Small-Diameter Artificial Arteries Engineered In Vitro

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Abstract—Although the need for a functional arterial replacement is clear, the lower blood flow velocities of small-diameter arteries like the coronary artery have led to the failure of synthetic materials that are successful for large-diameter grafts. Although autologous vessels remain the standard for small diameter grafts, many patients do not have a vessel suitable for use because of vascular disease, amputation, or previous harvest. As a result, tissue engineering has emerged as a promising approach to address the shortcomings of current therapies. Investigators have explored the use of arterial tissue cells or differentiated stem cells combined with various types of natural and synthetic scaffolds to make tubular constructs and subject them to chemical and/or mechanical stimulation in an attempt to develop a functional small-diameter arterial replacement graft with varying degrees of success. Here, we review the progress in all these major facets of the field. (Circ Res. 2006;98:25-35.)

Key Words: tissue engineering ■ artery ■ collagen ■ elastin ■ vascular graft

In 2002, more than 500,000 surgical procedures were performed involving replacement of small-caliber blood vessels.1 Despite a clear clinical need for a functional arterial graft, success has been limited to arterial replacements of large-caliber vessels such as the thoracic and abdominal aorta, arch vessels, iliac, and common femoral arteries; however, small-caliber (<6 mm) arterial substitutes, which account for a majority of the demand, have generally proved inadequate largely because of acute thrombogenicity of the graft, anastomotic intimal hyperplasia, aneurysm formation, infection, and progression of atherosclerotic disease.2 The lower blood flow velocities of smaller vessels pose a different set of design criteria and introduce a host of new problems not encountered in large-caliber arterial substitutes where Dacron and expanded polytetrafluoroethylene grafts have succeeded. Although autologous vessels, such as the saphenous vein, remain the standard for small diameter grafts, many patients do not have a vessel suitable for use because of vascular disease, amputation, or previous harvest. Moreover, this method requires a second surgical procedure to obtain the vessel. Tissue engineering has emerged as a promising approach to address the shortcomings of current options. Investigators have explored the use of arterial tissue cells combined with various types of natural and synthetic scaffolds to make tubular constructs and subject them to chemical and/or mechanical stimulation in an attempt to develop a functional small-diameter arterial replacement graft with varying degrees of success. There have been several reviews of vascular tissue engineering studies in recent years.3–6 Here, we review the progress in
all major facets of the field based on in vitro and ex vivo approaches; in vivo approaches are reviewed elsewhere.7,8

Many design criteria have been proposed for the development of a functional small-diameter arterial replacement graft.2,5,6,9–13 It must be biocompatible, ie, nonthrombogenic, nonimmunogenic, and resistant to infection, all of which are associated with a confluent, quiescent, nonactivated endothelium. Furthermore, it must induce an acceptable healing response that does not result in inflammation, hyperplasia, or fibrous capsule formation, and, ideally, leads to the integration of the graft into the body such that it eventually becomes indistinguishable from a native vessel. It must possess appropriate mechanical properties, which include physiological compliance, the ability to withstand long-term hemodynamic stress without failure, and no susceptibility to permanent creep that can lead to aneurysm formation. It must possess appropriate permeability to water, solutes, and cells. It must exhibit physiological properties, such as vasoconstriction/relaxation responses, insofar as these responses indicate a physiological SMC phenotype (systemic blood pressure is largely determined by cardiac output and peripheral resistance, which is governed by vessel diameter and/or anatomic location14). Finally, ease of handling and suturability are crucial for such vessels to be viable from a surgical standpoint.

The specific values for parameters that measure these target properties may depend on the nature of the artificial artery used. For example, the minimum acceptable burst pressure at the time of implantation, which is clearly a critical design criterion, might be lower for an artificial artery that is a mature tissue following in vitro culture (and will presumably undergo a slower and less extensive remodeling in vivo) as opposed to an artificial artery that is still largely an unremodeled scaffold and may degrade faster than new tissue forms in vivo. It must also be considered that the exquisite design of the native artery will probably not need to be reproduced to have a tissue-engineered artery that meets these criteria at implantation. In fact, the success of the field relies on this assumption. Moreover, remodeling of these living tissue constructs will occur in response to the local arterial and systemic vascular conditions, and a large body of literature on remodeling of native arteries will be relevant in understanding the outcomes. Because of the remodeling that will ensue, it is doubtful that the constructs will need to be fabricated for specific implantation sites other than to possess the required caliber.

