Published in final edited form as:

Cell Mol Life Sci. 2014 June ; 71(11): 2103–2118. doi:10.1007/s00018-013-1546-3.

Engineering of arteries *in vitro*

Angela H. Huang¹ and Laura E. Niklason^{1,2}

¹Department of Biomedical Engineering, Yale University, New Haven, CT, 06511, USA

²Department of Anesthesiology, Yale University, New Haven, CT, 06511, USA

Abstract

This review will focus on two elements that are essential for functional arterial regeneration *in vitro*: the mechanical environment and the bioreactors used for tissue growth. The importance of the mechanical environment to embryological development, vascular functionality, and vascular graft regeneration will be discussed. Bioreactors generate mechanical stimuli to simulate the biomechanical environment of the arterial system. This system has been used to reconstruct arterial grafts with appropriate mechanical strength for implantation by controlling the chemical and mechanical environments in which the grafts are grown. Bioreactors are powerful tools to study the effect of mechanical stimuli on extracellular matrix (ECM) architecture and the mechanical properties of engineered vessels. Hence biomimetic systems enable us to optimize chemo-biomechanical culture conditions to regenerate engineered vessels with physiological properties similar to those of native arterial vessels. In addition, this review will introduce and examine various approaches and techniques that have been used to engineer biologically-based vascular grafts, including collagen-based grafts, fibrin-gel grafts, cell sheet engineering, biodegradable polymers, and decellularization of native vessels.

Keywords

bioreactor; biaxial stretching; mechanical conditioning; tissue engineering; vascular grafts; engineered vessel; biomaterials

Introduction

Over 570,000 coronary artery bypasses are performed each year [1]. As a result, there is a critical demand for small-diameter (<6mm) vascular grafts. However, very few of the currently available blood vessel substitutes can completely exhibit the structural and functional properties of native vessels [2]. Synthetic materials such as polytetrafluoroethylene, DacronTM, and polyurethane have been used as large arterial substitutes (>6 mm) for thoracic and abdominal aorta, iliac, and common femoral arteries [3, 4]. These large arterial prostheses have been clinically effective, demonstrating durable patency, and requiring few repeat procedures [3]. Unfortunately, these synthetic materials

Corresponding author: laura.niklason@yale.edu, Phone: (203) 737-1422, Fax: (203)737-1484.

Mailing Address:

P.O. Box 208051, New Haven, CT 06520, angela.huang@yale.edu

have met with little success when used as small-diameter arterial grafts (< 6mm). The small-diameter synthetic prostheses inevitably have led to the development of anastomotic intimal hyperplasia, coagulation, and thrombogenesis [5, 6]. The ultimate goal of vascular engineering is to generate biologically-based vascular grafts that exhibit the biological and mechanical properties of native arteries. In this review, we will i) explore a number of different methodologies that are used to regenerate small-diameter engineered vessels, ii) focus on the importance of applying mechanical conditioning to vascular engineering, and iii) establish bioreactors as a promising device to study the effect and the impact of mechanical conditions on extracellular matrix (ECM) organization and the mechanical properties of engineered vessels.

Biomechanical Forces Impact Vascular Formation and Remodeling during Embryogenesis

Biomechanical stimuli are pivotal regulators of the developing cardiovascular system [7, 8]. In vertebrate embryos, the cardiovascular system is one of the first organs to develop [7]. The earliest vascular network, known as the capillary plexus, is formed from expanding endothelial cells (ECs) in the embryonic yolk sac [9]. The blood circulation created by the pulsing heart is established subsequent to the formation of microvascular plexus [7]. It has been shown that exposure of ECs to hemodynamic shear stress upregulates and downregulates arterial and venous markers, respectively, strongly implicating that biomechanical force is also involved in embryonic arterial differentiation [10]. Recruitment of perivascular cells to the vascular walls is also directed by hemodynamic forces and further contributes to the arterialization and stabilization processes [10, 11]. Additionally, blockage of blood flow leads to vascular developmental arrest at the capillary plexus stage [12, 13] and disrupts the remodeling of the heart tube into a multi-chambered structure [14, 15]. Vessels having little or no flow have been shown to regress during embryonic development [16].

Biomechanical forces exerted on embryonic vessels also elicit vascular remodeling to optimize the geometric and mechanical properties of the microvasculature in order to maintain homeostasis [17]. Over a century ago, Thomas concluded that the size of the microvascular lumen was regulated by blood flow rate in developing chick embryos. Similarly, in 1918, Clark observed that the growing tail of the frog larvae imposed an axial tension on the resident vasculature, which in turn modulated microvascular length and growth [16]. Clark's work suggested that axial strain on the microvasculature induced structural adaptation in order to re-establish vascular homeostasis. Furthermore, luminal pressure regulates embryonic vascular remodeling by modulating the thickness of vascular wall, similar to processes taking place in adult vasculature. Blood pressure increases progressively during prenatal development but increases sharply in the early postnatal weeks. Increases in medial thickness [18, 19] and medial lamellar units occur in parallel with the increase in blood pressure during early postnatal life [20]. The embryonic dorsal aorta in chick embryos adapts and responds to arterial load by altering its mechanical properties and collagen content. An increase in arterial load via vitelline artery ligation was shown to significantly increase aortic collagen type I and III content [21]. The altered blood

pressure and flow also impact arterial compliance and impedance of the embryonic chick dorsal aorta [22]. Since biomechanical forces are critical for vascular formation and maintenance during embryogenesis, many investigators have explored biophysical cues for the regeneration of cardiovascular tissues *in vitro* [23].

Vascular Biology and Biomechanics

Biomechanics of Arterial Vessels

Blood vessels belong to a class of soft tissues known as bioviscoelastic solids that demonstrate both viscous and elastic behaviors [24]. At low pressure, elastic fibers and contractile smooth muscle cells (SMCs) alone are responsible for vascular mechanical strength and the linear elastic behavior of arteries [24–26]. At high pressures, especially those greater than physiological blood pressures, recruitment of aligned collagen fibers accounts for the observed non-linear stress-strain relationship and primarily contributes to ultimate tensile strength (UTS) and stiffness of native arteries [26, 27], elastic modulus, respectively. Native arteries thus are compliant and elastic at low pressures and strong at high pressures.

3D Collagen Architecture and Remodeling across Vascular Wall Strata

The collagen fiber structure in native blood vessels is three-dimensional. Three families of collagen fibers have been identified in native arterial vessels: circumferential, helical, and axial collagen fibers [28–30]. In native vessels, collagen architecture changes from the media to the adventitia layers [31]. In the tunica media, collagen fibers predominately are aligned towards the circumferential direction and in parallel to SMCs [31]. The media layer may also contain helically oriented collagen fibers that can strengthen vascular mechanics in both circumferential and axial directions [29, 32]. In contrast, the collagen fibers are aligned more axially in the adventitia layer [31]. As luminal pressure increases, the helical collagen fibers become more circumferentially oriented, thus playing a major role in circumferential mechanical properties of arteries at high stresses [32].

Native vessels remodel circumferentially and axially in order to reestablish homeostasis in response to mechanical cues [33]. Relatively few studies have examined the effect of axial stretching on the biology and remodeling of blood vessels, as compared to the effect of circumferential strain or shear stress [34–37]. Likewise, little is known about the impact of simultaneous biaxial stretching (circumferential and axial stretching) on 3D extracellular matrix (ECM) microstructure remodeling and mechanical properties of native or engineered vessels [34]. Therefore, biomimetic systems that simulate multiple physiological forces can be essential to enhance our understanding of the impact of biomechanical forces on vascular remodeling and mechanics.

