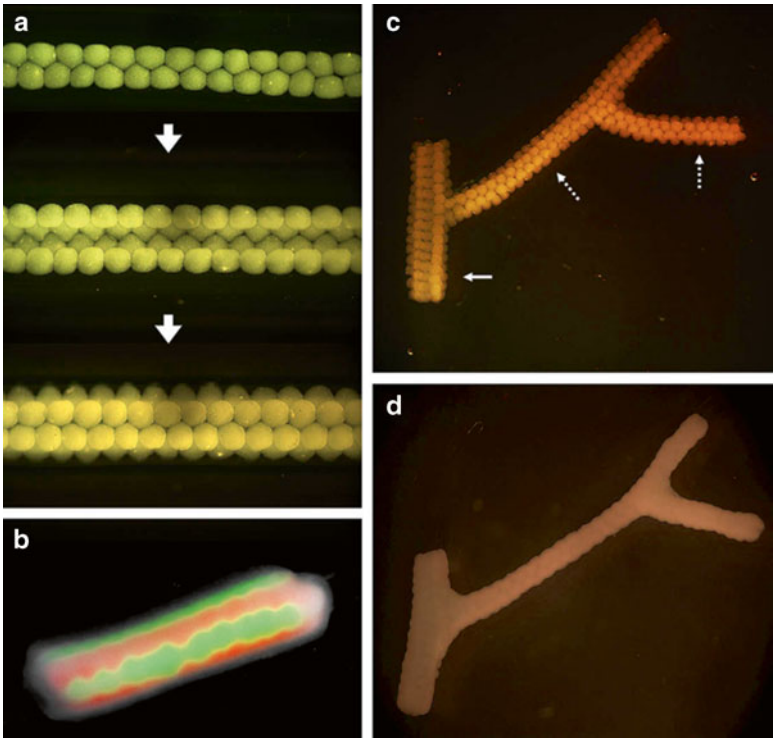

Fig. 1.6 Microgel assembly to form vascular networks. (**a**, **b**) Fabrication and assembly of 3D concentric microgels. (**c**, **d**) Complexity of assembled hydrogels [101]

In another study, McGuigan et al. adopted a biomimetic approach to fabricate microvascularized tissue by assembling micro-sized collagen rods [103]. In this approach, they microfabricated collagen gels containing HepG2 cells using automated cutters (Fig. 1.7). A confluent cell layer was obtained on the surface of the collagen modules before assembling them into a tube. The assembled module was then perfused with media or whole blood that was assisted by the interstitial spaces between collagen modules. Apart from high cell viability in the percolating scaffold, they observed that the endothelial cells prolonged clotting time and retained their non-thrombogenic phenotype.

Most of the microassembly techniques employ assembly of micro building blocks though physical forces and most of these physical forces are weak and unstable. To overcome this approach Hao et al. developed DNA glues to direct self-assembly of microfabricated structures. This DNA glue has complementary strands of single strand of DNA that are wrapped around microgel structure. One of the most intrigue aspects of this technique is the ability to fabricate complex self-assembled structures from the micrometer length scale to the macrometer length scale. The technology can be used to direct hydrogel microstructures to self-assemble in a programmable approach to design complex macroscale tissue architecture.

