11

MICRO- AND NANOTECHNOLOGY FOR VASCULAR TISSUE ENGINEERING

RYAN S. STOWERS AND LAURA J. SUGGS

Laboratory for Cardiovascular Tissue Engineering, Department of Biomedical Engineering, University of Texas at Austin, Austin, TX, USA

11.1 INTRODUCTION

Vascular tissue engineering aims to regenerate functional blood vessels through the use of biomaterial scaffolds either preseeded with cells or designed to recruit host cells for remodeling. The field can be divided according to two overall objectives: to develop small-diameter vascular grafts with long-term patency and to generate microvascular capillary networks within large tissue-engineered scaffolds.[1,2] Both of these distinct goals benefit from recent applications of micro- and nanotechnologic innovations. Early attempts in vascular tissue engineering, especially in regard to vascular grafts, have not met expectations in part because little was known about how micro- and nanofeatures could guide cellular regeneration. As it became apparent that cells interact with their environment at multiple length scales, engineers looked to fabricate scaffolds with micro- and nanoscale architectures. This chapter discusses recent micro- and nanotechnologic approaches to tissue-engineered vascular graft design and biomaterial-driven microvascular formation.

Micro and Nanotechnologies in Engineering Stem Cells and Tissues, First Edition. Edited by Murugan Ramalingam, Esmaiel Jabbari, Seeram Ramakrishna, and Ali Khademhosseini.

11.2 CONVENTIONAL VASCULAR GRAFTS

Synthetic vascular grafts have been successfully used as replacements for large vessels for several decades.[3] These grafts are typically composed of either expanded polytetrafluroethylene (ePTFE) or Dacron.[4] Despite their success as large vessel replacements, several complications arise when they are used to replace vessels with a diameter less than 6 mm. The primary reasons for failure are anastomotic intimal hyperplasia and thrombogenicity of the graft surface.[5] Intimal hyperplasia may be caused by a disruption in flow at the interface of the graft and native vessel, leading to a buildup of neotissue at the anastomosis site. Over time, this process can occlude the graft. Thrombosis on the graft surface can occur from fibrin deposition that begins immediately after implantation because of the lack of an endothelial lining. Ideally, a vascular graft would be fully covered by a confluent endothelial layer upon implantation, but synthetic grafts have shown less than 10% coverage even after several months.[6] Tissue ingrowth across the anastomotic site extends only 1–2 cm in humans and is insufficient to adequately cover a graft.[7] These two mechanisms account for nearly all the long-term failures in synthetic vascular grafts.[5] Despite much work toward overcoming these challenges, clinical results remain largely unchanged over the past few decades.

11.3 TISSUE-ENGINEERED VASCULAR GRAFTS

At a minimum, a clinically successful tissue-engineered vascular graft must meet two requirements: a confluent endothelium and a surrounding matrix for mechanical support.[1] The endothelium is essential to long-term patency to avoid platelet adhesion, coagulation, and stenosis. In native vessels, endothelial cells (ECs) form a confluent, interconnected monolayer generally aligned with the direction of blood flow. The endothelial layer is supported by the vascular basement membrane, which provides an anchorage site for ECs and can influence cellular functions through signaling pathways.[8] The basement membrane is made of extracellular matrix (ECM) protein nanofibers in a matrix of polysaccharides.[9] Tissue-engineered vascular grafts must also have sufficient mechanical properties to withstand physiological conditions and ideally would have a similar composition to native vessels. The tunica media found in smaller arteries is composed of collagen and elastin fibers with concentric layers of aligned smooth muscle cells (SMCs).[10,11] Aligned SMCs within these layers are at angles with the neighboring layers in a herringbone structure.[12] Researchers have tried to mimic both the structure and composition of native arteries to develop the ideal vascular graft.

Weinberg and Bell presented one of the first attempts at a tissue-engineered vascular graft by mimicking each layer of the native artery. The group formed SMC-laden collagen tubes supported by Dacron and seeded ECs on the luminal surface and fibroblasts on the outer surface to mimic the adventitia.[13] Although the composition was similar to physiological vessels, the resulting construct was mechanically weak. Later, Niklason et al. fabricated a SMC-seeded scaffold from biodegradable

materials and conditioned it for 8 weeks on a pulsatile bioreactor.[14,15] Afterward, ECs were attached to the lumen, and the grafts were implanted into pigs. This approach led to grafts that could withstand physiological pressures and remain patent for up to 1 month. However, when this strategy was used with human cells, the grafts were much weaker and not able to withstand physiological flow. In the past decade, much work has been done to advance the field. Other biodegradable materials, such as poly(lactic acid) (PLA), polycaprolactone (PCL), and polyhydroxybutyrate (PHB) and copolymers of these, have been examined and optimized for vascular graft applications.[16–18] ECM proteins besides collagen have been formed into vascular grafts, and methods to improve the mechanical properties through cross-linking or mechanical stimulation have been developed.[19] Material surfaces have been engineered to promote cell adhesion, display or release soluble factors, enhance diffusion, and promote cell infiltration.[20–22] Micro- and nanotechnologies have become increasingly pivotal because of the ability to interact on a cellular or macromolecular size scale. Strategies to alter cell behavior using micro- and nanotopographic cues and vascular grafts fabricated from micro- or nanofibrous materials have provided hope for a clinically successful graft.

11.4 MICRO- AND NANOTOPOGRAPHY IN VASCULAR TISSUE ENGINEERING

11.4.1 Micro- and Nanotopographies to Mimic Native Architecture

The basement membrane is a complex network of nanofibers; thus, it is not surprising that ECs have been shown to behave differently when cultured on materials with nanotopographies.[23–25] Polymer demixing techniques were used to create 13-, 35-, or 95-nm-tall islands on polystyrene-based materials in a study by Dalby et al.[26] ECs exhibited an elongated, arcuate morphology on nanotopographic surfaces compared with a flat and round shape on smooth polystyrene. The effect on cell shape was most prominent on the 13-nm islands. Chung et al. created nanoscale roughness on polyurethane (PU) films by conjugating arginine-glycine-aspartic acid (RGD)–functionalized poly(ethylene glycol) (PEG) molecules with either uniform chain length or a mixed chain length to the surface.[27] Human umbilical vein endothelial cells (HUVECs) cultured on the nano-rough films adhered and proliferated faster than on smooth surfaces. Bettinger et al. found that endothelial progenitor cell (EPC) morphology was altered by nanogrates of 600 or 1200 nm period (Fig. 11.1).[28] Whereas EPCs were more aligned and elongated, migration was enhanced compared with smooth surfaces. However, this study did not find any significant changes in endothelial gene expression, indicating that more can be done to fully promote functional endothelial layers for clinical applications.

It is likely necessary to incorporate nano- to microscale topographies into the interior surface of tissue-engineered vascular graft design. Nanofiber meshes can support confluent, interconnected EC monolayers formed from either HUVECs or outgrowth ECs from EPCs.[29] Furthermore, these monolayers showed signs of

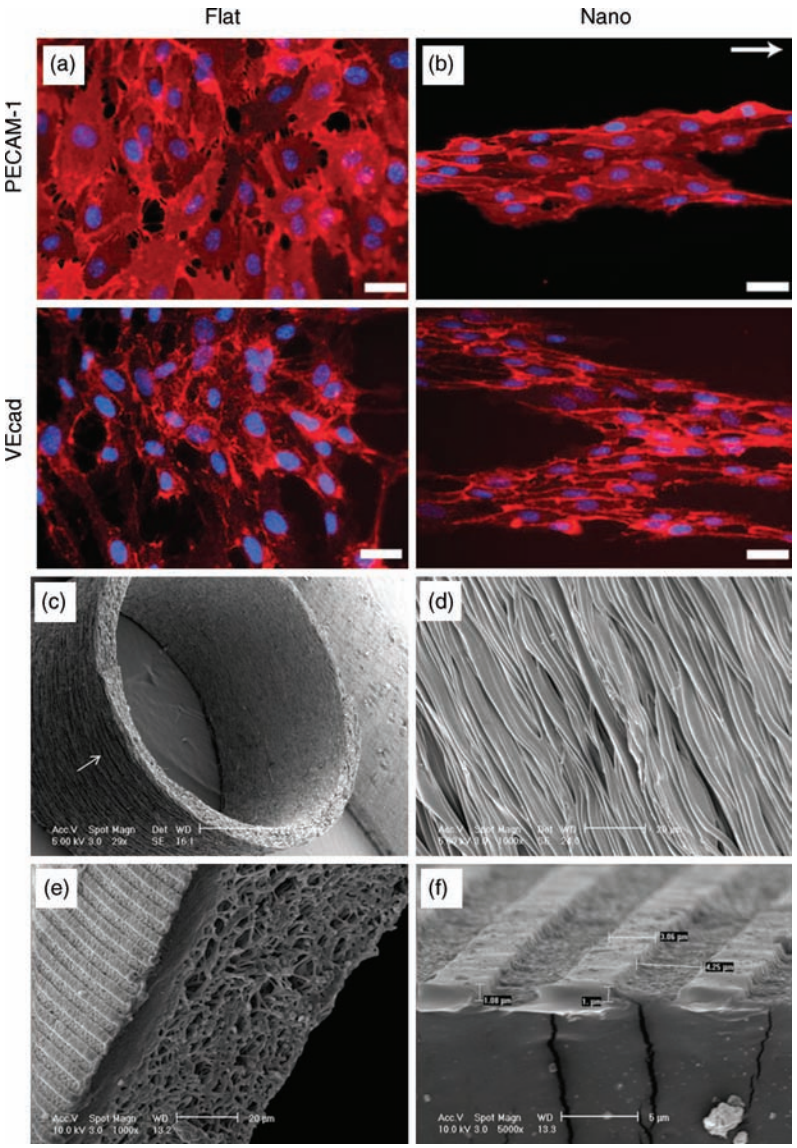

FIGURE 11.1 Endothelial progenitor cells (EPCs) cultured for 6 days on flat substrates (a). The cells form confluent layers with random orientation. EPCs cultured on nanotopography for 6 days align into multicellular structures in the direction of the topography (*arrow*) (b). The cells were stained for PECAM-1 and vascular endothelial cadherin (VEcad); scale bars are 50 μm. Adapted with permission from Ref. [28]. Scanning electron microscope (SEM) images of an electrospun PU graft with circumferentially aligned fibers (c) that are tightly packed (d). SEM images of a hybrid graft with microgrooves on the lumen surface and microfibers on the exterior (e and f). Adapted with permission from Ref. [23].