These design criteria are quite challenging given the demanding mechanical environment of the cardiovascular system. Although different approaches attempt to meet these criteria in different ways, it is widely held that 3 components are necessary for these criteria to be met: (1) a biocompatible component with high tensile strength to provide mechanical support (collagen fibers or their analogue); (2) a biocompatible elastic component to provide recoil and prevent aneurysm formation (elastin fibers or their analogue); and (3) a nonactivated, confluent endothelium to prevent thrombosis.5 Currently, there are 4 main approaches being investigated that attempt to meet these criteria:12 (1) decellularized tissues; (2) synthetic polymer scaffolds; (3) cell sheets via the “tissue self-assembly” method; and (4) hydrogels or biopolymer scaffolds (the method used in our laboratory). Each has associated advantages and disadvantages that will be discussed below.

Fabrication Methods and Materials

Decellularized Tissues

Decellularized tissues have the advantage of being entirely composed of natural extracellular matrix (ECM), giving them numerous advantages in mechanical properties and biocompatibility.15 Unlike the other main approaches, these grafts are typically implanted without cells present with the assumption that they will be recellularized by host cells following implantation. These tissues can be either vascular or nonvascular (eg, small intestinal submucosa [SIS]) in origin. Decellularization is typically accomplished by treating tissues with a combination of detergents, enzyme inhibitors, and buffers.16–18 The vascular tissue benefits from grossly retaining the structure and composition of a native vessel following decellularization; however, decellularization can adversely impact the tissue, resulting in reduced ultimate tensile strength and compliance.19 Significant shrinkage is typically observed in decellularized vessels, presumably as a result of proteoglycans being removed from the tissues by the detergent treatment.20 Decellularized xenografts undergo aneurysm formation, infection, and thrombosis.4 In addition, their residual antigenicity can impair subsequent reendothelialization.21

Another decellularized tissue that has been used for tissue engineering with a good deal of success is the matrix derived from SIS. SIS is prepared by mechanically removing layers of mucosa and muscle from the small intestine, lysing the native cells with a hypotonic solution, and treating the material with peracetic acid and saline buffer to remove the cells.22 This process results in an ECM material composed of approximately 90% collagen (primarily type I), fibronectin, growth factors, glycosaminoglycans, proteoglycans, and glycoproteins.22 The primary advantage of this tissue is its ability to promote site-specific remodeling and regeneration by the host. On implantation, rapid neovascularization and infiltration of host cells occur followed by structural and compositional remodeling of the SIS tissue.22–24 Patency rates for both autologous and xenogeneic vascular grafts made with SIS in a canine model are comparable to those for saphenous veins25,26; however, their thrombogenic properties are still poorly understood.15

Biodegradable Polymer Scaffolds

Several biodegradable synthetic polymer scaffolds have been investigated for their suitability in vascular tissue engineering applications. The basic idea in all of these approaches is to seed cells onto a degradable polymeric scaffold that supports tissue growth and remodeling. Because the conditions used to create them are too harsh for the cells to survive, cells cannot be directly entrapped during scaffold formation. Cellularization is subsequently accomplished by relying on cell-invasion or cell-seeding techniques that may lead to suboptimal cell distribution.27 Ideally, the polymer will be slowly resorbed in culture or after implantation, leaving only the tissue generated by the cells. These polymers are advantageous because their
microstructure, mechanical properties, and resorption rates can be carefully controlled via chemical composition in an attempt to enhance tissue growth and remodeling. Furthermore, these scaffolds can provide initial mechanical function for the graft in vitro and/or in vivo until the cells have the opportunity to synthesize significant amounts of ECM; however, premature implantation is a major risk because a failure of the cells to produce the requisite ECM before polymer degradation would be catastrophic. Polyglycolic acid (PGA) has been the most widely used polymer for tissue engineering applications. However, PGA is rapidly resorbed, which can lead to premature weakening of the tissues before the cells have the opportunity to completely remodel it. In attempts to improve tissue construct mechanical properties and further regulate cell phenotype via interactions with the polymer, numerous other polymers have been copolymerized with PGA, such as poly-L-lactic acid, polyhydroxyalkanoate, poly-4-hydroxybutyrate, polycaprolactone–co-polyactic acid, and polyethylene glycol. Watanabe et al used a hybrid scaffold consisting of a PGA sheet and a polycaprolactone–co-polyactic acid copolymer and seeded it with a mixed population of cells derived from the femoral vein of a dog. The scaffold was cultured for 7 days, at which point it was implanted into the inferior vena cava of the same dog. The polymer scaffold was completely degraded after 3 months, and the graft remained patent for up to 13 months, with no evidence of dilation or stenosis. Using the same polymer, this group performed the first successful clinical application of a tissue-engineered graft in an attempt to repair an occluded pulmonary artery in a 4-year-old girl. The graft was prepared by seeding a polymer scaffold with cells isolated from a peripheral vein of the patient. After 7 months, the graft showed no signs of stenosis or aneurysm formation. Subsequent clinical studies of reperative reconstructive cardiovascular surgery with tissue-engineered vessels made with bone marrow–derived cells on 22 patients with congenital heart disease showed 100% patency with no signs of thrombogenic complications, stenosis, or obstruction. Unfortunately, postimplantation remodeling of the constructs cannot be monitored in human patients, and, therefore, potential long-term complications such as calcification cannot be assessed. Furthermore, these constructs were implanted into the relatively low-pressure pulmonary circulation (≈20 to 30 mm Hg during systole), which is less demanding than the higher-pressure environment of the coronary artery (≈100 to 140 mm Hg during systole). Other studies using synthetic polymers in combination with mechanical conditioning are included below.