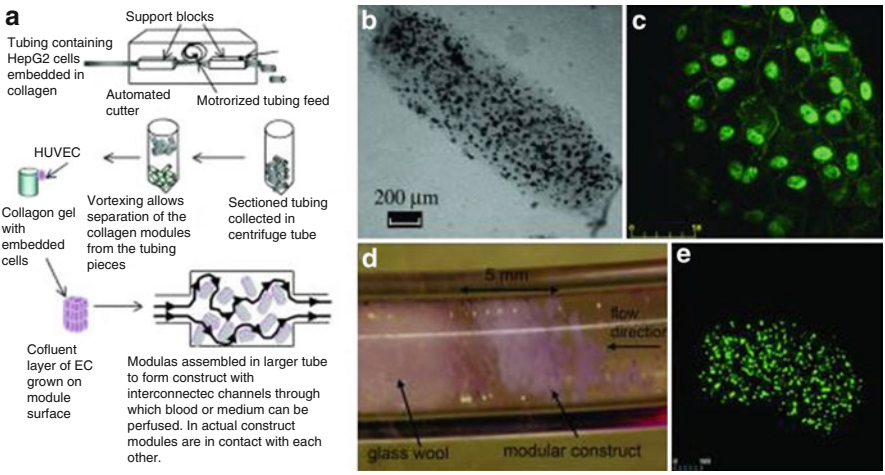

Fig. 1.7 Microassembly of collagen microgels containing HepG2 cells using automated cutters. (**a**) Collagen, HepG2 module tube fabrication process. (**b**) Collagen, HepG2 module before HUVEC seeding. (**c**) Seven days after seeding HUVECs, a confluent layer formed on the collagen module's surface. (**d**) Collagen modules randomly assembled into tube structure within a flow circuit and perfused with media or whole blood. (**e**) Collagen, HepG2, HUVEC module retrieved from tubular construct 7 days after perfusion [103]

5 Future Directions and Conclusion

Tissue engineering techniques have been applied to many tissue types; however, the most pressing challenge is for vascularization. Thus far research approaches have been on the micron level; microfabrication enhances and dictates the macroscale architecture and mechanics. Going forward we must delve an order of magnitude deeper by investigating the influence of nanoarchitectures on microscale materials. Developing nanoscale techniques and materials is a pressing challenge for engineers.

As hierarchical constructs, organs and tissue structures are difficult to replicate on all levels. Although there have been recent advances from the micro to macro scale, nanoscale techniques will become the new paradigm for these constructs [4, 7, 14, 104–106]. There has been an increase in the use of nanocomposites within hydrogels to incorporate this next level [107–110]. Carrow et al. reviewed a range of nanocomposite materials that are currently utilized in the area of tissue engineering and drug delivery [111]. Further, the emergence of 3D printing will elicit the demand for individualized printed organs [112]. Here, a delicate balance between material properties, cell survivability through the printing process, and cell adherence will determine the overall success. Current knowledge on well-developed materials will be instrumental in the adaptation of 3D printing to meet the demands of complex tissue formation.

Until nanoscale methods are commonplace, microfabrication techniques will remain the norm. The challenges with microfabrication techniques such as cell–cell

interactions and oxygen transport and availability must be considered when engineering scaffolds aimed to reproduce the body's architectural and geometrical intricacies. Our inability to generate a functional vasculature that can supply the tissue with nutrients and the inability to mimic complex cell–microenvironment will determine the success of such techniques while driving the development of nanoscale methods. Current approaches to engineer complex tissue structures are based on encapsulating cells within a porous scaffold while providing external molecular clues to facilitate formation of tissue structure [1, 2]. Extracellular matrices have been developed to assist in cellular organization; yet our inability to generate vasculature alongside the ECM has proven detrimental [2]. The full potential of tissue engineering cannot come to fruition without the formation of intrinsic vasculature [1, 3].

As one of the primary challenges in tissue engineering is to fabricate vascularized networks, we have presented two major approaches that have been utilized: "top-down" and "bottom-up." The top-down approach involves the use of a porous scaffold to promote the formation of a vascularized structure within a three-dimensional scaffold [6]. The bottom-up approach controls spatial and temporal distribution of cells, therefore directing cell–cell and cell–matrix interactions [8, 9]. The use of bottom-up methods at the nanoscale will become increasingly important as the demand for organs and tissues is rising faster than donor lists. Lab-generated complex tissues hold promise through presenting the correct cues at all levels to a selective mixture of co-cultures and materials.