Shear Stress on Endothelial Cells

Vascular endothelial cells (ECs) play an important role in maintaining homeostasis, metabolic activities, and proper functionality of the arterial system [38]. ECs are important in the regulation of thrombosis, vascular wound healing, chronic inflammation, and the pathogenesis of atherosclerosis [39]. Hemodynamic shear stress on ECs is essential in

mediating the phenotype, orientation, metabolic activities, and homeostasis of vascular endothelium [39, 40]. The arterial wall is covered with a confluent mono-layer of spindle-shaped ECs that are oriented in the direction of the blood flow [41]. Shear stress redistributes the centrally located stress fibers of polygonal ECs to stress fibers that are parallel to the direction of the flow in elongated ECs [42, 43]. Many studies have shown that shear stress is one of the most powerful stimuli for the release of vasodilator nitric oxide (NO) from ECs [44, 45]. NO is a key mediator for atheroprotective function of ECs through modulation of platelet aggregation [44, 45]. The hemodynamic shear stress on ECs also retains SMCs in a low synthetic and quiescent state, thus preventing neointimal formation and luminal narrowing [46]. Functional EC markers such as platelet endothelial cell adhesion molecule, VE-cadherin, and vascular endothelial growth factor receptor 2 are closely regulated and enhanced by hemodynamic shear stress on ECs [47, 48].

Cyclic Stretching on Smooth Muscle Cells

Mechanical stress on SMCs plays an important role in modulation of vascular injury, inflammatory responses, and pathogenesis [49]. Vascular SMCs maintain and regulate blood pressure, vascular tone, and blood flow distribution [50]. Cyclic stretching on the arterial wall modulates proliferation, differentiation, and ECM synthesis by vascular SMCs [51, 52].

Biomechanical signaling regulates the switching between the contractile and synthetic phenotypes of SMCs [50]. Cyclic strain enhances the contractile SMC phenotype and the expression of SMC contractile markers such as SM α -actin [53], calponin-1 [54], and smooth muscle myosin heavy chain (SMMHC) [55] at both the mRNA and protein levels. The synthesis rate of ECM proteins such as collagen, hyaluronan, and chondroitin 6-sulfate, is significantly increased by cyclic stretching on SMCs [56]. Cyclic strain also induces the expression of TGF β -1 signaling through SMAD pathways, which leads to elevation in the synthesis of collagen, elastin and other ECM proteins [57]. In particular, cyclic strain elevates type III collagen synthesis and the ratio of type III/I collagen at the mRNA and protein levels [58]. The half-lives of collagen and elastin in an arterial wall are 60–70 days [59] and 40 years [60–62], respectively, under normal physiological conditions. In adult vasculature, the ECM undergoes constant remodeling and turns over to renew the matrix and ensure its proper biochemical and biomechanical performance. Cyclic strain facilitates the turnover of ECM by increasing the expression of matrix metalloproteinases (MMP2 and MMP9) and tissue inhibitors of metalloproteinases-1, for example [63, 64].

Collagen Gel-based Engineered Vessels

Bell and Weinberg pioneered the *in vitro* regeneration of blood vessels by embedding vascular cells in collagen gels [65] (Fig. 1B). The bio-structure of these first biological vascular grafts resembled the concentric structure of native blood vessels. However, collagen gel-based tissue engineered vessels (TEVs) commonly suffer from low cell density and low levels of collagen cross-links, resulting in insufficient mechanical strength.

The weak mechanical properties of collagen-gel based TEVs could be attributed to several factors. First, collagen gel is usually formed from denatured protein, which has a disorganized structure and is different from the aligned fibrillar structure that provides the

mechanical strength of native collagen fibers (Fig. 1B). In addition, some studies have shown that collagen gel attenuates collagen synthesis by fibroblasts and SMCs, resulting in limited mechanical strength [66, 67]. Finally, the production of mature elastic fibers has not been reported in these collagen-based TEVs.

To improve the mechanical integrity of collagen-based TEVs, glutaraldehyde was used to cross-link the collagen fibrils. Glutaraldehyde is a common cross-linking reagent for collagen [68] and but cross-links other proteins as well [69]. However, the cytotoxicity of glutaraldehyde [70] led to the exploration of other collagen cross-linking reagents such as lysyl oxidase (LO) [71] and transglutaminase [72]. In addition, collagen cross-links have been achieved from a photo-crosslinking mechanism via reacting acrylated collagen with photoinitiator [73, 74]. Transglutaminase significantly increased the burst pressures of collagen-gel constructs from 46 ± 3 mmHg (noncrosslinked) to 71 ± 4 mmHg [72]. LO-enhanced collagen constructs showed increased elastic modulus and UTS from 40kPa to 75kPa and 25kPa to 45kPa, respectively [71]. Photochemically cross-linked collagen gels resulted in a 2-fold tensile strength increase from 200Pa to 400Pa [75]. Alternatively, cyclic stretching has been used to regenerate collagen-based TEVs [76]. UTS and elastic modulus of mechanically conditioned collagen-based TEVs were 58kPa and 142kPa, respectively, which corresponded to an increase of 240% and 108% compared to statically-grown controls. However, despite these improvements in the mechanical properties of collagen-gel based TEVs, the mechanical strength of collagen constructs is still inferior to that of native blood vessels (45MPa and 6.58Mpa for porcine artery elastic modulus and UTS, respectively) [77].

Engineered Vascular Grafts Based upon Fibrin Gels

Fibrin is a naturally occurring biodegradable matrix protein that has been used to engineer 3D constructs in a wide range of tissue engineering applications (Fig. 1C). The advantages of using fibrin for vascular engineering are its tunability, biocompatibility, and availability. The chemical and mechanical properties of fibrin, such as the polymerization rate and compliance, are tunable by varying the concentration of fibrinogen and thrombin mixture [78].

Compared to collagen-based TEVs, fibrin-based TEVs achieved significantly more collagen production [79] and promoted elastin synthesis [80] (Fig. 1B,C). The study showed that SMCs in fibrin produced 3.2–4.9 times the amount of collagen produced by SMCs in collagen gels [79]. After 6 weeks of culture, fibrin-based TEVs composed of rat SMCs attained an elastic modulus and UTS of 15.4MPa and 14kPa, respectively, which are comparable to those of native rat abdominal aorta (8.4MPa and 21kPa) [81]. In a large animal model, ovine SMC-derived fibrin TEVs were implanted into jugular veins in lambs (a low-pressure system) for 15 weeks [82]. These fibrin-based TEVs integrated well with the surrounding tissues and exhibited a significant amount of elastin deposition. The TEVs underwent matrix remodeling and increased in mechanical strength and vascular reactivity. Explanted TEVs demonstrated significant contraction in response to KCl with maximal forces of 6.7g/g, which were comparable to those of the adjacent downstream host vessel (10.8 g/g). The break tension of explanted TEVs also increased from 1,700g/g to 5,941g/g

post implantation and was 25% of the value for native lamb carotid artery. However, weak structural and mechanical integrity have limited fibrin-based TEVs to implantation at venous sites, which experience significantly lower physiological pressure than that found in the arterial environment. An alternative approach is required to engineer fibrin-based TEVs that can be implanted into and withstand arterial-pressure systems. This will be discussed in the next section.