polarization and enhanced expression of integrin β1, similar to cells on physiological basement membranes. Electrospinning has been extensively used to create nanofibrous matrices for vascular grafts, and examples are discussed later in this chapter. Readers should use caution when comparing studies of EC behavior on various topographies. Liliensiek et al. studied the effects of nanoscale geometries on various types of human ECs: HUVECs, dermal microvascular ECs, aortic ECs, and saphenous vein ECs.[30] Although all cell types did respond to the rough topography, differences were seen in proliferation and migration, primarily between microvascular cells and those derived from larger vessels.

11.4.2 Microengineered Cell Sheets

Generation of a multilayered tunica media to support the vascular construct is necessary, and ideally the layers would be arranged in a herringbone fashion as seen in native vessels. Cell sheet technology is one strategy with the potential for success. L'Heureux et al. presented the first report of a vascular graft made entirely of rolled cell sheets.[31] SMCs and fibroblasts were cultured for 30 days to develop a confluent sheet that could be removed and wrapped around a mandrel. ECs were seeded onto the surface of the construct. Although only 50% patency was demonstrated after 1 week in dogs, the grafts had burst strengths similar to physiological values (∼2200 mm Hg). Further refinement of this technique led to a graft in clinical trials for arteriovenous fistula.[32]

Researchers have attempted to build upon this success by using microfabrication techniques in the production of cell sheets. Conventional cells grown on sheets are randomly oriented on the planar surface. Wong and coworkers sought to adapt their work with aligned SMCs on microgrooved surfaces to transferable cell sheets.[33,34] Williams et al. developed a technique to align SMCs on micropatterned thermoresponsive poly(*N*-isopropylacrylamide (PIPAAm) using microcontact printing.[35] Importantly, transfer of the cell sheets from the PIPAAm films did not alter the cellular morphology. A recent study demonstrated the ability to remove and stack these cell sheets in a herringbone pattern resembling native architecture using a technique called gelatin stamping.[36] It should be noted that the adhesion strength between sheets was not tested and should be optimized to function as a supporting structure for a graft. If delamination can be overcome, one could easily imagine a construct consisting of an endothelial sheet[37] surrounded by multiple, aligned SMC sheets, all rolled around a mandrel to form a cellularized vascular graft.

11.4.3 Conclusion

It is clear that topographical cues influence vascular cell behavior to a great extent. A wealth of knowledge about cellular interactions with material interfaces has been gained and is currently being applied to vascular graft design. There is much hope that nanoscale topography will allow endothelialization of graft lumens and full infiltration and remodeling of the medial layer. One area in need of investigation is

translating the fabrication techniques to vascular graft materials and geometries. These techniques must be adaptable to biodegradable or natural polymers in addition to the ability to be scaled up for manufacturing.

11.5 MICRO- AND NANOFIBROUS SCAFFOLDS IN VASCULAR TISSUE ENGINEERING

11.5.1 Nanofibrous Scaffolds

The ECM is primarily composed of nanofibers on the order of 10–500 nm in diameter and up to several micrometers in length. The nanofibers are made of several types of proteins covered in cell adhesive ligands that can regulate cell behavior. The fibers are interwoven, providing a porous mesh for cells to inhabit. The ECM plays a crucial role in differentiation, morphogenesis, and many other cellular phenomena.[38] Ideally, tissue engineering scaffolds would recapitulate the ECM to promote proper tissue regeneration. Although much research has been done on electrospinning, thermally induced phase separation and molecular self-assembly are two other methods used to produce nanofibers. However, phase separation can only be used for polymers with crystalline structure, significantly limiting its applicability in tissue engineering.[39] Also, self-assembly techniques typically do not produce materials with adequate mechanical properties or allow for control of fiber alignment.[40] Thus, the discussion will focus on recent advances in creating tissue-engineered vascular graft using electrospun fibers.

11.5.2 Electrospun Fibers

Electrospinning is an attractive approach to produce nanofibrous grafts because of its simplicity, low cost, and potential for scale-up. Fibers on the order of 50 nm to several micrometers in diameter can be formed in either random meshes or aligned using a rotating collection mandrel. The properties of the fibers such as tensile strength and diameter can be tailored by adjusting several spinning parameters such s polymer viscosity, collection plate size, geometry, and rotational speed.[41] Electrospinning has been adapted to many types of synthetic polymers, including PCL, PLA, poly(glycolic acid) (PGA), poly(lactide-*co*-glycolide) (PLGA), and polydioxane (PDO).[41–45] Vascular grafts can be created from these nanofibrous meshes (Fig. 11.1c and d) and have, in some instances, improved preclinical outcomes compared with conventional synthetic graft materials. For example, slowly degrading electrospun PCL fibers were optimized on the basis of tensile strength, fiber size, and graft morphology.[46] These grafts were tested in rats against conventional grafts made from ePTFE for 24 weeks.[47] No stenosis was seen in the electrospun PCL grafts; however, two conventional grafts were stenotic as early as 18 weeks. Both graft types demonstrated comparable neointimal formation; however, electrospun PCL grafts had significantly better endothelial coverage, immune response, and neovascularization.

Natural polymers have also been electrospun into nanofibers, including collagens types I–IV, gelatin, elastin, fibrinogen, hemoglobin, and myoglobin.[48,41,49–52] Most electrospinning approaches for natural polymers are animal derived and thus carry the limitation of immune response in the clinical arena. Human proteins have been isolated and electrospun, but the limited supply is an issue for scale up. McKenna et al. sought to develop an electrospun graft from recombinant human tropoelastin to mitigate the possibility of adverse immune reactions and supply issues.[50] ECs were able to adhere and remain viable on the scaffolds. The mechanical properties were not different from extracted elastin, providing an attractive alternative for clinical applications. However, the authors state that the mechanical properties of the elastin scaffold alone are not sufficient for implantation in a graft model.

11.5.3 Synthetic and Natural Hybrid Nanofibers

Recently, researchers have begun to create hybrid nanofibrous grafts made from a combination of synthetic and natural materials. Synthetic nanofibers generally have superior mechanical properties and are more tailorable than natural polymers. However, synthetic polymers lack key biorecognition moieties that may be imperative to promote formation of a confluent endothelium and achieve a nonthrombogenic surface. The hybrid approach is taken to yield a material with optimal characteristics. Kwon and Matsuda developed a method to co-spin poly(L-lactide-*co*-caprolactone) (PLCL) with type I collagen by mixing the two components together before spraying.[53] They assessed the properties of the grafts with a range of collagen mass fractions (0–100%). It was demonstrated that as the fraction of collagen within the mix increased, the mechanical properties of the scaffolds decreased. This result is expected given the generally superior mechanical properties of synthetic polymers. Additionally, fiber diameter was inversely proportional to collagen content, decreasing from 520 nm in 100% PLCL scaffolds to 120 nm in 100% collagen scaffolds. Cellular studies demonstrated that HUVECs could attach, become well spread, and elongate in the direction of fibers in scaffolds with 5% or 10% collagen. In a similar approach, He et al. fabricated PLA-*co*-PCL:collagen blended hybrid nanofibers with diameters in the range of 100–200 nm.[54] They found that collagen blending enhances the viability, attachment, and spreading of human coronary artery ECs. Gene expression profiles of the EC markers endothelial leukocyte adhesion molecule 1, platelet EC adhesion marker-1 (CD31), intercellular adhesion marker 1, and vascular cell adhesion marker 1 in addition to von Willebrand factor (vWF) were not significantly different from controls, indicating maintenance of the EC phenotype. Using similar grafts in a later study, the group sought to evaluate the *in vivo* performance of an acellular graft in a rabbit superficial epigastric vein model.[55] After 7 weeks, the grafts showed no macroscopic deformation. The inflammatory response was mild, with a fibrous encapsulation around the graft but no host cell infiltration. No coagulation on the lumen surface was seen; however, there were no ECs present, either. The authors stated that coagulation could have occurred at a later time point because no endothelium had developed but stressed that

implantation of cell-seeded grafts would provide a better opportunity for formation of a confluent endothelial layer. Lee et al. have developed a PCL–collagen nanofibrous scaffold with superior burst strength than native vessels or PCL fibers alone.[56] The combination of the two materials gives a higher yield strength for the composite than for a pure PCL material, which translates to a higher burst strength. When seeded with ECs and SMCs, the ECs localized to the inner luminal surface, forming a confluent monolayer, but the SMCs infiltrated the periphery of the graft. This result is encouraging because previously it had been a challenge to develop sufficiently strong nanofiber grafts with high porosity for cell infiltration.