References

1. Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926
2. Khademhosseini A, Vacanti JP, Langer R (2009) Progress in tissue engineering. Sci Am 300:64–71
3. Langer R, Tirrell DA (2004) Designing materials for biology and medicine. Nature 428: 487–492
4. Gaharwar AK, Peppas NA, Khademhosseini A (2014) Nanocomposite hydrogels for biomedical applications. Biotechnol Bioeng 111:441–453
5. Singh A, Peppas NA (2014) Hydrogels and scaffolds for immunomodulation. Adv Mater 26:6530–6541
6. Place ES, Evans ND, Stevens MM (2009) Complexity in biomaterials for tissue engineering. Nat Mater 8:457–470
7. Khademhosseini A, Langer R, Borenstein J, Vacanti JP (2006) Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci U S A 103:2480
8. Griffith LG, Swartz MA (2006) Capturing complex 3D tissue physiology in vitro. Nat Rev Mol Cell Biol 7:211–224
9. Kaully T, Kaufman-Francis K, Lesman A, Levenberg S (2009) Vascularization – the conduit to viable engineered tissues. Tissue Eng Part B Rev 15:159–169
10. Lovett M, Lee K, Edwards A, Kaplan DL (2009) Vascularization strategies for tissue engineering. Tissue Eng Part B Rev 15:353–370
11. Phelps EA, Garcia A (2010) Engineering more than a cell: vascularization strategies in tissue engineering. Curr Opin Biotechnol 21:704–709

12. Naito Y, Shinoka T, Duncan D, Hibino N, Solomon D, Cleary M et al (2011) Vascular tissue engineering: towards the next generation vascular grafts. Adv Drug Deliv Rev 63:312–323
13. Santos MI, Reis RL (2010) Vascularization in bone tissue engineering: physiology, current strategies, major hurdles and future challenges. Macromol Biosci 10:12–27
14. Khademhosseini A, Langer R (2007) Microengineered hydrogels for tissue engineering. Biomaterials 28:5087–5092
15. Zorlutuna P, Annabi N, Camci-Unal G, Nikkhah M, Cha JM, Nichol JW et al (2012) Microfabricated biomaterials for engineering 3D tissues. Adv Mater 24:1782–1804
16. Patel RG, Purwada A, Cerchietti L, Inghirami G, Melnick A, Gaharwar AK et al (2014) Microscale bioadhesive hydrogel arrays for cell engineering applications. Cell Mol Bioeng 7(3):394–408
17. Lanza RP, Vacanti J (2007) Principles of tissue engineering. Academic Press, New York
18. Giuliani M, Moritz W, Bodmer E, Dindo D, Kugelmeier P, Lehmann R et al (2005) Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. Cell Transplant 14:67–76
19. Larrea X, Buechler P, Büchler P, Buchler P (2009) A transient diffusion model of the cornea for the assessment of oxygen diffusivity and consumption. Invest Ophthalmol Vis Sci 50:1076–1080
20. Cook CA, Hahn KC, Morrissette-McAlmon JBF, Grayson WL (2015) Oxygen delivery from hyperbarically loaded microtanks extends cell viability in anoxic environments. Biomaterials 52:376–384
21. Risau W, Flamme I (1995) Vasculogenesis. Annu Rev Cell Dev Biol 11:73–91
22. Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–674
23. Folkman J, Shing Y (1992) Angiogenesis. J Biol Chem 267:10931
24. Jain RK (2003) Molecular regulation of vessel maturation. Nat Med 9:685–693
25. Pepper MS (1997) Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity. Cytokine Growth Factor Rev 8:21–43
26. Flamme I, Frölich T, Risau W (1997) Molecular mechanisms of vasculogenesis and embryonic angiogenesis. J Cell Physiol 173:206–210
27. Patan S (2000) Vasculogenesis and angiogenesis as mechanisms of vascular network formation, growth and remodeling. J Neurooncol 50:1–15
28. Singer AJ, Clark R (1999) Cutaneous wound healing. N Engl J Med 341:738–746
29. Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF et al (1992) Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. J Exp Med 176:1375
30. Seghezzi G, Patel S, Ren CJ, Gualandris A, Pintucci G, Robbins ES et al (1998) Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. J Cell Biol 141:1659
31. Sato Y, Endo H, Okuyama H, Takeda T, Iwahashi H, Imagawa A et al (2011) Cellular hypoxia of pancreatic β-cells due to high levels of oxygen consumption for insulin secretion in vitro. J Biol Chem 286:12524–12532
32. Nichol JW, Khademhosseini A (2009) Modular tissue engineering: engineering biological tissues from the bottom up. Soft Matter 5:1312–1319
33. Griffith LG, Naughton G (2002) Tissue engineering – current challenges and expanding opportunities. Science 295:1009
34. West JL, Moon JJ (2008) Vascularization of engineered tissues: approaches to promote angiogenesis in biomaterials. Curr Top Med Chem 8:300–310
35. Khan OF, Sefton MV (2011) Endothelialized biomaterials for tissue engineering applications in vivo. Trends Biotechnol 29(8):379–387
36. Rouwkema J, Rivron N, van Blitterswijk C (2008) Vascularization in tissue engineering. Trends Biotechnol 26:434–441
37. Sukmana I, Vermette P (2010) Polymer fibers as contact guidance to orient microvascularization in a 3D environment. J Biomed Mater Res A 92A:1587–1597