Cell Sheets for Vascular Engineering

L'Heureux constructed the first implantable biological-based TEVs that were free of any synthetic biomaterials by rolling sheets of human vascular SMCs and dermal fibroblasts around a mandrel in concentric layers [83] (Fig. 1D). This research group also reported the first clinical use of cell sheet engineered TEVs for high pressure arterial implantation as arteriovenous grafts for dialysis access. In this initial clinical trial, autologous engineered arterial grafts were created for patients suffering from end-stage renal disease using the cell sheet engineering technique. The resulting TEVs displayed burst pressures of 3490 ± 892 mmHg and suture retention strength of 152 ± 50 gmf, comparable to those of native internal mammary artery (3196 ± 1264 mmHg and 138 ± 50 gmf, respectively) [84]. The autologous engineered vessels were implanted in patients as arteriovenous shunts for clinical time points beyond 21 months [84] and showed a patency of 60% 6 months after implantation [85]. TEV compliance prior to implantation was $3.4 \pm 1.6\%$ /100 mmHg. By 6 month after implantation, ultrasound examinations demonstrated an increase in TEV compliance to $8.8 \pm 4.2\%$ /100 mmHg, similar to the compliance of internal mammary artery ($11.5 \pm 3.9\%$ /100 mmHg). However, dilation and aneurysm formation at early time points in this clinical study were reported [85, 86]. Recently, L'Heureux and colleagues have explored the use of allogeneic TEVs (constructed from sheets of fibroblasts) as hemodialysis shunts in a clinical study [87]. This short-term study (1–11 months) in 3 patients showed no immune reactions, vascular wall degradation, or aneurysm. However, thrombogenic failures occurred in 2 patients at 3 months and 5 months, respectively. All allogeneic grafts developed stenoses by 11 months, some as early as by 1 month [87]. Cell sheet engineering is a promising means to reconstruct vascular grafts for the replacement of small and medium-caliber vessels. However, the high cost and long production time required for autologous TEVs (28 weeks) limit widespread clinical application of this technique.

Electrospinning of Scaffolds for Vascular Engineering

Electrospinning is a fabrication technique that results in deposition of well-aligned fibers, which closely imitates the microstructure of native ECM (Fig. 1E). Electrospinning of blended polymers or copolymers has gained recent popularity in vascular engineering. The mechanical and biological properties of electrospun vascular grafts can readily be tailored by altering the composition and combination of the blended polymers or copolymers [88]. The structural properties, such as polymer fiber size and scaffold thickness, can be controlled by adjusting electrospinning settings (voltage, speed, time, and concentration of the polymer solutions) [88, 89]. Highly porous biodegradable polymers with cell-sized pore diameters have excellent biocompatibility and integration in many *in vivo* studies [90]. However, to attain the mechanical strength of native blood vessels, the scaffolds created via

electrospinning are inevitably composed of dense layers that can lead to poor cell infiltration *in vivo* [91, 92] (Fig. 1E). The small pore size and low porosity of some electrospun scaffolds further impede cell migration and possibly matrix deposition, thus making it even more difficult for the scaffolds to be remodeled and integrated with the host environment. Although electrospun scaffolds have been shown to remain patent and display no sign of severe hyperplasia, small voids of the scaffolds can restrain cell migration and concomitant capillaries ingrowth within the graft walls [93].

Biodegradable Polymers Scaffolds

Recent studies have examined the potential of transforming biodegradable polymer TEVs into neovessels through complete degradation and remodeling of the scaffolds *in vivo*. In a clinical study, Shinoka and colleagues seeded autologous bone marrow cells onto degradable composite polymer scaffolds [94] (Fig. 1F). The composite polymer consisted of two parts i) a tube composed of a copolymer of L-lactide and ϵ -caprolactone (PCL/LA 50:50) with 80% porosity and pore diameters ranging from 2 to 100 μ m and ii) a polyglycolic acid (PGA) woven fabric. The polymer scaffolds were seeded with autologous bone marrow cells and implanted into 23 patients as extracardiac total cavopulmonary connection grafts on the same day. The ages of the patients ranged from 1 to 24 years and those patients received anticoagulation therapy for 3 to 6 months after the surgery. There was no evidence of aneurysm formation [95], calcification, thrombosis or obstruction of the TEVs over the course of up to 31.6 months of follow-up with cineangiography or computed tomography. All implanted vascular grafts remained patent and the diameter of the grafts increased with time to 110% of the implanted size. The autologous bone marrow cells used in this study were freshly obtained, isolated, and seeded onto the scaffolds on the day of surgery, making this approach attractive and practical for clinical applications. This work was originally executed in Japan, although confirmatory studies are now underway in the U.S.

Using a similar method, Breuer and colleagues showed that the inflammatory process can facilitate and orchestrate the integration and transformation of mononuclear cell-seeded scaffolds into neovessels *in vivo* [96]. hBMC (human bone marrow mononuclear cells) were seeded onto PGA-P(CL/LA) tubular scaffolds and implanted into the inferior vena cava of immuno-deficient mice. During the remodeling process, the polymer scaffolds were first repopulated with the host monocytes and then later, by host SMCs and ECs. Over a 6-month time course, the scaffolds were degraded and replaced by cellular and ECM components. All implanted TEVs remained patent and gradually integrated into the host blood vessels. Even though these studies established PGA-P(CL/LA) scaffolds as an efficient approach of vascular replacement for low-pressure systems, this approach maybe not be applicable for implantation in a high-pressure arterial system.

In a recent study, Wei and colleagues examined host remodeling of cell-free polymeric grafts through a fast degradation process of the scaffolds [97]. Fast-degrading polymeric scaffolds, a composite material of poly(glycerol sebacate) and PCL, were directly implanted as interposition grafts in rat abdominal aortas for 3 months. The grafts were integrated into and remodeled by the host environment as well as showed an overall patency of 80.9%. The

newly formed arteries were compliant ($11 \pm 2.2\%$ in 80–120mmHg range) and achieved a burst pressure of 2360 ± 673 mmHg. However, weak initial mechanical strength of the composite scaffolds (suture retention strength of < 50 g, elastic modulus = 536kPa) makes it challenging to translate this work to arterial-site implantation in large animal models.

Decellularized Vascular Scaffolds for Vascular Engineering

Allogeneic or xenogeneic tissues have been used as an alternative source to repair and restore blood vessels. Since the cellular components of allogeneic or xenogeneic tissues trigger immune responses, masking and eliminating all immunogenic constituents prior to transplantation is crucial to minimize inflammatory and immune responses.

Xenografts from large animals like cows [98] and pigs [99] are clinically viable only after they have been treated and cross-linked with glutaraldehyde to reduce immunogenicity. However, at the expense of attenuated immunological response, GA treatment has been shown to compromise the mechanical properties of tissues and to induce cytotoxicity and calcification [68].

Small intestinal submucosa (SIS) is a cell-free collagen matrix derived from small intestine [100]. The feasibility of using SIS as an acellular small-diameter arterial graft has been investigated. SIS was implanted as small-diameter arterial grafts (4.3 mm) into both the carotid and femoral arteries of 18 dogs for 8 weeks [101]. The SIS arterial grafts developed aneurysmal dilation in 4 of 36 SIS grafts (11%). Overall, graft patency was 75% and patent grafts had no evidence of infection, propagating thrombus, or intimal hyperplasia. However, the grafts tended to develop a dense organized collagenous connective tissue with little or minimum trace of endothelial cell lining on the luminal surface. This implicated inefficient cellular infiltration and poor remodeling of SIS vascular grafts within the host environment. The inability of SIS grafts to form functionalized and activated EC linings suggests that SIS is not ideal for arterial replacement.