**Cell Sheets**

Perhaps the most impressive completely biological tissue-engineered graft design thus far, at least in terms of burst pressure achieved with human cells, is the cell sheet approach developed by Auger and colleagues. In this approach, sheets of low passage human neonatal SMCs were grown on culture plates in the presence of elevated ascorbic acid (to induce significant collagen synthesis). After sufficient time a sheet was removed from its culture plate and wrapped around a porous, tubular mandrel to form the media of the tissue construct (the porous mandrel allowed transport of nutrients to the inner surface of the tissue layer by flowing culture medium through the mandrel lumen). In the same manner, a sheet of fibroblasts was grown and wrapped around the media to produce the adventitia. After several weeks of maturation, these 2 layers fused into a single cohesive layer. At this time, the tubular constructs were removed from the mandrels and seeded with ECs on the luminal surface by cannulating the ends, filling the lumen with a solution of Ecs, and then slowly rotating them overnight. The resulting construct showed well-defined multilayer organization in addition to abundant ECM deposition; however, the ECM fibers did not exhibit circumferential alignment, which is believed to confer the artery with its unique mechanical properties. SMCs demonstrated a reversion to the contractile phenotype by reexpressing desmin (a marker lost under culture conditions). The endothelium expressed von Willebrand factor, incorporated acetylated-LDL, produced prostacyclin, and inhibited platelet adhesion in vitro, all of which are performed by the endothelium of native arteries. Tissue constructs had burst strengths of 2594±501 mm Hg, which is significantly higher than human saphenous veins (1680±307 mm Hg), which remain the standard for bypass grafts.

However, although these tissue-engineered blood vessels are more compliant than expanded polytetrafluorethylene grafts, they are apparently much less compliant than the small-caliber vessels they are designed to replace, which could potentially lead to complications related to the issue of compliance mismatch, which often leads to anastomotic intimal hyperplasia. This lack of compliance may be attributable to insufficient elastic fiber deposition, which may also lead to irreversible creep predisposing aneurysm formation. In spite of this, short-term grafting experiments in a canine model were extremely encouraging. In a subsequent study, vessels made in this manner were shown to have marked vasoactive responses to numerous agonists such as histamine, bradykinin, ATP, and UTP.

**Biopolymer Scaffolds**

Motivated by the pioneering work of Weinberg and Bell, a number of researchers have investigated the prospect of seeding Ecs onto the luminal surface of a tube of reconstituted type I collagen gel populated and compacted by SMCs to construct a completely biological vascular graft. The concept of constructing such a graft, termed a “bioartificial artery” (BAA), in this manner is extremely appealing. In the case of collagen, it is the most abundant protein in the human body and is the major ECM component in most tissues, including the arterial wall, making it a natural cell substrate. Bovine type I collagen is already an approved material for implantation and used in the US Food and Drug Administration–approved bioartificial skin product Apligraf (Organogenesis Inc). Fibrin, another commonly used biopolymer, is the major structural protein in blood clots and plays a vital role in the subsequent wound healing response, being remodeling into cell-derived ECM. In clinical practice, fibrin is widely used as a wound sealant in place of sutures where appropriate. Both collagen and fibrin allow direct cellulariza-
tion by cell entrapment during fibrillogenesis (also known as gelation), because this occurs under physiological conditions. This is in stark contrast to synthetic polymer systems in which cells must be seeded after fabrication because of the harsh conditions required to fabricate the polymer scaffolds. Furthermore, as first suggested by L’Heureux et al.,50 it is possible to generate fibril and SMC alignment in BAAs by applying an appropriate mechanical constraint to compaction, which yields circumferential alignment of fibrils and cells comparable to that found in the native artery.48,53 (see Figure 1). As elucidated by Barocas et al, a nonadhesive mandrel induces an anisotropic strain field when the entrapped SMCs contract the initially isotropic network of fibrils.48 When combined with the assumption that fibrils align in the direction of extension and perpendicular to the direction of compression, the anisotropic strain associated with free longitudinal compaction and constrained circumferential compaction yields the desired circumferential alignment. It is this last feature that makes the biopolymer approach to vascular tissue engineering so attractive. This follows from 2 axioms: (1) that native artery function, particularly mechanical function, depends on structure (alignment of ECM and cells) as much as it depends on composition19–22 and (2) that the tissue-engineered artery should serve as a functional remodeling template, such that the biopolymer scaffold provides a directional template to guide the remodeling process into a functional bioartificial artery at implantation.12