38. Gaharwar AK, Nikkhah M, Sant S, Khademhosseini A (2015) Anisotropic poly (glycerol sebacate)-poly (ϵ-caprolactone) electrospun fibers promote endothelial cell guidance. Biofabrication 7:015001
39. Sant S, Iyer D, Gaharwar AK, Patel A, Khademhosseini A (2013) Effect of biodegradation and de novo matrix synthesis on the mechanical properties of valvular interstitial cell-seeded polyglycerol sebacate-polycaprolactone scaffolds. Acta Biomater 9:5963–5973
40. Patel ZS, Mikos AG (2004) Angiogenesis with biomaterial-based drug-and cell-delivery systems. J Biomater Sci Polym Ed 15:701–726
41. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H et al (1999) VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 18:3964–3972
42. Silva EA, Mooney DJ (2010) Effects of VEGF temporal and spatial presentation on angiogenesis. Biomaterials 31:1235–1241
43. Asahara T, Chen D, Takahashi T, Fujikawa K, Kearney M, Magner M et al (1998) Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. Circ Res 83:233
44. Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Yung S et al (2006) VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 124: 175–189
45. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9:669–676
46. Salcedo R, Wasserman K, Young HA, Grimm MC, Howard O, Anver MR et al (1999) Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: in vivo neovascularization induced by stromal-derived factor-1 {alpha}. Am J Pathol 154:1125
47. Tabata Y, Ikada Y (1999) Vascularization effect of basic fibroblast growth factor released from gelatin hydrogels with different biodegradabilities. Biomaterials 20:2169–2175
48. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M (2005) Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev 16:159–178
49. Bikfalvi A, Klein S, Pintucci G, Rifkin DB (1997) Biological roles of fibroblast growth factor-2. Endocr Rev 18:26
50. Roberts AB (2000) Molecular and cell biology of TGF. Miner Electrolyte Metab 24:111–119
51. Bergsten E, Uutela M, Li X, Pietras K, Östman A, Heldin CH et al (2001) PDGF-D is a specific, protease-activated ligand for the PDGF -receptor. Nat Cell Biol 3:512–516
52. LaRochelle WJ, Jeffers M, McDonald WF, Chillakuru RA, Giese NA, Lokker NA et al (2001) PDGF-D, a new protease-activated growth factor. Nat Cell Biol 3:517–521
53. Stiles CD (1983) The molecular biology of platelet-derived growth factor. Cell 33:653
54. Koblizek TI, Weiss C, Yancopoulos GD, Deutsch U, Risau W (1998) Angiopoietin-1 induces sprouting angiogenesis in vitro. Curr Biol 8:529–532
55. Melero-Martin JM, De Obaldia ME, Kang S-Y, Khan ZA, Yuan L, Oettgen P et al (2008) Engineering robust and functional vascular networks in vivo with human adult and cord blood-derived progenitor cells. Circ Res 103:194–202
56. Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC et al (2005) Engineering vascularized skeletal muscle tissue. Nat Biotech 23:879–884
57. Phelps EA, Landázuri N, Thulé PM, Taylor WR, García AJ (2010) Bioartificial matrices for therapeutic vascularization. Proc Natl Acad Sci 107:3323–3328
58. Vozzi G, Flaim C, Ahluwalia A, Bhatia S (2003) Fabrication of PLGA scaffolds using soft lithography and microsyringe deposition. Biomaterials 24:2533–2540
59. Wang GJ, Hsueh CC, Hsu S, Hung HS (2007) Fabrication of PLGA microvessel scaffolds with circular microchannels using soft lithography. J Micromech Microeng 17:2000
60. Sodha S, Wall K, Redenti S, Klassen H, Young MJ, Tao SL (2011) Microfabrication of a three-dimensional polycaprolactone thin-film scaffold for retinal progenitor cell encapsulation. J Biomater Sci Polym Ed 22(4–6):443–456