Elastin is a major contributor to the mechanical properties of native vessels.[57] Several groups have attempted to fabricate elastin nanofiber graft or incorporate elastin within a hybrid blend.[58–60] Although pure elastin grafts lack sufficient mechanical strength to be used clinically, recent developments with blended materials hold promise. A bilayer scaffold was created by sequentially spinning elastin and PCL around a rotating mandrel.[61] This approach yielded reduced platelet adhesion and decreased thrombogenicity, which was measured by plasma clotting time. After implantation as a rabbit carotid interposition graft for 1 month, the bilayer grafts showed no reduction in size, burst strength, or compliance. Han et al. created co-spun fibers from three components, PLGA, gelatin, and elastin.[62] By altering the ratios of each component, the researchers were able to tailor key features such as fiber size, swelling characteristics, and mechanical properties. A 3:2:1 volume ratio of PLGA:gelatin:elastin yielded the smallest diameter fibers (317 nm) with the highest mechanical strength. All the variants tested were able to support cellular attachment and spreading. ECs were able to form a functional nonthrombogenic monolayer as assessed by gene expression and clotting assays.

11.5.4 Release from Nanofibers

Electrospun nanofibers are able to mimic native ECM fibers in terms of size and composition; however to fully recapitulate the cellular microenvironment, soluble factors should also be presented. Chew et al. first demonstrated the feasibility of encapsulating growth factors into electrospun fibers.[63] Human β-nerve growth factor (NGF) in a carrier protein, bovine serum albumin (BSA), was electrospun in a copolymer of PCL and poly(ethyl ethylene phosphate) (PCLEEP). Bioactive NGF was released over a period of 3 months via diffusion from the fibers. In a subsequent application to vascular grafts, heparin was incorporated in PCL nanofibers and released over a period of 14 days in a bioactive form. One limitation of incorporating the soluble factor directly into the fiber is the initial burst release. Wei et al. incorporated platelet-derived growth factor BB (PDGF-BB)–loaded microspheres into a PLLA electrospun scaffold to achieve sustained growth factor release up to 60 days.[64] The release kinetics were dependent on microsphere degradation and therefore inherently independent of the properties of the scaffold.

A more recent approach to loading nanofibers with soluble factors uses coaxial spinning technology, wherein a core material is spun inside a shell of a separate

material using two outlets. Zhang et al. demonstrated the feasibility of this approach to encapsulate proteins for release from the fibers.[65] Fluorescein isothiocyanate–BSA (FITC–BSA) was incorporated in water-soluble PEG in the core, and PCL was used as the shell material. The release properties were dependent on fiber size, with smaller fibers releasing faster because of increased ratios of surface area to volume. The study demonstrated continuous release for up to 5 months. Liao et al. extended this approach to release PDGF-BB from a BSA-core/PCL-shell nanofiber.[66] Lu et al. designed a PCL-core/cationized gelatin-shell fiber. FITC-heparin was adsorbed onto the gelatin layer, providing a platform to deliver heparin-binding growth factors within vascular grafts.[67] The group demonstrated this capability by releasing vascular endothelial growth factor (VEGF) over 15 days in a controlled manner. Future work in this area should include the release of multiple growth factors[68] with independently controlled rates and *in vivo* studies to determine the functional impact of growth factor incorporation.

11.5.5 Antithrombogenic Nanofibers

A clinically successful graft requires antithrombogenic properties to be presented until a confluent endothelium can be established on the graft lumen. Researchers have recently recognized the opportunity to present antithrombogenic signals on nanofibers to prevent platelet adhesion and coagulation within small diameter vascular grafts. Poly(ether urethane urea) (PEUU) has been electrospun with a bio-inspired phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylcholine-*co*-methacryloyloxyethyl butylurethane (PMBU), to reduce its thrombogenicity.[69] These fibers were formed in 1.3 mm grafts and implanted in a rat aortic interposition model and found to increase patency and reduce thrombogenicity compared with PEUU controls. EC attachment was still allowed and a confluent monolayer formed within the 8-week time course of the study. Soletti et al. immobilized a similar phospholipid polymer (PMA, 70% 2-methacryloyloxyethyl phosphorylcholine: 30% methacrylic acid) to PEUU after electrospinning as an antithrombogenic surface functionalization.[70] The grafts were again studied as aortic replacements in rats and reduced platelet adhesion by 10-fold compared with untreated PEUU controls. Significantly more PMA-PEUU scaffolds were patent after 8 weeks (92%) compared with PEUU alone (40%).[71] Histologic evaluation of the scaffolds revealed neotissue formation with aligned collagen and elastin, confluent ECs aligned with blood flow, and SMCs in the interior of the scaffold. Recently, Liu et al. sulfated silk fibroin to mimic the highly sulfated heparin molecules found on native endothelium.[72] This biomimetic strategy reduced platelet adhesion and thrombogenicity compared with nonsulfated silk fibroin scaffolds. ECs and SMCs were cultured in the scaffolds and found to organize into confluent luminal monolayers and multilayered structures, respectively. It is critical to the success of an implanted graft to maintain an antithrombotic lumen until ECs adhere and form a confluent monolayer. These strategies are promising to reduce thrombus formation; however, it is also important to quickly recruit ECs.

11.5.6 Cell-Adhesive Nanofibers

Nanofibers can be modified by adding cell adhesive ligands to the surface to promote attachment and spreading. Kim and Park fabricated nanofibers from PLGA and PLGA-*b*-PEG-NH_2 to form amino groups on the fiber surface, to which GRGDY peptide sequences were conjugated.[73] NIH 3T3 fibroblasts were able to adhere, spread, and proliferate better on the RGD-modified fibers. A similar approach was taken by Grafahrend et al. using PEG-*b*-PDLLA electrospun fibers.[74] When RGD sequences were covalently linked, 100% of seeded cells survived after 24 h, but no living cells were found in the unmodified controls. RGD immobilized to nanofiber surfaces has also been shown in PU,[75] PCL, and P(LLA-CL)[76] with similar increases in cell adhesion, spreading, and proliferation. Other biomolecules have been conjugated to electrospun scaffolds, including type I and IV collagen, marine collagen, and chitosan of various molecular weights.[77]

11.5.7 Future Work and Conclusion

Results with electrospun nanofibrous vascular grafts offer much promise of a clinically relevant solution to small-diameter graft problems. The ability to mimic the native ECM topography, tailor the mechanical properties, release signaling molecules, and reduce thrombogenicity through antithrombogenic and EC adhesive surfaces gives them potential for future research. The field is not without limitations, however. Current methods of producing electrospun fibers have a lower size limit of around 50 nm,[9] and scaffolds are made from fibers of a few hundred nanometers in diameter.[41] This is at the upper limit of native ECM fibers, and it may be necessary to more accurately mimic the native components with smaller fibers. The fabrication process is lauded for its simplicity and potential for scale up, but it requires conditions that are not favorable for many biological entities. Harsh organics, high voltages, and processing steps can denature natural proteins. To this end, researchers have developed a method to electrospin collagen with ethanol and PBS to avoid denaturation and unwanted changes in structure.[78] Finally, the low porosity of most electrospun scaffolds limits the cellular infiltration needed for development of a truly regenerated medial layer. Numerous studies have investigated methods to increase the scaffold porosity, but most techniques significantly reduce the mechanical properties of the scaffold. Recently, groups have shown that cells can be electrosprayed in the core of a coaxial electrospinning cone to form fibers with encapsulated cells.[79–81] This approach, which was shown to maintain cellular viability, negates the need for cellular infiltration because the cells can be uniformly distributed throughout the construct. However, cell-mediated remodeling of the graft structure is also dependent on porosity. Therefore, simply seeding cells throughout a construct may not be sufficient to establish a functional medial layer. Others have developed bi- and trilayered scaffold to optimize the properties of each layer toward specific goals (Fig. 11.1e and f). Ju et al. developed a scaffold with an inner layer made from 270-nm PCL fibers surrounded by a highly porous outer layer of micron-

sized fibers.[82] ECs attached to the inner nanoscale fibers in a confluent monolayer, which was confirmed by CD31 staining. SMCs were shown to infiltrate the more porous outer layer, with α-smooth muscle actin (SMA) present throughout.

Electrospinning nanofibers is a simple and controllable method to produce ECM-mimicking fibers to fabricate tissue-engineered vascular grafts. Engineers must continue to adapt electrospinning techniques to overcome the current limitations. Additionally, progress had been made by combining electrospun fibers with other micro- and nanofabrication techniques. Researchers should continue to look for synergistic combinations to produce clinically successful tissue-engineered vascular grafts.