In recent years, decellularization has emerged as a new approach for functional vessel replacement (Fig. 1G,H). Commercially available decellularized xenografts, such as the Artegraft Bovine Carotid Arteries[™], demonstrated significantly higher patency rates (60.5% with 26 patients) than polytetrafluoroethylene grafts (10.1% with 27 patients) at 1 year post implantation [102]. To enhance integration and remodeling of decellularized scaffolds, cells are often seeded onto the decellularized scaffolds prior to implantation [103]. Cho and colleagues implanted decellularized canine carotids arteries that were reseeded with autologous BMCs into canine recipients [104]. The seeded grafts remained patent for up to 8 weeks, demonstrated well-preserved collagen and elastin matrix, and maintained intimal, medial, and adventitial layers.

To minimize potential immunogenicity triggered by any remaining antigenic components in decellularized xenografts, decellularized human vessels are considered to be a more appropriate choice for clinical applications. Off-the-shelf (decellularized) native human vascular grafts (CryoVein[®] and CryoArtery[®]) are commercially available from CryoLife[®], ranging from saphenous veins to descending thoracic aorta. CryoLife[®] uses SynerGraft[®] Technologies, a patented decellularization technology, to create acellular grafts and have

reported in over 22 published clinical studies. These cryopreserved allografts are resistant to reinfection, thrombosis, or aneurysmal dilatation in midterm follow-up (20 months post implantation) of a clinical study [105].

We have shown that human umbilical arteries that were decellularized remained mechanically intact and patent up to 8 weeks when implanted into nude rats as abdominal aorta interposition grafts [106]. In a canine model, Schaner and colleagues implanted decellularized human saphenous veins as carotid interposition grafts. The grafts integrated well into the host environment without anastomotic complications or rupture [107].

Decellularized human vessels are a promising scaffold material for vascular engineering and regenerative medicine. However, the limited availability and accessibility of human vessels that are free of diseases could be a hurdle to widespread clinical implementation. This limitation and inadequate availability of suitable dimensions of blood vessels could restrict the clinical application of decellularized human TEVs. The advantages and disadvantages of above TEV fabrication methods are summarized in Table 1.

Application of Mechanical Conditions and Biomimetic Systems

Biomimetic systems imitating the chemo-mechanical environment of native blood vessels have been used to study and understand *in vivo* biochemical and mechanical signaling in tissue development and remodeling processes. Flow chambers and bioreactors are two principal biomimetic systems implemented in vascular engineering applications. Major attributes that establish biomimetic systems as useful tools to study functional TEV development and regeneration at the molecular, cellular, and tissue levels are:

- I. The capability to investigate how different mechanical and biochemical forces impact the alignment, organization, and architecture of the cells and ECM
- II. The ability to provide insight into the interplay between the cells and ECM during growth and remodeling in response to mechanical and biochemical stimuli
- III. The capacity to study the impact of cellular and ECM alignment, organization, and interaction on mechanical properties of engineered constructs
- IV. The feasibility to optimize the *in vitro* biochemical and mechanical environment to regenerate engineered tissues with properties that closely resemble those of native tissues

Effect of Shear Stress on Endothelial Cells in Parallel Plate System

Shear stress stimulates the production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) in ECs. NO maintains the quiescent and non-proliferative phenotype of vascular SMCs and thus suppresses narrowing of the arterial lumen, intimal hyperplasia, and thrombosis [108, 109]. In a flow chamber study, Chien and co-workers demonstrated that shear stress with disturbed and oscillatory flow causes prolonged molecular signaling of pro-inflammatory pathways [110]. The release of atherogenic factors such as vascular cell adhesion protein 1, monocyte chemotactic protein-1, endothelin-1, connective tissue growth factor, intercellular adhesion molecule 1, and E-/P-selectin induces vascular constriction

and increases recruitment, adhesion, and permeability of circulating inflammatory cells [111]. On the other hand, laminar shear stress enhances the expression of anti-inflammatory, antithrombotic, anticoagulant molecules such as [111] eNOS, and prostacyclin [112].

To delay and prevent platelet adhesion and blood clotting processes, the lumen of synthetic grafts is often seeded with a confluent mono-layer of ECs. EC seeding leads to attenuated thrombogenicity and improves patency [113–115]. Pasic and co-workers implanted EC-seeded 4mm Dacron grafts end-to-end at the carotid position in a canine model for 6 to 12 months [116]. All seeded Dacron grafts were patent. The thrombus-free area on the luminal surfaces of explants at both 6 months and 12 months was 99.6%. However, rapid detachment and loss of ECs from the surfaces of seeded grafts upon exposure to acute arterial shear stresses pose as a major problem [117, 118]. Shear stress pretreatment has been shown to strengthen functional EC adherence to prosthetic grafts upon exposure to acute *in vivo* shear stress [119], which, in turn, reduces intimal hyperplasia and thrombogenicity. To perform shear stress preconditioning, specially-designed flow chambers have been used to i) coat the lumens of prosthetic grafts with a confluent layer of ECs and ii) functionalize the ECs by applying hemodynamic shear stress to strengthen EC alignment in the direction of flow [120], enhance differentiation, and upregulate anti-thrombotic factor expression [121]. Density of Weibel-Palade bodies was nearly 40-fold higher in aortic endothelial cells exposed to arterial shear stress than in the cells not exposed to the stress [121, 122]. Hence, preconditioning EC-seeded TEVs in a flow chamber with arterial shear stress is a promising method and important procedure to functionalize the EC mono-layer by enhancing EC differentiation.

Bioreactor and Mechanical Conditioning on Engineered Tissues

In addition to shear stress, blood vessels constantly experience cyclic circumferential distensions and axial stretch. Mooney and colleagues [123] investigated the effect of cyclic strain on SMC-seeded 3D constructs. Rats aortic SMCs were seeded onto collagen sponges and subjected to 7% cyclic strain (1Hz) for 20 weeks inside a tissue culture chamber. Mechanically conditioned constructs achieved a 12- and 34-fold increase in UTS and elastic modulus, respectively, as compared to the non-strained constructs. Type I collagen and tropoelastin mRNA levels were significantly upregulated in the cyclically strained constructs as comparing to the non-strained group. This study demonstrated that long-term cyclic strain strongly regulated the ECM production of SMCs and exerted an impact on engineered tissue mechanics.

Advancing this technique to reconstruct 3D tubular-shaped tissues, our group developed a bioreactor that applies cyclic pulsatile strain [124] to generate implantable biological vascular grafts (Fig. 2A). The bioreactor is connected to a closed flow system where a peristaltic pump administers cyclic pulsatile strain to cell-seeded PGA scaffolds. The luminal circumferential extension exerted on PGA scaffolds closely simulates the cyclic circumferential stretching imposed on native vessels by the heart.