61. Armani DK, Liu C (2000) Microfabrication technology for polycaprolactone, a biodegradable polymer. J Micromech Microeng 10:80
62. Bettinger CJ, Orrick B, Misra A, Langer R, Borenstein JT (2006) Microfabrication of poly (glycerol-sebacate) for contact guidance applications. Biomaterials 27:2558–2565
63. Guillemette MD, Park H, Hsiao JC, Jain SR, Larson BL, Langer R et al (2010) Combined technologies for microfabricating elastomeric cardiac tissue engineering scaffolds. Macromol Biosci 10(11):1330–1337
64. Bettinger CJ, Weinberg EJ, Kulig KM, Vacanti JP, Wang Y, Borenstein JT et al (2006) Three dimensional microfluidic tissue engineering scaffolds using a flexible biodegradable polymer. Adv Mater 18:165–169
65. Neeley WL, Redenti S, Klassen H, Tao S, Desai T, Young MJ et al (2008) A microfabricated scaffold for retinal progenitor cell grafting. Biomaterials 29:418–426
66. Zhang H, Patel A, Gaharwar AK, Mihaila SM, Iviglia GI, Mukundan S et al (2013) Hyperbranched polyester hydrogels with controlled drug release and cell adhesion properties. Biomacromolecules 14(5):1299–1310
67. Nikkhah M, Eshak N, Zorlutuna P, Annabi N, Castello M, Kim K et al (2012) Directed endothelial cell morphogenesis in micropatterned gelatin methacrylate hydrogels. Biomaterials 33:9009
68. Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S, Khademhosseini A (2010) Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials 31:5536–5544
69. Oh J, Kim K, Won S, Cha C, Gaharwar A, Selimovic S et al (2013) Microfluidic fabrication of cell adhesive chitosan microtubes. Biomed Microdevices 15(3):465–472
70. Mihaila SM, Gaharwar AK, Reis RL, Marques AP, Gomes ME, Khademhosseini A (2013) Photocrosslinkable kappa-carrageenan hydrogels for tissue engineering applications. Adv Healthc Mater 2(6):895–907
71. Chiu Y-C, Larson JC, Perez-Luna VH, Brey EM (2009) Formation of microchannels in poly(ethylene glycol) hydrogels by selective degradation of patterned microstructures. Chem Mater 21:1677–1682
72. Heckele M, Schomburg W (2004) Review on micro molding of thermoplastic polymers. J Micromech Microeng 14:R1
73. Kim E, Xia Y, Whitesides GM (1996) Micromolding in capillaries: applications in materials science. J Am Chem Soc 118:5722–5731
74. Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP, Langer R, Wang Y (2005) Endothelialized microvasculature based on a biodegradable elastomer. Tissue Eng 11:302–309
75. Zheng Y, Henderson PW, Choi NW, Bonassar LJ, Spector JA, Stroock AD (2011) Microstructured templates for directed growth and vascularization of soft tissue in vivo. Biomaterials 32:5391–5401
76. Diez M, Schulte VA, Stefanoni F, Natale CF, Mollica F, Cesa CM et al (2011) Molding micropatterns of elasticity on PEG based hydrogels to control cell adhesion and migration. Adv Eng Mater 13(10):B395–B404
77. Bianchi F, Rosi M, Vozzi G, Emanueli C, Madeddu P, Ahluwalia A (2007) Microfabrication of fractal polymeric structures for capillary morphogenesis: applications in therapeutic angiogenesis and in the engineering of vascularized tissue. J Biomed Mater Res B Appl Biomater 81B:462–468
78. Vozzi G, Previti A, De Rossi D, Ahluwalia A (2002) Microsyringe-based deposition of two-dimensional and three-dimensional polymer scaffolds with a well-defined geometry for application to tissue engineering. Tissue Eng 8:1089–1098
79. Lewis JA (2006) Direct ink writing of 3D functional materials. Adv Funct Mater 16: 2193–2204
80. Lewis JA, Gratson GM (2004) Direct writing in three dimensions. Mater Today 7:32–39
81. Therriault D, White SR, Lewis JA (2003) Chaotic mixing in three-dimensional microvascular networks fabricated by direct-write assembly. Nat Mater 2:265–271
82. Therriault D, Shepherd RF, White SR, Lewis JA (2005) Fugitive inks for direct write assembly of three dimensional microvascular networks. Adv Mater 17:395–399