The method of BAA fabrication developed by Weinberg and Bell46 has been improved on by numerous researchers including the authors. The common approach involves the injection of a collagen monomer solution with suspended SMCs into a tubular mold and allowing collagen fibrillogenesis to occur. The resulting SMC-populated collagen gel is compacted around a mandrel by SMC traction forces forming a construct termed a media-equivalent (ME), which could be subsequently endothermalized to form a BAA. In the case of Weinberg and Bell, a Dacron mesh sleeve was placed around the construct to provide additional mechanical support as the constructs were only capable of supporting burst pressures on the order of 90 mm Hg, and by alternating 3 collagen layers with 2 Dacron mesh layers, the burst pressure was increased to a value of 323 ± 31 mm Hg. The collagen fibril alignment and SMC orientation in these constructs were found to be in the axial direction, which is perpendicular to the circumferential alignment found in the native artery. In addition, these constructs did not incorporate any elastin. Despite the use of the Dacron mesh and incorrect alignment of SMCs and collagen, the work of Weinberg and Bell represented an important first step in the development of a BAA.

Hirai and Matsuda49 sought to improve on this technique by investigating the compaction of a SMC-containing, type I collagen gel around a mandrel, but with an important difference first suggested by L’Heureux et al50 and more thoroughly examined by our group.48 This difference relates to the constraint of compaction around a nonadhesive mandrel, as discussed above. This reorganization places the fibrils in the primary loading direction (ie, circumferential), the consequence of which is an improvement in mechanical properties.48 Unfortunately, circumferential alignment alone was not enough to generate constructs with appropriate mechanical strength to withstand the pressure of the arterial environment. Several approaches have been undertaken to further enhance the mechanical properties of these constructs including non-enzymatic cross-linking (glycation) using ribose54 and enzymatic cross-linking using lysyl oxidase.55 Although the results from these studies were encouraging, the mechanical properties of collagen-based BAAs remained inadequate.

In an attempt to address the shortcomings of collagen-based BAAs yet still retain the attractive aspects of the biopolymer-based approach (namely the high degree of circumferential fibril and cell alignment and direct cellularization of the constructs), our group has investigated the use of fibrin as a viable alternative to collagen.56,57 SMCs and fibroblasts entrapped in collagen gel were shown to suppress ECM synthesis relative to monolayer cultures,58 whereas fibroblasts entrapped in fibrin gel were stimulated to secrete ECM relative to fibroblasts entrapped in collagen gel.59 The fabrication technique for fibrin-based constructs remains largely the same as for collagen, except here a suspension of SMCs in fibrinogen is mixed with a solution containing thrombin (which catalyzes the formation of fibrin fibrils from fibrinogen monomers)60 before injection into a tubular mold. The fibrin fibrils can vary from 40 to 400 nm in diameter, which is the range of ECM fibers that cells interact with in vivo. The entrapped neonatal rat aortic SMCs were shown to compact and align the fibrin gels in a manner similar to...
containing abundant collagen fibers (stain green) and an inner layer containing other fibers, likely including elastic fibers (fibrin stains a bright red that is not evident here). B, Fiber alignment map generated from a polarized light method that reveals strong circumferential alignment in the tissue on the left. The red segments indicate the local direction and strength of fiber alignment, and the sample is oriented with its longitudinal axis being vertical after being sliced and splayed open. The dark area near the left edge is the burst location.