The mechanical environment of the pulsatile bioreactor enabled us to generate engineered vessels with significantly higher collagen content and superior mechanical strength as compared to the static controls. Engineered vessels generated under pulsatile culture

conditions at 5% strain and frequency of 165 beats/minute expressed moderate amounts of SMC contractile markers such as smooth muscle myosin heavy chain in the outer layers of TEVs, implying some biological contractile characteristics. Vessels also showed contractility in the presence of serotonin, endothelin-1, and prostaglandin-F2 α [124]. The absolute magnitudes of contraction in response to prostaglandin-F2 α were about 5% of rabbit abdominal aorta and 15% of excised vein grafts [125]. A substantial amount of collagen deposition was found in 8-week pulsed TEVs ($50 \pm 5\%$ of dry weight), similar to native bovine arteries ($45 \pm 9\%$). In contrast, non-pulsed vessels showed significantly lower amounts of collagen ($35 \pm 3\%$). High collagen deposition in pulsed vessels was also accompanied by higher suture retention strength ($91 \pm 26\text{g}$) than non-pulsed counterparts ($22 \pm 8\text{g}$). The burst pressures of 8-week pulsed vessels ($2150 \pm 709\text{ mmHg}$ [126]) were greater than the value reported of native saphenous vein ($1680 \pm 307\text{ mmHg}$) [127]. EC-coated and preconditioned grafts were successfully implanted into saphenous arteries of miniature pigs and remained patent for up to 4 weeks without evidence of stenosis or dilation.

Bioreactors Designed to Study the Effect of Axial Loading on Collagen Remodeling

The importance of circumferential strain on the collagen matrix and mechanical properties of TEVs has been well characterized. However, the effect of axial stretch on collagen matrix development and deposition has not been investigated in detail. Although the effect of axial stretch on the mechanical and biological properties of native vessels has been explored [34, 35, 37], researchers have only recently started to examine the effect of axial loading on mechanical behaviors and ECM remodeling of engineered blood vessels.

A perfusion bioreactor with the capacity for axial strain was developed by Mironov and colleagues in 2003 [128]. This was one of the earliest attempts to study and apply axial strain to arterial constructs. The perfusion bioreactor could apply axial stretches ranging from 0% to 200% to silicone tubing and to native bovine carotid arteries, in addition to applying circumferential strains. The bioreactor supported recording of the diameter and pressure during both static and dynamic regimens by incorporating a digital TV camera and pressure transducers to a computer-controlled system. This system was a valuable prototype for biaxial stretching bioreactors but did not demonstrate the ability to regenerate or support long-term growth of biological TEVs.

To study the effect of circumferential and axial stretching on biological-based engineered vessels, Zaucha and co-workers designed a computer-controlled bioreactor and biomechanical testing system [129]. The bioreactor system (i) enabled simultaneous and independent control of circumferential pressure and axial load on TEVs; and (ii) performed biaxial mechanical testing on the same vessel at multiple time-points in culture. In addition to mechanical stimulation and testing, the apparatus was designed to achieve intermittent non-invasive imaging of the ECM synthesis and organization within the bioreactor in a real-time setting. A multi-photon microscope was incorporated into the testing device to perform second harmonic generation imaging on the collagen matrix of live vessels under multiple loading conditions. However, the effect of collagen matrix orientation, alignment, and organization on the mechanical properties of TEVs was not discussed in this study.

Although this system was built to study mechanically induced collagen growth and remodeling of TEVs, the feasibility of supporting a long-term tissue growth or mechanical conditioning was not demonstrated.

To rectify the lack of mechanical strength in fibrin-based engineered vessels, Tranquillo's group incorporated mechanical conditioning into fibrin-based TEV culture. The addition of mechanical conditioning improved the mechanical properties of fibrin-based TEVs by enhancing collagen production [130]. This group achieved implantable and robust arterial grafts by culturing neonatal human dermal fibroblasts-seeded fibrin TEVs in a bioreactor that was capable of applying cyclic distension, transmural flow, and axial shortening to engineered vessels (Fig. 2B). The fibrin TEVs exhibited burst pressures in the range of 1400–1600mmHg and compliance comparable to native femoral arteries after 7–9 weeks in the bioreactor [131]. Our recent study shows that mechanical conditioning enables fibrin TEVs to develop mature elastic fibers that are consisted of elastin core (cross-linked tropoelastin) as well as microfibril template [132].

In the same study, the impact of axial retraction on the mechanical properties of TEVs was examined. TEVs with unfixed-ends exhibited 60% axial shortening during culture (Fig. 2B). 60% axial retraction significantly improved the mechanical properties of TEVs. In comparison to the fixed-length TEVs (circumferential elastic modulus $\approx 400\text{kPa}$, circumferential UTS = $350 \pm 106\text{ kPa}$), axially retracted TEVs showed a remarkable increase in circumferential elastic modulus and UTS (elastic modulus $\approx 2600\text{kPa}$, UTS = $1761 \pm 206\text{kPa}$). The group attributes the observed improvement in mechanical properties to two causes: i) increase in collagen concentration due to tissue volume reduction and ii) circumferential collagen fiber alignment. Axially retracted TEVs were reported to exhibit a circumferential collagen alignment pattern, while the fixed-length TEVs showed axial alignment. However, the mechanism by which the axial retraction realigns collagen fibers toward the circumferential direction remains unaddressed. Due to limited spatial resolution of the imaging performed [133], salient collagen alignment at the individual fiber level could not be confirmed in this study. Ultra-structural analysis of the individual collagen fiber organization would be useful to understand the effect of axial shortening on collagen realignment and the improved mechanical properties of fibrin-based TEVs.

Bose® sells ElectroForce® BioDynamic® Test Instruments that can simulate cyclic torsion, axial loads, and pulsatile perfusion on vascular constructs to characterize the mechanical properties. However, these expensive devices are optimized and customized primarily to run mechanical tests on vascular constructs, not specifically designed for the production of engineered arterial grafts. Table 2 summarizes the advantages and disadvantages of different vascular bioreactors that apply circumferential and axial loading to vascular constructs.

Assessment of Collagen Remodeling and Growth of TEVs

Our group examined the effect of mechanical conditions on collagen fibril ultra-structure as well as the impact of collagen fiber ultra-structure in the mechanical properties of engineered vessels [28]. Transmission electron microscopy (TEM) was used to analyze 3D collagen fiber distribution, orientation, and ultra-structure in both native and engineered vessels. Collagen banding periodicity was comparable between engineered vessels and

native vessels at $59.2 \pm 0.7\text{nm}$ and $59.3 \pm 2.1\text{nm}$, respectively. However, both collagen fibril and fiber diameter were significantly greater in native vessels than in engineered vessels ($80 \pm 11\text{nm}$ vs. $43 \pm 1\text{nm}$ and $5.1 \pm 1.6\mu\text{m}$ vs. $1.8 \pm 0.3\mu\text{m}$). All three families of collagen fibers (axial, helical, and circumferential) were present in both engineered and native arteries. In both engineered and native vessels, slightly less than half of the collagen fibrils were in a helical orientation. However, engineered vessels had significantly fewer circumferentially aligned collagen fibrils and more axially aligned collagen fibrils than the native vessels.

Collagen fiber alignment has been shown to increase with the magnitude of applied strain and to reorient toward to the direction of applied stretch [134]. As luminal pressure increases, helical collagen fibers become more circumferentially oriented and contribute to circumferential mechanical properties of blood vessels [135]. In comparison to the 1.5% strain that was applied to culture the engineered vessels, native vessels can experience up to 35% cyclic circumferential strain within a 70–120mmHg physiological pressure range [136]. Thus, it was hardly surprising that both burst pressure and elastic modulus were significantly higher in native vessels ($443 \pm 55\text{kPa}$, $45.1 \pm 16.8\text{MPa}$, respectively) than in engineered vessels ($107 \pm 14\text{kPa}$, $11.8 \pm 2.7\text{MPa}$) [77].