83. Wu W, DeConinck A, Lewis JA (2010) Omnidirectional printing of 3D microvascular networks. Adv Mater 23(24):H178–H183
84. Xavier JR, Thakur T, Desai P, Jaiswal MK, Sears N, Cosgriff-Hernandez E et al (2015) Bioactive nanoengineered hydrogels for bone tissue engineering: a growth-factor-free approach. ACS Nano 9:3109–3118
85. Whitesides GM (2006) The origins and the future of microfluidics. Nature 442:368–373
86. Choi NW, Cabodi M, Held B, Gleghorn JP, Bonassar LJ, Stroock AD (2007) Microfluidic scaffolds for tissue engineering. Nat Mater 6:908–915
87. Borenstein JT, Terai H, King KR, Weinberg EJ, Kaazempur-Mofrad MR, Vacanti JP (2002) Microfabrication technology for vascularized tissue engineering. Biomed Microdevices 4:167–175
88. King KR, Wang CCJ, Kaazempur-Mofrad MR, Vacanti JP, Borenstein JT (2004) Biodegradable microfluidics. Adv Mater 16:2007–2012
89. Borenstein JT, Megley K, Wall K, Pritchard EM, Truong D, Kaplan DL et al (2010) Tissue equivalents based on cell-seeded biodegradable microfluidic constructs. Materials 3: 1833–1844
90. Wang Y, Ameer GA, Sheppard BJ, Langer R (2002) A tough biodegradable elastomer. Nat Biotech 20:602–606
91. Wang J, Bettinger CJ, Langer RS, Borenstein JT (2010) Biodegradable microfluidic scaffolds for tissue engineering from amino alcohol-based poly (ester amide) elastomers. Organogenesis 6:212
92. Borenstein J, Tupper M, Mack P, Weinberg E, Khalil A, Hsiao J et al (2010) Functional endothelialized microvascular networks with circular cross-sections in a tissue culture substrate. Biomed Microdevices 12:71–79
93. Golden AP, Tien J (2007) Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element. Lab Chip 7:720–725
94. Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen D-HT, Cohen DM et al (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater 11(9):768–774
95. Bellan LM, Pearsall M, Cropek DM, Langer R (2012) A 3D interconnected microchannel network formed in gelatin by sacrificial shellac microfibers. Adv Mater 24:5187–5191
96. Sakaguchi K, Shimizu T, Horaguchi S, Sekine H, Yamato M, Umezu M et al (2013) In vitro engineering of vascularized tissue surrogates. Sci Rep 3
97. Aubin H, Nichol JW, Hutson CB, Bae H, Sieminski AL, Cropek DM et al (2010) Directed 3D cell alignment and elongation in microengineered hydrogels. Biomaterials 31:6941–6951
98. Fernandez JG, Khademhosseini A (2010) Micro-masonry: construction of 3D structures by microscale self-assembly. Adv Mater 22:2538–2541
99. Du Y, Lo E, Ali S, Khademhosseini A (2008) Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs. Proc Natl Acad Sci 105:9522–9527
100. Du Y, Ghodousi M, Qi H, Haas N, Xiao W, Khademhosseini A. Sequential assembly of cell-laden hydrogel constructs to engineer vascular-like microchannels. Biotechnol Bioeng 108(7):1693–1703
101. Jakab K, Norotte C, Damon B, Marga F, Neagu A, Besch-Williford CL et al (2008) Tissue engineering by self-assembly of cells printed into topologically defined structures. Tissue Eng Part A 14:413–421
102. Jakab K, Norotte C, Marga F, Murphy K, Vunjak-Novakovic G, Forgacs G (2010) Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2:022001
103. McGuigan AP, Sefton MV (2006) Vascularized organoid engineered by modular assembly enables blood perfusion. Proc Natl Acad Sci 103:11461
104. Paul A, Hasan A, Kindi HA, Gaharwar AK, Rao VT, Nikkhah M et al (2014) Injectable graphene oxide/hydrogel-based angiogenic gene delivery system for vasculogenesis and cardiac repair. ACS Nano 8:8050–8062
105. Gaharwar AK, Avery RK, Assmann A, Paul A, McKinley GH, Khademhosseini A et al (2014) Shear-thinning nanocomposite hydrogels for the treatment of hemorrhage. ACS Nano 8:9833–9842