collagen gels, with the cell-synthesized collagen fibrils being laid down in the same (circumferential) direction as the fibrin fibrils (see Figure 2). Optimizing the concentrations of transforming growth factor-β1 (TGF-β1) and insulin in the culture medium yielded a 20-fold increase in collagen production in fibrin gels as compared with collagen gels and uniaxial tensile strength and modulus that were comparable to those of the rat abdominal aorta. Furthermore, significant elastogenesis with SMCs has been observed in the fibrin-based constructs, which does not occur in collagen-based constructs. Gene expression studies over the course of the first five weeks of culture showed marked increases in mRNA levels of tropoelastin, collagen, and lysyl oxidase that correlated with measured quantities of ECM deposition and improvement in mechanical properties. Using a similar system, Swartz et al produced ovine-based fibrin BAA scaffolds that demonstrated strong vasoactive responses to potassium chloride, norepinephrine, U46619, and sodium nitroprusside. After approximately 3 weeks in culture with minimal in vitro fibrin remodeling, endothelialized constructs were implanted into the low-pressure external jugular vein of sheep and remained patent for up to 15 weeks. Examination of the vessels following implantation revealed significant remodeling of the fibrin scaffold and large increases in mechanical strength.

In addition to the ability of fibrin to stimulate collagen and elastic fiber deposition by SMCs, the initial fibrin gel can be manipulated in a number of ways, including fibril diameter and network structure via thrombin concentration, calcium ion concentration, and ionic strength. Factor XIII and other transglutaminases can be used to crosslink the fibrinopeptides that self-assemble to form the fibrils and thereby modulate the network stiffness and the ability of entrapped cells to contract and align the fibrillar network, as well as modulate the rate of fibrinolysis. Inhibitors of fibrinolysis, such as aprotinin and aminocaproic acid, can also be used to modulate fibrin degradation by cell-activated plasmin. Fibrin binds a number of ECM components (eg, fibronectin) and growth factors (eg, fibroblast growth factor and vascular endothelial growth factor), making it a cell substrate of high information content, although these constituents and others are present in variable concentrations in commercial preparations. Certain preparations are approved by the US Food and Drug Administration as tissue sealants, another beneficial feature of fibrin as a tissue scaffold. Finally, autologous fibrin can be generated, if ultimately necessary, directly from the blood of a patient.

**Novel Biomimetic Materials**

A new generation of biomaterials is being developed that attempts to mimic the structure and characteristics of native ECM, such as fibrillar structure, viscoelasticity, cell adhesion domains, growth factor binding, and proteolytic sensitivity. Such materials are attractive because, in principle, their properties can be readily controlled while mimicking many of the critical biological functions of the native ECM, which are largely lacking from synthetic polymers such as PGA. The technique of electrospinning has been used to produce fibers with diameters on the order of those found in native ECM from polyester as well as biopolymers such as collagen and fibrinogen. Although directly yielding circumferential alignment (or a prescribed alignment pattern), cells cannot be entrapped during electrospinning and must be seeded in the scaffolds postfabrication. Other approaches to achieving a fiber diameter that is in the range of ECM fibers are based on self-assembly of oligopeptides that have alternating hydrophobic and charged residues and peptide-lipid amphiphiles; however, these approaches have only yielded fibers of 5 to 10 nm in diameter to date. We are exploring the use of amphiphilic diblock copolymers that also self-assemble into networks of cylindrical micelles but allow larger diameter fibers to be obtained. Mann and colleagues...
showed that SMCs grown on photopolymerizable polyethylene glycol hydrogels modified with adhesion ligands had reduced rates of proliferation, migration, and ECM synthesis with increasing ligand density.78,79; however, this suppressive effect of the adhesion domains could be overcome by the incorporation of proteolytically degradable domains80 and a tethered growth factor (TGF-β),81 demonstrating that it is possible to tailor such materials to alter cell function. In an attempt to develop materials for facilitating arterial healing following balloon angioplasty, Seliktar et al were able to enhance EC migration and adhesion on polyethylene glycol hydrogels that incorporated cell-adhesion domains and domains that were sensitive to matrix metalloproteinase (MMP)-2 cleavage in addition to tethering vascular endothelial growth factor, which promotes MMP-2 expression in ECs,82 to the polymer backbone.83 A similar hydrogel has been developed that incorporates cell-adhesion and plasmin-degradable domains based on fibrinogen and anti–thrombin III, as well as heparin-binding sites to encourage the deposition of heparin and, in turn, heparin-binding growth factors.84 Although all these techniques are promising, these materials have yet to be put into practice as potential scaffolds for vascular tissue engineering.

Mechanical Conditioning via Bioreactors

Despite many advances in vascular tissue engineering, investigators have yet to develop a functional arterial replacement graft that not only meets the requirements for mechanical strength and stiffness but also mimics the biological functionality of a native vessel. Numerous approaches have been taken to address these issues including, in the case of biopolymer-based fabrication, nonenzymatic cross-linking (glycation) using ribose,54,85 enzymatic cross-linking using lysyl oxidase,55 and fabricating fibrin-based MEs with TGF-β1 and insulin56,57 culture medium supplementation as another means of stimulating ECM synthesis and cross-linking. Although such approaches have shown great promise, none of them has produced a construct that meets all the criteria outlined above.