11.6 MICROVASCULAR TISSUE ENGINEERING

11.6.1 Need for Microvascular Networks in Tissue Engineering

Apart from designing macroscale vascular constructs, vascular tissue engineering is focused on *de novo* development of microvascular networks. Thin or avascular tissue engineering products have been successful in the clinic,[83–85] but bulk constructs have proven challenging because cells seeded within the scaffolds must rely on diffusion to provide oxygen and nutrients necessary for viability, proliferation, and remodeling. Thus, the thickness of the construct becomes the limiting factor. Studies have shown that cells cannot survive more than a few hundred micrometers from a capillary source. [2,86] Creating or developing robust vascular networks within tissue engineering scaffolds is critical to the continued success of the field. Toward this goal, microfluidics and microfabrication techniques have been used to gain precise control of geometry, architecture, and flow within a construct.

11.6.2 Microfluidics

As awareness of the need to vascularize tissue-engineered constructs became apparent, researchers looked to the field of microfluidics as an attractive system for controlling size, branching, and flow in a precise manner. The initial contributions in this field were made by Bornstein and Vacanti. Bifurcated patterns were fabricated using photolithography in silicon and Pyrex, and ECs and hepatocytes were cultured in the device (Fig. 11.2a and b).[87,88] The cells were lifted from the surface as a monolayer and maintained their proliferative capacity and functionality. The lifted ECs also aligned to form branched networks, reminiscent of native capillary structures. Further studies were performed using soft lithography to mold polydimethylsiloxane (PDMS) on silicon wafers.[89]

Although this pioneering work demonstrated the ability to engineer microfluidic systems and successfully culture cells within them, the materials used did not lend themselves to tissue engineering applications because of their limited biocompatibility and nonbiodegradable nature.[90] King et al. used the biodegradable polymer PLGA to form microfluidic systems.[91] However, PLGA is a brittle material

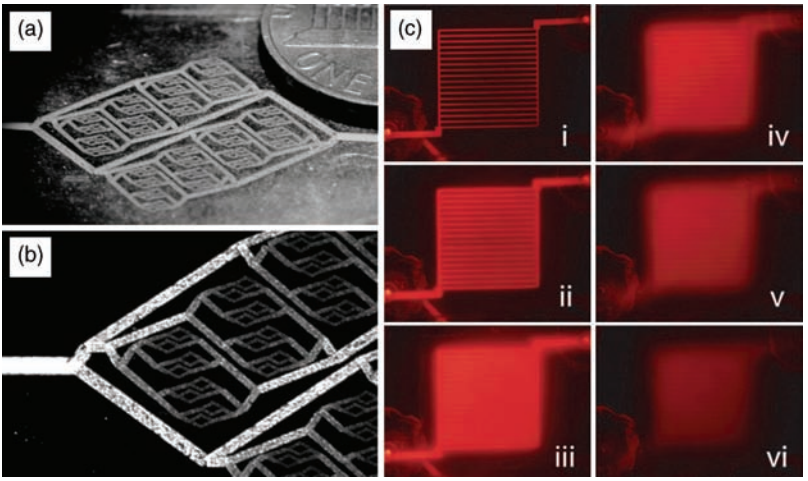

FIGURE 11.2 Multilevel microchannel network (a and b). Branch dimensions range from 130 to 660 μm. Reproduced with permission from Ref. [88]. Demonstration of spatiotemporal control of macromolecules in hydrogel networks (c). Microfluidic network formed in an alginate hydrogel showing assisted delivery (i–iii) and assisted extraction (iv–vi) of fluorescent conjugated dextran (70 kDa). Adapted with permission from Ref. [94].

that is not ideal for microvascular networks. Wang and coworkers were able to engineer microfluidic systems with elastomeric poly(glycerol sebacate) (PGS) as a more flexible alternative.[92]

Three-dimensional scaffolds are necessary to recapitulate the native environment and fully perfuse a scaffold. It still remains a challenge to develop 3D microfluidic systems, and most success has been had in stacking 2D films to create a 3D composite. Successful stacking was first demonstrated by King et al. in PLGA[91] and Bettinger et al. in PGS,[93] both using thermally bonding layers of 2D films. The PGS construct was able to exhibit maximum shear stress throughout each channel, making it attractive for microvascular systems. Unfortunately, stacking of films is not ideal because it is difficult to scale.

11.6.3 Microfluidic Hydrogels

Microfabrication techniques were recently applied to hydrogel materials to yield constructs amenable to cell culture and vascularization with precise control of architecture in three dimensions. Cabodi et al. first demonstrated the creation of a microfluidic hydrogel using soft lithographic techniques with calcium alginate, a commonly used biomaterial whose ionic cross-links can be reversed with a calcium chelator.[94] In this work, the group created slabs of alginate and bonded them together by first treating the surfaces with sodium citrate, a chelator, and then adding calcium chloride to seal the layers. This layer-by-layer approach yielded a microfluidic gel with minimum channel dimensions of 25 μm. The group also demonstrated that the gel was permeable to both small molecule and macromolecular solutes, as needed in

tissue engineering applications (Fig. 11.2c). The Stroock group later demonstrated the ability to fabricate these gels in the presence of cells and maintain their viability.[95] Capitalizing on the microfluidic channels and permeability of the gels, the group demonstrated supreme spatial and temporal control of both large and small solutes. Additionally, multiple independent networks could be used as sources or sinks to establish and sustain concentration gradients within the constructs. It has been shown that many angiogenic processes are enhanced or even dependent on the presence of concentration gradients. These seminal studies established a basis for growth, and recently microfluidic and microfabrication approaches have been extended into a variety of hydrogel and 3D tissue engineering constructs.[96–101]

11.6.4 Micropatterning

Patterning microchannels into scaffolds has been proposed as an attractive mechanism to drive vascular formation and infiltration deep within a construct. Bryant et al. created porous poly(hydroxyethylmethacrylate) (polyHEMA) scaffolds with patterned channels between 200 and 500 μm using photo-patterning techniques.[96] In this work, a photomask was used to block UV irradiation of a polymer precursor solution, which could be washed away from masked regions after irradiation. However, construct thickness is limited by the relatively shallow penetration of conventional UV patterning techniques. Other limitations of this approach include detrimental effects from exposure of cells to UV light and solvents. Another study from the Ratner laboratory showed the fabrication of a porous poly(HEMA)-*co*-(methacrylic acid) scaffold with channels by using sacrificial polycarbonate fibers embedded within the construct upon initial polymerization.[97] The fibers were dissolved afterward, leaving parallel channels of the diameter of the fiber, which allows for precise control of the diameter and spacing of the channels, with uniform properties throughout the depth of the scaffold. Scaffolds with 60 μm channels were implanted into rat myocardium for 4 weeks and found to enhance the neovascular response. By perfusing the rats before sacrifice, the group demonstrated functional vessels throughout the scaffold that successfully inosculated with the host. Additionally, SMCs were found surrounding ECs, suggesting mature vasculature had formed.

The Stroock group sought to transition to materials that could be remodeled by cells because they are more usable in tissue engineering applications. Collagen gels were selected because they would allow adhesion, proliferation, and modification by ECs. The group chose to use dense collagen gels to achieve superior mechanical properties without altering the structure or function of collagen. Micromolding was used to create patterned channels within the gels and could be successfully performed on gel concentrations as low as 0.3%.[98] Again, diffusion of large macromolecules (dextran 70 kDa) was shown. HUVECs that were seeded onto the channels showed attachment and the ability to remodel the matrix through either displacement or degradation. Tube formation was evident within 3 days, and networks grew over time. Finally, the study demonstrated that HUVECs could

invade the gels, with invasion distance and speed inversely proportional to collagen concentration. Zheng et al. recently created microstructured pores, slots, or networks within collagen and calcium alginate gels and investigated the vascularization response in a subcutaneous model in rats.[99] Circular channels of 100–400 μm diameter and 400 μm depth, microslots 100 μm wide and 400 μm deep, or a double-layered combination of the two could be fabricated in bulk gel constructs that were 1 mm thick and 8 mm in diameter. Vascularization was seen all the way into the depths of the channels or slots, with lateral invasion into the gel constructs by day 14. Even double-layered structures were vascularized throughout the gels; however, no vascularization was seen in nonstructured control gels at the same time point. CD31, SMA, and presence of blood cells indicated that the vessels were mature, stable, and inosculated with the host. The invading cell density was found to decrease with increasing structure size. This work demonstrated successful guidance of cell ingrowth and vascular formation with spatial control to 100 μm *in vivo*.