106. Dvir T, Timko BP, Kohane DS, Langer R (2011) Nanotechnological strategies for engineering complex tissues. Nat Nanotechnol 6:13–22
107. Gaharwar AK, Schexnailder PJ, Kline BP, Schmidt G (2011) Assessment of using laponite cross-linked poly(ethylene oxide) for controlled cell adhesion and mineralization. Acta Biomater 7:568–577
108. Gaharwar AK, Schexnailder P, Kaul V, Akkus O, Zakharov D, Seifert S et al (2010) Highly extensible bio-nanocomposite films with direction-dependent properties. Adv Funct Mater 20:429–436
109. Gaharwar AK, Kishore V, Rivera C, Bullock W, Wu C-J, Akkus O et al (2012) Physically crosslinked nanocomposites from silicate-crosslinked PEO: mechanical properties and osteogenic differentiation of human mesenchymal stem cells. Macromol Biosci 12:779–793
110. Gaharwar AK, Rivera CP, Wu C-J, Schmidt G (2011) Transparent, elastomeric and tough hydrogels from poly(ethylene glycol) and silicate nanoparticles. Acta Biomater 7: 4139–4148
111. Carrow JK, Gaharwar AK (2015) Bioinspired polymeric nanocomposites for regenerative medicine. Macromol Chem Phys 216:248–264
112. Schuurman W, Khristov V, Pot MW, van Weeren PR, Dhert WJA, Malda J (2011) Bioprinting of hybrid tissue constructs with tailorable mechanical properties. Biofabrication 3(2):021001