Recently, several groups have investigated the use of mechanical conditioning as a means of influencing the development of a tissue-engineered arterial. Vascular tissues are subject to 4 principal hemodynamic forces: (1) shear stresses, tangential frictional forces acting on ECs as a result of blood flow and on SMCs as a result of transmural interstitial flow; (2) luminal pressure, a cyclic normal force attributable to blood pressure; (3) mechanical stretch, a cyclic circumferential stress caused by blood pressure; and (4) tension in the longitudinal direction. All of these forces have been shown to act both independently and synergistically to modulate the behavior of vascular tissues.87–90 Forces associated with vessel distension have direct influences on both ECs and SMCs,88 whereas shear stresses exhibit their direct influence on the ECs lining the vessel wall.93 However, these stresses can also modulate SMC behavior via secondary signals released by the ECs.87

To expose tissues to such mechanical stimulation, a wide array of bioreactors systems has been developed that allow investigators to study tissue growth and behavior under physiological conditions. Bioreactors are not limited to providing mechanical stimulation but can be used to enhance nutrient and oxygen delivery as well as facilitate long-term culture and scale-up.91–94 Such systems can be simple devices used to mimic 1 aspect of the cardiovascular environment, such as cyclic distension of the vascular wall,95,96 or more elaborate systems that seek to mimic the vascular environment.97–99 All such systems have demonstrated their ability to enhance tissue growth by enhancing ECM synthesis and/or increasing cell proliferation, as well as at least partially restoring cells to their normal in vivo phenotypes.

Cyclic Stretching/Distension of Constructs

Because vascular SMCs are not exposed directly to shear stress induced by blood flow under normal conditions, the pulsatile distension of the arterial media during the cardiac cycle is by far the dominant mechanical stimulus that these cells perceive. Mechanical stretching of SMCs cultured on distensible substrata or entrapped in collagen gels has been shown to have profound effects on SMC phenotypic state,99–106 orientation,101,106–109 ECM deposition,9,96,110–119 growth factor release,120–122 proliferation,100,123,124 and vascular tone,125,126 leading several groups to investigate these mechanisms as a means of enhancing the development and maturation of tissue-engineered arteries. In particular, Kanda et al showed that adult bovine SMC-based collagen MEs that were cyclically loaded at 1 Hz with a strain amplitude of 10% for up to 4 weeks exhibited increases in contractile components such as myofilaments, dense bodies, and basement membranes,106 indicating that cyclic stretching induces SMC reversion to a more contractile phenotype. They also reported changes in the orientation of cells and fibrils in response to cyclic stretching.127 Seliktar et al found that cyclically distending (CD) adult rat aortic SMC-based collagen MEs during the compaction period at 1 Hz and a strain of 10% for 8 days yielded increases in ultimate tensile strength (3-fold) and tensile modulus (3.5-fold),37 as well as an increase in MMP-2 activity.128 Using the same system as Seliktar et al,37 Stegemann et al did not observe any changes in smooth muscle α-actin expression in response to 10% cyclic strain.129 Taken together, these results clearly indicate that cyclic loading of engineered tissues can be used to influence their mechanical properties by altering SMC behavior; however, the results are difficult to interpret because of 2 concurrent confounding factors: cell induced gel compaction/fibril alignment and irrecoverable creep. By using only highly compacted, ribose cross-linked constructs, and thus avoiding the issues of fiber alignment and creep that can be induced by CD, our group was able to isolate the effects of cyclic mechanical loading on adult rat aortic SMC-based collagen ME development.95 Furthermore, our studies focused on the effects of long-term (5 weeks) CD of MEs as opposed to the short-term (several days) of previous studies. We found that in all cases involving CD, MEs were both stronger and stiffer than their static counterparts (increases of up to 2.4- and 2.7-fold, respectively), whereas loading parameters such as strain, stretch time, and relaxation time all influence ME
mechanical properties. In addition, we found deposition of significant amounts of insoluble elastin in our MEs, which was a surprising finding given that the adult rat aortic SMCs used in this study typically lack significant elastogenic potential in static culture.\(^{130,131}\)