In another recent contribution, PDMS molds were used to form collagen gels within microchannels.[100] Microvascular cells could be seeded within the gels by centrifugation and were shown to form tubes between 24 and 48 h in culture with media containing basic fibroblast growth factor (bFGF) and VEGF. The group was able to control tubulogenesis dynamics and tube diameters by altering the channel geometries and collagen concentrations. Wider, elliptical shaped channels formed larger tubes, as did channels filled with higher concentrations of collagen. Tubes formed more rapidly in channels filled with lower concentrations of collagen. Branched structures were created, and the researchers demonstrated that tubes could be formed to the shape of the mold, with patent lumens throughout the branches, illustrated by staining. Finally, the study showed that the tubes could be encapsulated into a biological matrix by applying ungelled collagen over the channels and cross-linking it, resulting in a bulk structure with spatially controlled EC tubes throughout. The implications in this approach for design of bulk tissue engineering constructs are apparent. However, because of the planar nature of the design, a layer-by-layer approach would be necessary for 3D constructs, and scale up would be difficult. Gillette et al. demonstrated the formation of branched networks within bulk phase natural polymer hydrogels.[101] These channels were filled with another patterned phase hydrogel that is anchored to the bulk hydrogel through *in situ* collagen fiber assembly (Fig. 11.3). Using this technique, a variety of material combinations of collagen, fibrin, alginate, or matrigel can be used to fabricate cell-seeded branched networks within large tissue engineering matrices.

11.6.5 Hybrid or Advanced Approaches

Sundararaghavan et al. sought to combine the ECM-mimicking properties of electrospun fibers with the geometrical control afforded by photopatterning.[102] They spun methacrylated hyaluronic acid (HA) with poly(ethylene oxide) (PEO) into random or aligned nanofibrous mats. The mats were polymerized with UV irradiation and could be patterned with a photomask to either 165 or 333 μm diameter channels. Subcutaneous implants of the scaffolds revealed large vascular

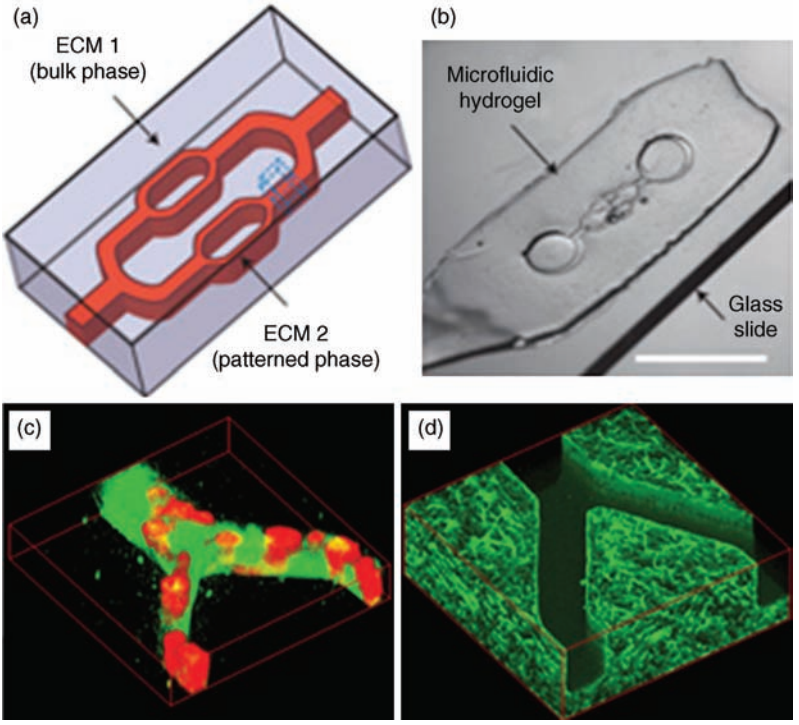

FIGURE 11.3 Schematic of microfluidic hydrogel with two phases of ECM proteins (bulk and patterned) (a). Image of corresponding microfluidic hydrogel; scale bar is 5 mm (b). Confocal reflectance microscopy of patterned collagen (*green*) seeded with HUVECs (*red*) demonstrates complete filling of the channel and formation of sharp boundaries (c). Box dimensions are 230 μm × 230 μm × 30 μm. Collagen–alginate bulk phase before patterned collagen is added, demonstrating precise geometry and uniform distribution of bulk phase fibers (d). Box dimensions are 230 μm × 230 μm × 75 μm. Adapted with permission from Ref. [101].

structures within the channels after 1 week but found no vascularization in non-patterned scaffolds.

Others have recently found unconventional methods to generate patterned structures for microvascular formation. Sadr et al. attached HUVECs to gold microrods using an oligopeptide self-assembled monolayer (SAM) that was electrochemically cleavable.[103] Methacrylated gelatin (GelMA) was poured over the rods and cross-linked with UV light. By applying an electric potential across the rods, the SAMs were cleaved resulting in efficient transfer of HUVEC monolayers to the walls of channels formed around the rods in the GelMA constructs. The group also demonstrated the ability to form a layer of NIH 3T3 fibroblast support cells around the HUVEC monolayer by dip coating the HUVEC covered rods in a solution of GelMA:3T3s. Other work in the Khademhosseini laboratory has focused on the sequential assembly of microgels to form vascular-like constructs.[104] Doughnut-shaped PEG microgels loaded or coated with HUVECs could be assembled into

tubes by sequentially detaching them from a surface in a hydrophobic medium. Because of the nature of sequential assembly, the inner diameters of the channels can be modified, and bifurcations can even be introduced, with resolution proportional to the thickness of the microgels. Concentric microgels were also formed with SMCs on the external surface and HUVECs on the inner surface.

Huang et al. created tree-like structures in poly(methyl methacrylate) (PMMA) blocks using electron beam lithography generate a charge within the material and then grounding it to induce a rapid discharge.[105] The intense discharge leads to vaporization and fracture within the block, resembling branched microvascular networks with a thick trunk around 1 mm that tapers to many endpoints on the order of 10 μm in diameter. By forming multiple nucleation sites before discharge, interconnected networks could be created with "ports" at several locations. The group also demonstrated the feasibility of this technology in biodegradable PLA blocks; however, a narrower channel range was found (20–300 μm). Cellular studies still need to be performed to assess the practicability of this technique for vascularization.

11.6.6 Nanofiber Gels

Recapitulation of the ECM is also desirable to induce microvascular formation. As previously discussed, electrospinning is a simple and convenient technique to form nanofibers. However, electrospinning is only capable of producing fibers down to approximately 50 nm. ECM fibers can be as small as 10 nm, prompting researchers to develop other techniques to reach the lower end of the spectrum. The self-assembly approach is attractive for its ability to produce fibers on the order of 10 nm and incorporate biomimetic moieties.

The Stupp laboratory has pioneered the field of self-assembling peptide amphiphiles (PAs). The group has designed the PAs around four basic functional units: (1) a hydrophobic moiety, (2) a β-sheet forming peptide sequence, (3) one to three charged amino acids, and (4) a bioactive signaling epitope that is displayed on the surface of the fiber.[106] Each of these domains can be tailored to fit a particular application. For example, the gel's mechanical properties, gelation kinetics, and nanostructure are largely dependent on the particular β-sheet unit used. The bioactive signaling unit is not necessary for fiber formation but is used to direct cells. Using this PA approach, fibers can be formed between 6 and 12 nm wide and up to several micrometers in length. The resulting nanofiber gels have a storage modulus around 10 kPa.

Narmoneva et al. developed hydrogels from ionic self-complementary peptides that underwent self-assembly.[107] Cardiomyocytes were cultured in these gels alone, with ECs, or with ECs that were cultured for 24 h before cardiomyocyte seeding to develop "prevascularized" networks. Both EC co-culture groups also promoted expression of the gap junction protein connexin 43. Preformed EC networks demonstrated a functional contribution by increasing spontaneous contractility of the cardiomyocytes by three orders of magnitude. A separate study investigated the effects of these gels for *in vivo* vascularization in a myocardial injection model in mice.[108] The nanofiber gels were found to recruit EC progenitors and vascular SMCs

that formed vascular structures. Injected neonatal cardiomyocytes were able to survive and proliferate while recruiting endogenous cells.

Physical matrix induction cues can be enhanced with the addition of soluble factors. The Stupp group sought to bind and release proangiogenic molecules within the nanofiber gels to provide physical and chemical signals for the encapsulated cells. Rajangam et al. found that the Cardin–Weintraub heparin-binding domain could be incorporated into the PAs.[109] These PAs self-assembled into nanofiber gels in the presence of heparin and demonstrated prolonged release of bound protein for more than 10 days. The gels promoted significant neovascularization in a rat corneal angiogenesis assay when loaded with small amounts of VEGF or FGF-2. A dorsal skin fold chamber and a subcutaneous implant model were used to further analyze the performance of the gels *in vivo*.[110] In both models, neovascular formation was promoted, and the inflammatory response was minimal. Furthermore, the gels were shown to persist for at least 30 days in the subcutaneous environment.

Self-assembly nanofibers provide a technique to form small ECM-mimicking fibers with tailorable mechanical properties to promote vascularization. However, a challenge for the field has been the difficulty to form complex structures with this approach. Very recently, PA chemistry was also translated to form a peptide-based membrane construct to bind and release growth factors and promote cell adhesion.[111] HA and a cationic PA were combined to form a self-assembled PA-HA hybrid membrane. By incorporating the heparin-binding domain again, the group was able to bind heparin-binding growth factors within the membrane and release them over time. Additionally, mesenchymal stem cells (MSCs) could adhere and proliferate on the membranes. In a chick allantoic membrane model, the membranes induced rapid and robust angiogenesis when loaded with small amount of growth factors compared with unloaded membranes or soluble growth factors alone. Formation of complex structures is an important step for self-assembly nanofibers, and future work to create other structures using self-assembly holds much potential.