In a previous study, we have shown that engineered vessels that were cultured with cyclic circumferential strain exhibited higher collagen content and burst pressure than their static counterparts [124]. Ultra-structural analysis of collagen fibers strongly suggested that cyclic circumferential strain promoted the circumferential alignment of collagen, thus accounting for the improved circumferential mechanical strengths in pulsed engineered vessels. Therefore, applying larger circumferential strains during culture may increase circumferentially aligned collagen fibers and thus enhance the mechanical strength of engineered vessels.

In a more recent study, we have developed a novel pulsatile bioreactor system that supports non-invasive imaging of living engineered arteries via nonlinear optical microscopy (NLOM) [137]. High-resolution NLOM offers complementary information to traditional 2D histological and biochemical assessments of growing engineered tissues. NLOM has the capacity to image biological molecules, particularly collagen and elastin, through their specific nonlinear optical signals without the use of exogenous labels [138–141]. Collagen fibers and SMCs can be detected by second harmonic generation and two-photon fluorescence acquisitions, respectively, through an optical window positioned just above the engineered tissues. Combining NLOM with bioreactor systems offers spatial and temporal assessments of collagen matrix growth and remodeling in a real-time setting [129, 142].

NLOM imaging showed that at 2 weeks of culture, collagen matrix was sparse in engineered vessels [137]. By 4 weeks, more collagen had been deposited and aligned along the remaining PGA matrix, implying some effect of PGA fibers on collagen deposition during the early stages of tissue culture. However, at week 6, the influence of PGA on collagen orientation had waned due to PGA bulk degradation, which resulted in an increase cellular response to mechanical stimuli applied by the bioreactor. Consequently, collagen density and dispersion of orientation angles had increased and continued to the 8th week.

Quantification of collagen fiber orientation through the wall of 8-week vessels revealed that three predominant collagen fiber families were centered approximately at 0° , $\pm 45^\circ$, and 90° within the image plane (indicating, circumferentially, helically, and axially aligned collagen fibers, respectively). These orientations are consistent with our prior observations of collagen fibers in engineered vessels obtained by transmission electron microscopy [28]. The mass fractions for the three primary families of collagen fibers were similar at 8 weeks (33% axial, 37% circumferential, 30% for the axially symmetric helical families). Growth and remodeling mathematical models of engineered vessels cultured on PGA scaffolds also were generated to predict growing tissue morphology and mechanics after long periods of culture. If the basal collagen production rates of TEVs were chosen to yield the empirical wall thickness of the engineered vessels at 8 weeks [77], the model predicted that the steady state turnover of collagen fibers and hence the mechanics would not be achieved within 8 weeks. This non-steady state of turnover is indicated by much higher rates of collagen production and cell division in engineered vessels than in mature native arteries [124, 126].

Conclusions

The biomechanical environment has an immediate impact on the microstructure and organization of ECM in developing engineered vessels. Application of cyclic circumferential stretching to TEVs has resulted in a significant improvement in collagen fiber alignment and organization. The observed increase in mechanical strength of the engineered vessels in the circumferential direction might be attributed to the re-alignment of collagen fibers toward the circumferential orientation. However, the impact of long-term axial stretching on collagen fiber organization and mechanical properties of TEVs is not well understood. Hence, to generate strong and compliant TEVs that exhibit biological and mechanical properties similar to those of native vessels, it is essential to understand the impact of biaxial stretching (circumferential and axial stretching) on collagen matrix as well as on the mechanical properties of TEVs. Further studies are required to improve our understanding of ECM growth, remodeling, and adaptation processes in engineered vessels in the presence of biochemical or mechanical perturbation. Bioreactors are promising instruments to study the effects of different mechanical inputs on ECM remodeling and the mechanical properties of developing TEVs under a controlled environment. Finally, bioreactors allow us to achieve optimized chemo-biomechanical culture conditions to regenerate TEVs that behave like and can replace native vessels.

References

1. Dahl SLM, et al. Decellularized native and engineered arterial scaffolds for transplantation. *Cell Transplantation*. 2003; 12(6):659–666. [PubMed: 14579934]
2. Opitz F, et al. Tissue engineering of ovine aortic blood vessel substitutes using applied shear stress and enzymatically derived vascular smooth muscle cells. *Annals of Biomedical Engineering*. 2004; 32(2):212–222. [PubMed: 15008369]
3. Conte MS. The ideal small arterial substitute: a search for the Holy Grail? *Faseb Journal*. 1998; 12(1):43–45. [PubMed: 9438409]
4. Kannan RY, et al. Current status of prosthetic bypass grafts: A review. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*. 2005; 74B(1):570–581.
5. Pasic M, et al. Seeding with omental cells prevents late neointimal hyperplasia in small-diameter Dacron grafts. *Circulation*. 1995; 92(9):2605–16. [PubMed: 7586363]

6. Graham LM, et al. Effects of thromboxane synthetase inhibition on patency and anastomotic hyperplasia of vascular grafts. *J Surg Res.* 1989; 46(6):611–5. [PubMed: 2659898]
7. Lucitti JL, et al. Vascular remodeling of the mouse yolk sac requires hemodynamic force. *Development.* 2007; 134(18):3317–3326. [PubMed: 17720695]
8. Adamo L, et al. Biomechanical forces promote embryonic haematopoiesis. *Nature.* 2009; 459(7250):1131–U120. [PubMed: 19440194]
9. Jones EAV. Mechanical factors in the development of the vascular bed. *Respiratory Physiology & Neurobiology.* 2011; 178(1):59–65. [PubMed: 21458600]
10. Culver JC, Dickinson ME. The Effects of Hemodynamic Force on Embryonic Development. *Microcirculation.* 2010; 17(3):164–178. [PubMed: 20374481]
11. Obi S, et al. Fluid shear stress induces arterial differentiation of endothelial progenitor cells. *Journal of Applied Physiology.* 2009; 106(1):203–211. [PubMed: 18988767]
12. Chapman WB. The effect of the heart-beat upon the development of the vascular system in the chick. *American Journal of Anatomy.* 1918; 23(1):175–203.
13. Wakimoto K, et al. Targeted disruption of Na⁺/Ca²⁺ exchanger gene leads to cardiomyocyte apoptosis and defects in heartbeat. *Journal of Biological Chemistry.* 2000; 275(47):36991–36998. [PubMed: 10967099]
14. Hierck BP, et al. Fluid shear stress and inner curvature remodeling of the embryonic heart. Choosing the right lane! *The Scientific World Journal.* 2008; 8:212–22.
15. Huang CQ, et al. Embryonic atrial function is essential for mouse embryogenesis, cardiac morphogenesis and angiogenesis. *Development.* 2003; 130(24):6111–6119. [PubMed: 14573518]
16. Clark ER. Studies on the growth of blood-vessels in the tail of the frog larva - By observation and experiment on the living animal. *American Journal of Anatomy.* 1918; 23(1):37–88.
17. Wagenseil JE, Mecham RP. Vascular Extracellular Matrix and Arterial Mechanics. *Physiological Reviews.* 2009; 89(3):957–989. [PubMed: 19584318]
18. Gerrity RG, Cliff WJ. The aortic tunica media of the developing rat. I. Quantitative stereologic and biochemical analysis. *Lab Invest.* 1975; 32(5):585–600. [PubMed: 1127878]
19. Olivetti G, et al. Morphometric study of early postnatal development of the thoracic aorta in the rat. *Circ Res.* 1980; 47(3):417–24. [PubMed: 7408124]
20. Berry CL, Looker T, Germain J. The growth and development of the rat aorta. I. Morphological aspects. *J Anat.* 1972; 113(Pt 1):1–16. [PubMed: 4648481]
21. Lucitti JL, et al. Increased arterial load alters aortic structural and functional properties during embryogenesis. *American Journal of Physiology-Heart and Circulatory Physiology.* 2006; 291(4):H1919–H1926. [PubMed: 16648183]
22. Lucitti JL, Tobita K, Keller BB. Arterial hemodynamics and mechanical properties after circulatory intervention in the chick embryo. *Journal of Experimental Biology.* 2005; 208(10):1877–1885. [PubMed: 15879068]
23. Freed LE, et al. Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. *Tissue Eng.* 2006; 12(12):3285–305. [PubMed: 17518670]
24. Valdez-Jasso D, et al. Linear and Nonlinear Viscoelastic Modeling of Aorta and Carotid Pressure-Area Dynamics Under In Vivo and Ex Vivo Conditions. *Annals of Biomedical Engineering.* 2011; 39(5):1438–1456. [PubMed: 21203846]
25. Cheng J, Wagenseil J. Extracellular matrix and the mechanics of large artery development. *Biomechanics and Modeling in Mechanobiology.* :1–18.
26. Silver FH, Horvath I, Foran DJ. Viscoelasticity of the vessel wall: The role of collagen and elastic fibers. *Critical Reviews in Biomedical Engineering.* 2001; 29(3):279–301. [PubMed: 11730097]
27. Barra JG, et al. Assessment of smooth muscle contribution to descending thoracic aortic elastic mechanics in conscious dogs. *Circ Res.* 1993; 73(6):1040–50. [PubMed: 8222076]
28. Dahl SLM, Vaughn ME, Niklason LE. An ultrastructural analysis of collagen in tissue engineered arteries. *Annals of Biomedical Engineering.* 2007; 35:1749–1755. [PubMed: 17566861]
29. Canham PB, Finlay HM, Boughner DR. Contrasting structure of the saphenous vein and internal mammary artery used as coronary bypass vessels. *Cardiovascular Research.* 1997; 34(3):557–567. [PubMed: 9231039]