Other investigators have subjected synthetic polymer scaffolds seeded with SMCs to cyclic loading with enhancement of mechanical properties and alteration of SMC phenotype. The most promising results obtained thus far with the synthetic polymer approach have been those reported by Niklason et al.\(^9\) In this study, tubular PGA meshes were seeded with adult bovine aortic SMCs and placed around distensible silicone tubes for 8 weeks. The silicone tubes were cyclically inflated to 5% radial distension at a rate of 165 pulses per minute to simulate the fetal environment of large animals. During the 8-week culture period, SMCs produced significant quantities of collagen, and the polymer scaffold had been significantly resorbed. After this initial culture period, the silicone tubes were removed, and the constructs were seeded with ECs and perfused with culture medium for 3 days. The burst pressures of these constructs were 2150±709 mm Hg; however, the stiffness of the vessels was very high (ie, low compliance) and might present problems in terms of compliance mismatch, and a lack of elastic fibers could predispose these constructs to irrecoverable creep and aneurysm. The vessels showed measurable contractility in the presence of serotonin, endothelin-1, and prostaglandin-F\(_{2\alpha}\). Furthermore, SMCs expressed calponin and myosin heavy chain, both markers of the normal in vivo contractile SMC phenotype.\(^{43}\) Following implantation in miniature swine, the mechanically stimulated constructs were patent up to 4 weeks, as compared with nonstimulated grafts that thrombosed over the same period of time.

Using a technique similar to Niklason,\(^9\) Hoerstrup et al achieved a burst pressure of 326.3±24 mm Hg after 4 weeks in culture.\(^{33}\) In this study, a copolymer of PGA and poly-4-hydroxybutyrate was used as the scaffold because of its higher mechanical strength and elasticity than PGA alone. In addition, the PGA–poly-4-hydroxybutyrate copolymer is a thermoplastic, allowing it to be easily molded into nearly any shape. The higher initial mechanical strength of this scaffold allowed the constructs to be exposed to direct shear and cyclic distention immediately following cell seeding with adult ovine SMCs and ECs. Significant cell proliferation and collagen deposition were observed. The constructs also had suitable suture retention strength for implantation. Degradation of the scaffold was noted, but the extent of this degradation was not quantified.

Kim et al observed increases in mechanical stiffness and strength, collagen and elastin synthesis rates, and cell proliferation in response to mechanically stretching PGA scaffolds seeded with SMCs to a strain of 7% at 1 Hz for up to 20 weeks.\(^{96}\) These PGA scaffolds were cross-linked with poly-L-lactic acid before cell seeding, which likely imparts a degree of elasticity to the scaffolds. However, it is not clear whether creep is an issue with these or other synthetic polymer scaffolds because it has not been discussed in the literature as it pertains to cyclic mechanical loading. Similar results were also observed for SMCs grown on poly(lactide–co-caprolactone) meshes for 8 weeks under pulsatile conditions.\(^{97}\)

### Flow Conditioning of ECs

The range of vessel responses to flow-induced shear stresses is extremely broad and often complex.\(^{87}\) Although shear stress is directly perceived only by the endothelium, these signals are transmitted to other regions of the vessel wall and the blood by the ECs via a number of physical and chemical pathways. In this manner, the entire vessel is capable of responding to changes in shear stress. These signals can induce changes in vessel diameter and tone, SMC proliferation, lumen thrombogenicity, and ECM organization, all of which are critical to maintaining vascular homeostasis. Specifically, normal levels of shear stress decrease vessel thrombogenicity, maintain proper vascular tone, and inhibit SMC proliferation, whereas low levels of shear stress promote intimal thickening and increase thrombus formation on the vessel lumen.\(^{87,132–138}\) Although the mechanotransduction cascade is not well understood,\(^{87}\) it is clear that the cytoskeleton, particularly actin filaments, plays the principal role in the transmission of mechanical signals.\(^{139,140}\) ECs have been shown to align in the flow direction with marked changes in cytoskeletal and subendothelial matrix organization.\(^{141}\) These changes in cytoskeletal organization and morphology are likely to have a major impact on the ability of ECs to sense and transmit shear stress signals. Turbulent flow is incapable of inducing changes in EC orientation and, in most cases, has no effect on EC function. In contrast, laminar flows are capable of inducing changes in EC orientation, morphology, and function over a range of shear stresses.\(^{142}\) Finally, the ability to regulate transport of fluid and macromolecules across the vessel wall is largely controlled by the endothelium via a highly regulated process that involves both convective and diffusive pathways, which can be modulated by several biochemical and physical factors, particularly shear stress. In vitro studies have shown that 1 dyne/cm\(^2\) of shear stress causes a 4-fold increase in vascular permeability, and 10 dynes/cm\(^2\) increase permeability 10-fold, both of which are time dependent and reversible.\(^{143}\)