Natural materials can also be used to form nanofibrous gels with the ability to direct cell behavior. These gels can be modified to enhance their mechanical properties, load and release growth factors, or guide differentiation. Our laboratory has developed a chemically modified (PEGylated) fibrin gel (Fig. 11.4a) with tailorable properties such as fiber diameter and storage modulus based on the type of PEG used.[112,113] Notably, the initial fiber diameter and storage modulus of unmodified fibrin can be either increased or decreased (Fig. 11.4b–d). We have demonstrated differentiation of MSCs to an endothelial phenotype and the formation of tubes *in vitro* (Fig. 11.4e–g). The cellular response is dependent on the specific PEG used and is therefore tailorable. We have also demonstrated loading and release of multiple growth factors within the gel.[68] The growth factors can be loaded via physical affinity for the fibrin matrix or by covalent conjugation to the PEG chains.

11.6.7 Conclusion

Micro- and nanotechnology have provided techniques to make great strides in the formation of microvascular networks for tissue engineering scaffolds. Microfluidics

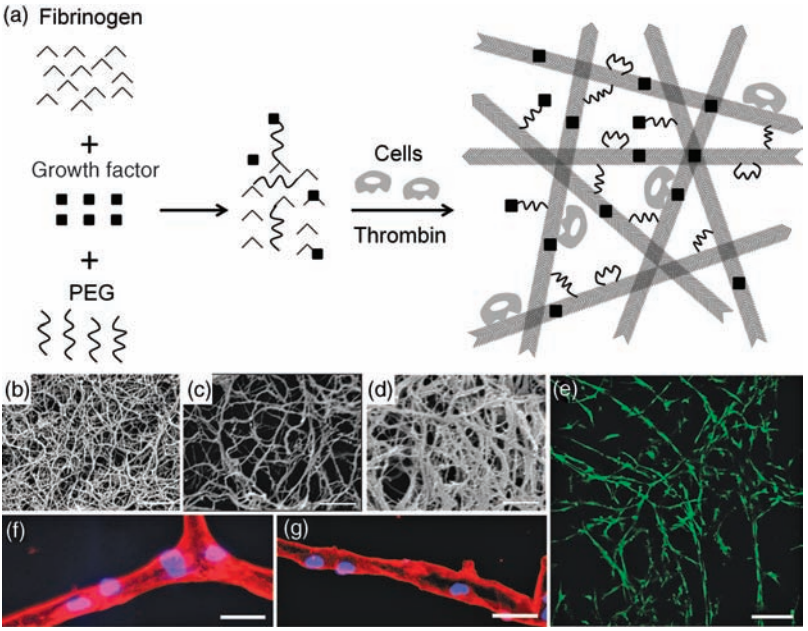

FIGURE 11.4 Schematic diagram of fibrin PEGylation, growth factor loading, and thrombin-mediated cross-linking in the presence of cells (a). Scanning electron microscope images of fibrous network demonstrating the ability to tune fiber diameter with PEG type. Fibrin-only fiber diameter was 175 nm (b), NHS-PEG was 130 nm (c), and SMB-PEG was 220 nm (d). Scale bar is 1 μm. Confocal microscopy Z-stack of calcein-AM stained human mesenchymal stem cell (hMSC) network in PEGylated fibrin gels demonstrating a robust tube network (e). Scale bar is 250 μm. Immunostaining for CD31 (f) and vWF (g) of hMSCs in PEGylated fibrin gels. Scale bar is 10 μm. Parts b, c, d, f, and g are adapted with permission from Ref. [113].

allow supreme spatial control of cell-laden channels and other conditions within the matrix. Microfabrication techniques allow researchers to direct tube formation *in vitro* or guide vascular invasion *in vivo*. Nanofiber gels have shown the ability to induce neovascularization, bind angiogenic molecules, and function as membranes for tissue engineering applications. Engineers must continue to adapt other technologies to vascular tissue engineering to achieve success.

11.7 CONCLUSIONS

Contributions to the field of vascular tissue engineering from micro- and nanotechnology are numerous. Toward the development of tissue-engineered vascular grafts, they include surface topography to direct vascular cells, micropatterned cell sheets, and nanofibrous matrices for scaffolds. Additionally, microfluidic systems to control flow and cell location have given us methods to study angiogenesis under

tightly regulated conditions. Finally, microfabricated scaffolds and nanofiber gels have been designed to enhance vascularization *in vivo*, bringing tissue engineering one step closer to clinical success.

REFERENCES

1. Mitchell SL, Niklason LE. Requirements for growing tissue-engineered vascular grafts. Cardiovasc Pathol 2003;12(2):59–64.
2. Moon JJ, West JL. Vascularization of engineered tissues: approaches to promote angiogenesis in biomaterials. Curr Top Med Chem 2008;8(4):300–310.
3. Szilagyi DE, Smith RF, Elliott JP, Allen HM. Long-term behavior of a Dacron arterial substitute: clinical, roentgenologic and histologic correlations. Ann Surg 1965; 162(3):453–477.
4. Brewster DC. Current controversies in the management of aortoiliac occlusive disease. J Vasc Surg 1997;25(2):365–379.
5. Zilla P, Bezuidenhout D, Human P. Prosthetic vascular grafts: wrong models, wrong questions and no healing. Biomaterials 2007;28(34):5009–5027.
6. Chlupác J, Filová E, Bacáková L. Blood vessel replacement: 50 years of development and tissue engineering paradigms in vascular surgery. Physiol Res 2009;58:S119–S139.
7. Berger K, Sauvage LR, Rao AM, Wood SJ. Healing of arterial prostheses in man: its incompleteness. Ann Surg 1972;175(1):118–127.
8. Nikolova G, Strilic B, Lammert E. The vascular niche and its basement membrane. Trends Cell Biol 2007;17(1):19–25.
9. Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. Nat Nanotechnol 2011;6(1):13–22.
10. Armentano RL, Levenson J, Barra JG, Fischer EI, Breitbart GJ, Pichel RH, et al. Assessment of elastin and collagen contribution to aortic elasticity in conscious dogs. Am J Physiol 1991;260(6 Pt 2):H1870–H1877.
11. Barra JG, Armentano RL, Levenson J, Pichel RH, Simon A. Assessment of smooth muscle contribution to descending thoracic aortic elastic mechanics in conscious dogs. Circ Res 1993;1040–1050.
12. O'Connell MK, Murthy S, Phan S, Xu C, Buchanan J, Spilker R, et al. The three-dimensional micro- and nanostructure of the aortic medial lamellar unit measured using 3D confocal & electron microscopy imaging. Matrix Biol 2008;27(3):171–181.
13. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. Science 1986;231(4736):397–400.
14. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, et al. Functional arteries grown *in vitro*. Science 1999;284(5413):489–493.
15. Niklason LE, Abbott W, Gao J, Klagges B, Hirschi KK, Ulubayram K, et al. Morphologic and mechanical characteristics of engineered bovine arteries. J Vasc Surg 2001; 33(3):628–638.
16. Stitzel JD, Pawlowski KJ, Bowlin GL, Wnek GE, Simpson DG. Arterial smooth muscle cell proliferation on a novel biomimicking, biodegradable vascular graft scaffold. J Biomater Appl 2001;16(1):22–33.

17. Serrano MC, Pagani R, Vallet-Regí M, Peña J, Rámila A, Izquierdo I, et al. *In vitro* biocompatibility assessment of poly(epsilon-caprolactone) films using L929 mouse fibroblasts. Biomaterials 2004;25(25):5603–5611.
18. Naito Y, Shinoka T, Duncan D, Hibino N, Solomon D, Cleary M, et al. Vascular tissue engineering: towards the next generation vascular grafts. Adv Drug Deliv Rev 2011; 63(4–5):312–323.
19. Stegemann JP, Nerem RM. Phenotype modulation in vascular tissue engineering using biochemical and mechanical stimulation. Ann Biomed Eng 2003;31(4):391–402.
20. Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials 2003;24(24):4385–4415.
21. Wissink MJ, Beernink R, Poot AA, Engbers GH, Beugeling T, van Aken WG, et al. Improved endothelialization of vascular grafts by local release of growth factor from heparinized collagen matrices. J Control Release 2000;64(1–3):103–114.
22. Theiler S, Mela P, Diamantouros SE, Jockenhoevel S, Keul H, Möller M. Fabrication of highly porous scaffolds for tissue engineering based on star-shaped functional poly (ε-caprolactone). Biotechnol Bioeng 2011;108(3):694–703.
23. Uttayarat P, Perets A, Li M, Pimton P, Stachelek SJ, Alferiev I, et al. Micropatterning of three-dimensional electrospun polyurethane vascular grafts. Acta Biomater 2010; 6(11):4229–4237.
24. Pezzatini S, Morbidelli L, Solito R, Paccagnini E, Boanini E, Bigi A, et al. Nano-structured HA crystals up-regulate FGF-2 expression and activity in microvascular endothelium promoting angiogenesis. Bone 2007;41(4):523–534.
25. Biggs MJP, Richards RG, Dalby MJ. Nanotopographical modification: a regulator of cellular function through focal adhesions. Nanomedicine 2010;6(5):619–633.
26. Dalby MJ, Riehle MO, Johnstone H, Affrossman S, Curtis ASG. *In vitro* reaction of endothelial cells to polymer demixed nanotopography. Biomaterials 2002; 23(14):2945–2954.
27. Chung T-W, Liu D-Z, Wang S-Y, Wang S-S. Enhancement of the growth of human endothelial cells by surface roughness at nanometer scale. Biomaterials 2003; 24(25):4655–4661.
28. Bettinger CJ, Zhang Z, Gerecht S, Borenstein JT, Langer R. Enhancement of *in vitro* capillary tube formation by substrate nanotopography. Adv Mater 2008;20(1): 99–103.
29. Bondar B, Fuchs S, Motta A, Migliaresi C, Kirkpatrick CJ. Functionality of endothelial cells on silk fibroin nets: comparative study of micro- and nanometric fibre size. Biomaterials 2008;29(5):561–572.
30. Liliensiek SJ, Wood JA, Yong J, Auerbach R, Nealey PF, Murphy CJ. Modulation of human vascular endothelial cell behaviors by nanotopographic cues. Biomaterials 2010;31(20):5418–5426.
31. L'Heureux N, Pâquet S, Labbé R, Germain L, Auger FA. A completely biological tissue-engineered human blood vessel. Faseb J 1998;12(1):47–56.
32. L'Heureux N, McAllister TN, de la Fuente LM. Tissue-engineered blood vessel for adult arterial revascularization. N Engl J Med 2007;357(14):1451–1453.
33. Sarkar S, Dadhania M, Rourke P, Desai TA, Wong JY. Vascular tissue engineering: microtextured scaffold templates to control organization of vascular smooth muscle cells and extracellular matrix. Acta Biomater 2005;1(1):93–100.