30. Canham PB, et al. Medial collagen organization in human arteries of the heart and brain by polarized light microscopy. *Connect Tissue Res.* 1991; 26(1–2):121–34. [PubMed: 1711950]
31. Bou-Gharios G, et al. Extra-cellular matrix in vascular networks. *Cell Proliferation.* 2004; 37(3): 207–220. [PubMed: 15144498]
32. Shadwick RE. Mechanical design in arteries. *Journal of Experimental Biology.* 1999; 202(23): 3305–3313. [PubMed: 10562513]
33. Humphrey JD. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. *Cell Biochemistry and Biophysics.* 2008; 50(2):53–78. [PubMed: 18209957]
34. Gleason RL, Wilson E, Humphrey JD. Biaxial biomechanical adaptations of mouse carotid arteries cultured at altered axial extension. *Journal of Biomechanics.* 2007; 40(4):766–776. [PubMed: 16750537]
35. Jackson ZS, Gotlieb AI, Langille BL. Wall tissue remodeling regulates longitudinal tension in arteries. *Circulation Research.* 2002; 90(8):918–925. [PubMed: 11988494]
36. Clerin V, et al. Tissue engineering of arteries by directed remodeling of intact arterial segments. *Tissue Engineering.* 2003; 9(3):461–472. [PubMed: 12857414]
37. Lawrence AR, Gooch KJ. Transmural pressure and axial loading interactively regulate arterial remodeling ex vivo. *Am J Physiol Heart Circ Physiol.* 2009; 297(1):22.
38. Cines DB, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood.* 1998; 91(10):3527–3561. [PubMed: 9572988]
39. Davies PF. Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nature Clinical Practice Cardiovascular Medicine.* 2009; 6(1):16–26.
40. Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *American Journal of Physiology-Heart and Circulatory Physiology.* 2007; 292(3):H1209–H1224. [PubMed: 17098825]
41. Yamamoto K, et al. Proliferation, differentiation, and tube formation by endothelial progenitor cells in response to shear stress. *Journal of Applied Physiology.* 2003; 95(5):2081–2088. [PubMed: 12857765]
42. Franke RP, et al. Induction of human vascular endothelial stress fibres by fluid shear stress. *Nature.* 1984; 307(5952):648–9. [PubMed: 6537993]
43. Wechezak AR, Viggers RF, Sauvage LR. Fibronectin and F-actin redistribution in cultured endothelial cells exposed to shear stress. *Lab Invest.* 1985; 53(6):639–47. [PubMed: 4068668]
44. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol.* 1998; 18(5):677–85. [PubMed: 9598824]
45. Vanhoutte PM. Endothelium and control of vascular function. State of the Art lecture. *Hypertension.* 1989; 13(6 Pt 2):658–67. [PubMed: 2661425]
46. Paszkowiak JJ, Dardik A. Arterial wall shear stress: observations from the bench to the bedside. *Vascular and endovascular surgery.* 2003; 37(1):47–57. [PubMed: 12577139]
47. Balligand JL, Feron O, Dessy C. eNOS Activation by Physical Forces: From Short-Term Regulation of Contraction to Chronic Remodeling of Cardiovascular Tissues. *Physiological Reviews.* 2009; 89(2):481–534. [PubMed: 19342613]
48. Tzima E, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Faseb Journal.* 2006; 20(5):A1378–A1378.
49. Shaw A, Xu Q. Biomechanical stress-induced signaling in smooth muscle cells: an update. *Curr Vasc Pharmacol.* 2003; 1(1):41–58. [PubMed: 15320852]
50. Owens GK. Regulation of differentiation of vascular smooth muscle cells. *Physiol Rev.* 1995; 75(3):487–517. [PubMed: 7624392]
51. Stegmann JP, Hong H, Nerem RM. Mechanical, biochemical, and extracellular matrix effects on vascular smooth muscle cell phenotype. *Journal of Applied Physiology.* 2005; 98(6):2321–2327. [PubMed: 15894540]
52. Gupta V, Grande-Allen KJ. Effects of static and cyclic loading in regulating extracellular matrix synthesis by cardiovascular cells. *Cardiovascular Research.* 2006; 72(3):375–383. [PubMed: 17010955]