Most of the studies mentioned above are in model systems; few studies report a detailed characterization of ECs seeded onto the luminal surface of tissue-engineered arteries following flow conditioning in vitro. Baguneid et al radiolabeled ECs before endothelialization of Dacron-supported collagen gels to quantify EC attachment and retention after exposure to fluid flow.\(^{144}\) Even with fibronectin precoating and flow preconditioning of the luminal surface, EC retention was below 80% after 10 minutes of flow. We have found that approximately 95% of the ECs seeded as a monolayer onto the luminal surface of fibrin-based BAAs fabricated as previously described elsewhere\(^{53,61}\) remain adherent at shear stresses of 10 dynes/cm\(^2\) in both steady and pulsatile laminar flow, and these ECs exhibit elongation and alignment in the flow direction.\(^{145}\) This high EC retention may be attributable to the deposition of fibronectin and laminin that were colocatalized on the luminal surface.
Meeting all criteria for the ideal tissue-engineered artery simultaneously remains a challenge. For example, high burst strength is often associated with compliance mismatch,\textsuperscript{38} which can lead to intimal hyperplasia at the suture line.\textsuperscript{44} Conversely, constructs that possess physiological compliance have lacked high burst strength.\textsuperscript{34} Notably, no approach has yet resulted in all the key features of the media, namely circumferential alignment of SMCs, collagen fibers, and elastin lamellae. In fact, mature (ie, cross-linked) elastin fibers have only been reported in the self-assembly approach, and in association with fibroblasts, not SMCs.\textsuperscript{38} The developmental downregulation of elastogenesis in SMCs creates the hurdle.\textsuperscript{130,131} Indeed, elastic recoil is critical to abolish permanent creep and is conferred by elastin lamellae in the artery. We have recently reported that elastogenesis can be realized in fibrin-based constructs remodeled by entrapped neonatal SMCs,\textsuperscript{61,63} so this hurdle may now be cleared; however, the structure of this elastin and its functional significance have yet to be ascertained. The use of neonatal SMC would generally entail a BAA with nonautologous cells, although this may not be problematic in the case of tissue cells (eg, Apligraf can be described as bioartificial skin and uses nonautologous neonatal fibroblasts). The use of adult stem cells that differentiate into “neonatal-like” elastogenic SMCs may prove to be an alternative. There is no imminent solution to the extreme immunogenicity of nonautologous ECs. Even if a construct could be prefabricated from nonautologous SMCs, it would still take many days to weeks to isolate and expand the ECs of a patient to the numbers required for seeding a construct of useful length, with circulating EC progenitor cells\textsuperscript{145–147} and blood outgrowth endothelial cells,\textsuperscript{148,149} both of which possess high proliferative capacity and can differentiate into mature ECs, being attractive options. The associated time lag, however, might limit the applicability of BAAs fabricated with these cell sources to patients with anticipated repeat procedures. The optimal sources for SMCs and ECs remain to be determined, but economic and regulatory considerations would favor prefabrication of BAAs from nonautologous, genetically unmodified cells.

Although certain combinations of cells (terminally differentiated or stem-cell derived), scaffolds (synthetic or natural), stimulation (chemical, mechanical, and possibly electromagnetic), and tissue culture/bioreactor systems (diffusive or convective transport) that are yet to be identified will no doubt lead to improvements, a major obstacle to the advancement of the field is the current use of invasive or destructive methods to monitor most tissue growth variables (ie, cellularly, localized ECM composition and microstructure, localized concentrations of soluble and bound factors, material and mechanical properties). The development of imaging methods to make these measurements noninvasively would greatly improve the productivity of such studies by revealing spatiotemporal growth patterns and relationships among these variables that are elusive with current methods. High-throughput models that accurately reflect tissue growth in constructs of the target size and geometry would also enhance productivity. Ultimately, a predictive basis for the optimal combination of cell source/scaffold/stimulation/bioreactor will hinge on a more complete understanding of how the cell integrates the various signals at the cellular and molecular level. This understanding will translate into biophysical models that relate cell cycle regulation and the production and assembly of ECM components in response to these integrated signals and ultimately into multiscale mechanical models that relate the evolving ECM at the molecular level to macroscopic mechanical and functional properties. There are recent continuum mechanical models of vascular growth and remodeling that are aimed in this direction.\textsuperscript{150–152}

In summary, although great advances have been made toward a small-diameter artificial artery, many open questions and obstacles remain. Answering these questions and overcoming these obstacles will require an interdisciplinary effort requiring critical contributions from biologists, engineers, and clinicians, with strong collaborations among these 3 fields being crucial to success. The enormity of the clinical need will ensure that such efforts will continue to grow and drive research toward the goal of producing a functional small-diameter vascular graft.

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