34. Sarkar S, Lee GY, Wong JY, Desai TA. Development and characterization of a porous micro-patterned scaffold for vascular tissue engineering applications. Biomaterials 2006;27(27):4775–4782.
35. Williams C, Tsuda Y, Isenberg BC, Yamato M, Shimizu T, Okano T, et al. Aligned cell sheets grown on thermo-responsive substrates with microcontact printed protein patterns. Adv Mater 2009;21(21):2161–2164.
36. Williams C, Xie AW, Yamato M, Okano T, Wong JY. Stacking of aligned cell sheets for layer-by-layer control of complex tissue structure. Biomaterials 2011;32(24): 5625–5632.
37. Elloumi Hannachi I, Itoga K, Kumashiro Y, Kobayashi J, Yamato M, Okano T. Fabrication of transferable micropatterned-co-cultured cell sheets with microcontact printing. Biomaterials 2009;30(29):5427–5432.
38. Tsang KY, Cheung MCH, Chan D, Cheah KSE. The developmental roles of the extracellular matrix: beyond structure to regulation. Cell Tissue Res 2010;339(1): 93–110.
39. Smith LA, Ma PX. Nano-fibrous scaffolds for tissue engineering. Colloids Surf B Biointerfaces 2004;39(3):125–131.
40. Ma Z, Kotaki M, Inai R, Ramakrishna S. Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Eng 2005;11(1–2):101–109.
41. Barnes CP, Sell SA, Boland ED, Simpson DG, Bowlin GL. Nanofiber technology: designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev 2007;59(14):1413–1433.
42. Reneker DH, Kataphinan W, Theron A, Zussman E, Yarin A L. Nanofiber garlands of polycaprolactone by electrospinning. Polymer 2002;43(25):6785–6794.
43. Boland E, Wnek G, Simpson D, Pawlowski K, Bowlin G. Tailoring tissue engineering scaffolds using electrostatic processing techniques: a study of poly(glycolic acid) electrospinning. J Macromol Sci Part A 2001;38(12):1231–1243.
44. Luu YK, Kim K, Hsiao BS, Chu B, Hadjiargyrou M. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA–PEG block copolymers. J Control Release 2003;89(2):341–353.
45. Boland ED, Coleman BD, Barnes CP, Simpson DG, Wnek GE, Bowlin GL. Electrospinning polydioxanone for biomedical applications. Acta Biomater 2005; 1(1):115–123.
46. Nottelet B, Pektok E, Mandracchia D, Tille JC, Walpoth B, Gurny R, et al. Factorial design optimization and *in vivo* feasibility of poly(epsilon-caprolactone)-micro- and nanofiber-based small diameter vascular grafts. J Biomed Mater Res Part A 2009; 89(4):865–875.
47. Pektok E, Nottelet B, Tille JC, Gurny R, Kalangos A, Moeller M, et al. Degradation and healing characteristics of small-diameter poly(epsilon-caprolactone) vascular grafts in the rat systemic arterial circulation. Circulation 2008;118(24):2563–2570.
48. Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Electrospinning of collagen nanofibers. Biomacromolecules 2002;3(2):232–238.
49. Zhang Y, Ouyang H, Lim CT, Ramakrishna S, Huang Z-M. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. J Biomed Mater Res B Appl Biomater 2005;72(1):156–165.

50. McKenna KA, Hinds MT, Sarao RC, Wu P-C, Maslen CL, Glanville RW, et al. Mechanical property characterization of electrospun recombinant human tropoelastin for vascular graft biomaterials. Acta Biomater 2011;1–9.
51. Wnek GE, Carr ME, Simpson DG, Bowlin GL. Electrospinning of nanofiber fibrinogen structures. Nano Lett 2003;3(2):213–216.
52. Barnes CP, Smith MJ, Bowlin GL, Sell SA, Matthews JA, Simpson DG, et al. Feasibility of electrospinning the globular proteins hemoglobin and myoglobin. J Eng Fiber Fabr 2006;1(2):16–29.
53. Kwon IK, Matsuda T. Co-electrospun nanofiber fabrics of poly(L-lactide-*co*-epsilon-caprolactone) with type I collagen or heparin. Biomacromolecules 2005;6(4): 2096–2105.
54. He W, Yong T, Teo WE, Ma Z, Ramakrishna S. Fabrication and endothelialization of collagen-blended biodegradable polymer nanofibers: potential vascular graft for blood vessel tissue engineering. Tissue Eng 2005;11(9–10):1574–1588.
55. He W, Ma Z, Teo WE, Dong YX, Robless PA, Lim TC, et al. Tubular nanofiber scaffolds for tissue engineered small-diameter vascular grafts. J Biomed Mater Res A 2009; 90(1):205–216.
56. Lee SJ, Liu J, Oh SH, Soker S, Atala A, Yoo JJ. Development of a composite vascular scaffolding system that withstands physiological vascular conditions. Biomaterials 2008;29(19):2891–2898.
57. Baxter BT, Mcgee GS, Shively VP, Drummond IAS, Dixit SN, Yamauchi M, et al. Elastin content, cross-links, and mRNA in normal and aneurysmal human aorta. J Vasc Surg 1992;16:192–200.
58. Li M, Mondrinos MJ, Gandhi MR, Ko FK, Weiss AS, Lelkes PI. Electrospun protein fibers as matrices for tissue engineering. Biomaterials 2005;26(30):5999–6008.
59. Li M, Mondrinos MJ, Chen X, Gandhi MR, Ko FK, Lelkes PI. Co-electrospun poly(lactide-*co*-glycolide), gelatin, and elastin blends for tissue engineering scaffolds. J Biomed Mater Res A 2006;79A:963–973.
60. Buttafoco L, Kolkman NG, Engbers-Buijtenhuijs P, Poot AA, Dijkstra PJ, Vermes I, et al. Electrospinning of collagen and elastin for tissue engineering applications. Biomaterials 2006;27(5):724–734.
61. Wise SG, Byrom MJ, Waterhouse A, Bannon PG, Weiss AS, Ng MKC. A multilayered synthetic human elastin/polycaprolactone hybrid vascular graft with tailored mechanical properties. Acta Biomater 2011;7(1):295–303.
62. Han J, Lazarovici P, Pomerantz C, Chen X, Wei Y, Lelkes PI. Co-electrospun blends of PLGA, gelatin, and elastin as potential nonthrombogenic scaffolds for vascular tissue engineering. Biomacromolecules 2011;12(2):399–408.
63. Chew SY, Wen J, Yim EKF, Leong KW. Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules 2005;6(4):2017–2024.
64. Wei G, Jin Q, Giannobile WV, Ma PX. Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB. J Control Release 2006;112(1):103–110.
65. Zhang YZ, Wang X, Feng Y, Li J, Lim CT, Ramakrishna S. Coaxial electrospinning of (fluorescein isothiocyanate-conjugated bovine serum albumin)-encapsulated poly (epsilon-caprolactone) nanofibers for sustained release. Biomacromolecules 2006; 7(4):1049–1057.

66. Liao IC, Chew SY, Leong KW. Aligned core-shell nanofibers delivering bioactive proteins. Nanomedicine 2006;1(4):465–471.
67. Lu Y, Jiang H, Tu K, Wang L. Mild immobilization of diverse macromolecular bioactive agents onto multifunctional fibrous membranes prepared by coaxial electrospinning. Acta Biomater 2009;5(5):1562–1574.
68. Drinnan CT, Zhang G, Alexander MA, Pulido AS, Suggs LJ. Multimodal release of transforming growth factor-β1 and the BB isoform of platelet derived growth factor from PEGylated fibrin gels. J Control Release 2010;147(2):180–186.
69. Hong Y, Ye SH, Nieponice A, Soletti L, Vorp DA, Wagner WR. A small diameter, fibrous vascular conduit generated from a poly(ester urethane)urea and phospholipid polymer blend. Biomaterials 2009;30(13):2457–2467.
70. Soletti L, Hong Y, Guan J, Stankus JJ, El-Kurdi MS, Wagner WR, et al. A bilayered elastomeric scaffold for tissue engineering of small diameter vascular grafts. Acta Biomater 2010;6(1):110–122.
71. Soletti L, Nieponice A, Hong Y, Ye SH, Stankus JJ, Wagner WR, et al. *In vivo* performance of a phospholipid-coated bioerodable elastomeric graft for small-diameter vascular applications. J Biomed Mater Res A 2011;96(2):436–448.
72. Liu H, Li X, Zhou G, Fan H, Fan Y. Electrospun sulfated silk fibroin nanofibrous scaffolds for vascular tissue engineering. Biomaterials 2011;32(15):3784–3793.
73. Kim TG, Park TG. Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly(D,L-lactic-*co*-glycolic acid) nanofiber mesh. Tissue Eng 2006;12(2):221–233.
74. Grafahrend D, Calvet JL, Klinkhammer K, Salber J, Dalton PD, Möller M, et al. Control of protein adsorption on functionalized electrospun fibers. Biotechnol Bioeng 2008;101 (3):609–621.
75. Choi WS, Bae JW, Lim HR, Joung YK, Park JC, Kwon IK, et al. RGD peptide-immobilized electrospun matrix of polyurethane for enhanced endothelial cell affinity. Biomed Mater 2008;3(4):044104.
76. Iucci G, Ghezzo F, Danesin R, Modesti M, Dettin M. Biomimetic peptide-enriched electrospun polymers: a photoelectron and infrared spectroscopy study. Polymer 2011;122:3574–3582.
77. Mattanavee W, Suwantong O, Puthong S, Bunaprasert T, Hoven VP, Supaphol P. Immobilization of biomolecules on the surface of electrospun polycaprolactone fibrous scaffolds for tissue engineering. ACS Appl Mater Interfaces 2009;1(5):1076–1085.
78. Dong B, Arnoult O, Smith ME, Wnek GE. Electrospinning of collagen nanofiber scaffolds from benign solvents. Macromol Rapid Commun 2009;30(7):539–542.
79. Stankus JJ, Guan J, Fujimoto K, Wagner WR. Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. Biomaterials 2006;27(5):735–744.
80. Stankus JJ, Soletti L, Fujimoto K, Hong Y, Vorp DA, Wagner WR. Fabrication of cell microintegrated blood vessel constructs through electrohydrodynamic atomization. Biomaterials 2007;28(17):2738–2746.
81. Sahoo S, Lee WC, Goh JCH, Toh SL. Bio-electrospraying: a potentially safe technique for delivering progenitor cells. Biotechnol Bioeng 2010;106(4):690–698.
82. Ju YM, Choi JS, Atala A, Yoo JJ, Lee SJ. Bilayered scaffold for engineering cellularized blood vessels. Biomaterials 2010;31(15):4313–4321.

83. Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. Nat Biotechnol 1999;17(2): 149–155.
84. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006;367(9518):1241–1246.
85. Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Rees LE, Cogan TA, et al. Clinical transplantation of a tissue-engineered airway. Lancet 2008;372(9655):2023–2030.
86. Rouwkema J, Rivron NC, van Blitterswijk CA. Vascularization in tissue engineering. Trends Biotechnol 2008;26(8):434–441.
87. Kaihara S, Borenstein J, Ravens M, Pien H, Cunningham B. Silicon micromachining to tissue engineer branched vascular channels for liver fabrication. Tissue Eng 2000; 6(2):105–117.
88. Lim D, Kamotani Y, Cho B, Mazumder J, Takayama S. Fabrication of microfluidic mixers and artificial vasculatures using a high-brightness diode-pumped Nd:YAG laser direct write method. Lab Chip 2003;3(4):318–323.
89. Borenstein JT, Terai H, King KR, Weinberg E, Kaazempur-Mofrad M, Vacanti J. Microfabrication technology for vascularized tissue engineering. Biomed Microdevices 2002;4(3):167–175.
90. Du Y, Cropek D, Mofrad MRK, Weinberg EJ, Khademhosseini A, Borenstein J. Microfluidic systems for engineering vascularized tissue constructs. In: *Microfluidics for Biological Applications* Berlin, Germany: Springer;2008. pp. 222–242.
91. King KR, Wang CCJ, Kaazempur-Mofrad MR, Vacanti JP, Borenstein JT. Biodegradable microfluidics. Adv Mater 2004;16(22):2007–2012.
92. Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP, Langer R, Wang Y. Endothelialized microvasculature based on a biodegradable elastomer. Tissue Eng 2005;11(1):302–309.
93. Bettinger CJ, Weinberg EJ, Kulig KM, Vacanti JP, Wang Y, Borenstein JT, et al. Three-dimensional microfluidic tissue-engineering scaffolds using a flexible biodegradable polymer. Adv Mater 2006;18(2):165–169.
94. Cabodi M, Choi NW, Gleghorn JP, Lee CSD, Bonassar LJ, Stroock AD. A microfluidic biomaterial. J Am Chem Soc 2005;127(40):13788–13789.
95. Choi NW, Cabodi M, Held B, Gleghorn JP, Bonassar LJ, Stroock AD. Microfluidic scaffolds for tissue engineering. Nat Mater 2007;6(11):908–915.
96. Bryant SJ, Cuy JL, Hauch KD, Ratner BD. Photo-patterning of porous hydrogels for tissue engineering. Biomaterials 2007;28(19):2978–2986.
97. Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, Cuy JL, et al. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. Proc Natl Acad Sci USA 2010;107(34):15211–15216.
98. Cross VL, Zheng Y, Won Choi N, Verbridge SS, Sutermaster BA, Bonassar LJ, et al. Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesis and vasculogenesis *in vitro*. Biomaterials 2010; 31(33):8596–8607.
99. Zheng Y, Henderson PW, Choi NW, Bonassar LJ, Spector JA, Stroock AD. Micro-structured templates for directed growth and vascularization of soft tissue *in vivo*. Biomaterials 2011;32(23):5391–5401.

100. Raghavan S, Nelson CM, Baranski JD. Geometrically controlled endothelial tubulogenesis in micropatterned gels. Lab Invest 2010;16(7).

101. Gillette BM, Jensen JA, Tang B, Yang GJ, Bazargan-Lari A, Zhong M, et al. In situ collagen assembly for integrating microfabricated three-dimensional cell-seeded matrices. Nat Mater 2008;7(8):636–640.

102. Sundararaghavan HG, Metter RB, Burdick JA. Electrospun fibrous scaffolds with multiscale and photopatterned porosity. Macromol Biosci 2010;10(3):265–270.

103. Sadr N, Zhu M, Osaki T, Kakegawa T, Yang Y, Moretti M, et al. SAM-based cell transfer to photopatterned hydrogels for microengineering vascular-like structures. Biomaterials 2011;32(30):7479–7490.

104. 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 2011;108(7):1693–1703.

105. Huang J-H, Kim J, Agrawal N, Sudarsan AP, Maxim JE, Jayaraman A, et al. Rapid fabrication of bio-inspired 3D microfluidic vascular networks. Adv Mater 2009; 21(35):3567–3571.

106. Matson JB, Zha RH, Stupp SI. Peptide self-assembly for crafting functional biological materials. Curr Opin Solid State Mater Sci 2011; 1–11.

107. Narmoneva DA, Vukmirovic R, Davis ME, Kamm RD, Lee RT. Endothelial cells promote cardiac myocyte survival and spatial reorganization: implications for cardiac regeneration. Circulation 2004;110(8):962–968.

108. Davis ME, Motion JPM, Narmoneva DA, Takahashi T, Hakuno D, Kamm RD, et al. Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. Circulation 2005;111(4):442–450.

109. Rajangam K, Behanna HA, Hui MJ, Han X, Hulvat JF, Lomasney JW, et al. Heparin binding nanostructures to promote growth of blood vessels. Nano Lett 2006;6(9): 2086–2090.

110. Ghanaati S, Webber MJ, Unger RE, Orth C, Hulvat JF, Kiehna SE, et al. Dynamic *in vivo* biocompatibility of angiogenic peptide amphiphile nanofibers. Biomaterials 2009;30 (31):6202–6212.

111. Chow LW, Bitton R, Webber MJ, Carvajal D, Shull KR, Sharma AK, et al. A bioactive self-assembled membrane to promote angiogenesis. Biomaterials 2011;32(6): 1574–1582.

112. Zhang G, Wang X, Wang Z, Zhang J, Suggs L. A PEGylated fibrin patch for mesenchymal stem cell delivery. Tissue Eng 2006;12(1):9–19.

113. Zhang G, Drinnan CT, Geuss LR, Suggs LJ. Vascular differentiation of bone marrow stem cells is directed by a tunable three-dimensional matrix. Acta Biomater 2010; 6(9):3395–3403.