53. Tock J, et al. Induction of SM- α -actin expression by mechanical strain in adult vascular smooth muscle cells is mediated through activation of JNK and p38 MAP kinase. *Biochemical and Biophysical Research Communications*. 2003; 301(4):1116–1121. [PubMed: 12589828]
54. Li X, et al. Uniaxial Mechanical Strain Modulates the Differentiation of Neural Crest Stem Cells into Smooth Muscle Lineage on Micropatterned Surfaces. *Plos One*. 2011; 6(10)
55. Reusch P, et al. Mechanical strain increases smooth muscle and decreases nonmuscle myosin expression in rat vascular smooth muscle cells. *Circulation Research*. 1996; 79(5):1046–1053. [PubMed: 8888698]
56. Leung DY, Glagov S, Mathews MB. Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. *Science*. 1976; 191(4226):475–7. [PubMed: 128820]
57. Beamish JA, et al. Molecular Regulation of Contractile Smooth Muscle Cell Phenotype: Implications for Vascular Tissue Engineering. *Tissue Engineering Part B-Reviews*. 2010; 16(5): 467–491. [PubMed: 20334504]
58. Carver W, et al. Collagen expression in mechanically stimulated cardiac fibroblasts. *Circ Res*. 1991; 69(1):116–22. [PubMed: 2054929]
59. Sheidaei A, et al. Simulation of abdominal aortic aneurysm growth with updating hemodynamic loads using a realistic geometry. *Medical Engineering & Physics*. 2011; 33(1):80–88. [PubMed: 20961796]
60. Rucker RB, Tinker D. Structure and metabolism of arterial elastin. *Int Rev Exp Pathol*. 1977; 17:1–47. [PubMed: 849882]
61. Lefevre M, Rucker RB. Aorta elastin turnover in normal and hypercholesterolemic Japanese quail. *Biochim Biophys Acta*. 1980; 630(4):519–29. [PubMed: 6772235]
62. Shapiro SD. Matrix metalloproteinase degradation of extracellular matrix: biological consequences. *Current Opinion in Cell Biology*. 1998; 10(5):602–608. [PubMed: 9818170]
63. Solan A, et al. Effect of pulse rate on collagen deposition in the tissue-engineered blood vessel. *Tissue Engineering*. 2003; 9(4):579–586. [PubMed: 13678437]
64. Asanuma K, et al. Uniaxial strain upregulates matrix-degrading enzymes produced by human vascular smooth muscle cells. *American Journal of Physiology-Heart and Circulatory Physiology*. 2003; 284(5):H1778–H1784. [PubMed: 12543633]
65. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. *Science*. 1986; 231(4736):397–400. [PubMed: 2934816]
66. Clark RA, et al. Collagen matrices attenuate the collagen-synthetic response of cultured fibroblasts to TGF- β . *J Cell Sci*. 1995; 108(Pt 3):1251–61. [PubMed: 7622608]
67. Thie M, et al. Aortic smooth muscle cells in collagen lattice culture: effects on ultrastructure, proliferation and collagen synthesis. *Eur J Cell Biol*. 1991; 55(2):295–304. [PubMed: 1935993]
68. Schmidt CE, Baier JM. Acellular vascular tissues: natural biomaterials for tissue repair and tissue engineering. *Biomaterials*. 2000; 21(22):2215–2231. [PubMed: 11026628]
69. Payne JW. Polymerization of proteins with glutaraldehyde. Soluble molecular-weight markers. *Biochem J*. 1973; 135(4):867–73. [PubMed: 4204954]
70. Charulatha V, Rajaram A. Influence of different crosslinking treatments on the physical properties of collagen membranes. *Biomaterials*. 2003; 24(5):759–767. [PubMed: 12485794]
71. Elbjearami WM, et al. Enhancing mechanical properties of tissue-engineered constructs via lysyl oxidase crosslinking activity. *Journal of Biomedical Materials Research Part A*. 2003; 66A(3): 513–521. [PubMed: 12918034]
72. Orban JM, et al. Crosslinking of collagen gels by transglutaminase. *Journal of Biomedical Materials Research Part A*. 2004; 68A(4):756–762. [PubMed: 14986330]
73. Brinkman WT, et al. Photo-cross-linking of type I collagen gels in the presence of smooth muscle cells: Mechanical properties, cell viability, and function. *Biomacromolecules*. 2003; 4(4):890–895. [PubMed: 12857069]
74. Ibusuki S, et al. Photochemically cross-linked collagen gels as three-dimensional scaffolds for tissue engineering. *Tissue Engineering*. 2007; 13(8):1995–2001. [PubMed: 17518705]

75. Mi SL, et al. Photochemical cross-linking of plastically compressed collagen gel produces an optimal scaffold for corneal tissue engineering. *Journal of Biomedical Materials Research Part A*. 2011; 99A(1):1–8. [PubMed: 21732526]
76. Seliktar D, et al. Dynamic mechanical conditioning of collagen-gel blood vessel constructs induces remodeling in vitro. *Annals of Biomedical Engineering*. 2000; 28(4):351–362. [PubMed: 10870892]
77. Dahl SLM, et al. Mechanical properties and compositions of tissue engineered and native arteries. *Annals of Biomedical Engineering*. 2007; 35(3):348–355. [PubMed: 17206488]
78. Shaikh FM, et al. Fibrin: A natural biodegradable scaffold in vascular tissue engineering. *Cells Tissues Organs*. 2008; 188(4):333–346. [PubMed: 18552484]
79. Grassl ED, Oegema TR, Tranquillo RT. Fibrin as an alternative biopolymer to type-I collagen for the fabrication of a media equivalent. *Journal of Biomedical Materials Research*. 2002; 60(4):607–612. [PubMed: 11948519]
80. Long JL, Tranquillo RT. Elastic fiber production in cardiovascular tissue-equivalents. *Matrix Biol*. 2003; 22(4):339–50. [PubMed: 12935818]
81. Grassl ED, Oegema TR, Tranquillo RT. A fibrin-based arterial media equivalent. *Journal of Biomedical Materials Research Part A*. 2003; 66A(3):550–561. [PubMed: 12918038]
82. Swartz DD, Russell JA, Andreadis ST. Engineering of fibrin-based functional and implantable small-diameter blood vessels. *American Journal of Physiology-Heart and Circulatory Physiology*. 2005; 288(3):H1451–H1460. [PubMed: 15486037]
83. L'Heureux N, et al. In vitro construction of a human blood vessel from cultured vascular cells: a morphologic study. *J Vasc Surg*. 1993; 17(3):499–509. [PubMed: 8445745]
84. Konig G, et al. Mechanical properties of completely autologous human tissue engineered blood vessels compared to human saphenous vein and mammary artery. *Biomaterials*. 2009; 30(8):1542–1550. [PubMed: 19111338]
85. McAllister TN, et al. Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study. *Lancet*. 2009; 373(9673):1440–1446. [PubMed: 19394535]
86. Garrido SA, et al. Haemodialysis access via tissue-engineered vascular graft Authors' reply. *Lancet*. 2009; 374(9685):201–201. [PubMed: 19616718]
87. Wystrychowski W, et al. First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access. *J Vasc Surg*. 2013; 5(13):01530–9.
88. Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng*. 2006; 12(5):1197–211. [PubMed: 16771634]
89. Sill TJ, von Recum HA. Electro spinning: Applications in drug delivery and tissue engineering. *Biomaterials*. 2008; 29(13):1989–2006. [PubMed: 18281090]
90. Mikos AG, et al. Prevascularization of porous biodegradable polymers. *Biotechnol Bioeng*. 1993; 42(6):716–23. [PubMed: 18613104]
91. 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):2096–105. [PubMed: 16004450]
92. Pham QP, Sharma U, Mikos AG. Electrospun poly(epsilon-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules*. 2006; 7(10):2796–805. [PubMed: 17025355]
93. Bergmeister H, et al. Electrospun small-diameter polyurethane vascular grafts: ingrowth and differentiation of vascular-specific host cells. *Artif Organs*. 2012; 36(1):54–61. [PubMed: 21848935]
94. Shin'oka T, et al. Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells. *Journal of Thoracic and Cardiovascular Surgery*. 2005; 129(6):1330–1338. [PubMed: 15942574]
95. Hibino N, et al. Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg*. 2010; 139(2):431–6. [PubMed: 20106404]
96. Roh JD, et al. Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(10):4669–4674. [PubMed: 20207947]

97. Wu W, Allen RA, Wang Y. Fast-degrading elastomer enables rapid remodeling of a cell-free synthetic graft into a neoartery. *Nat Med.* 2012; 18(7):1148–53. [PubMed: 22729285]
98. Fiddler GI, et al. Calcification of glutaraldehyde-preserved porcine and bovine xenograft valves in young children. *Ann Thorac Surg.* 1983; 35(3):257–61. [PubMed: 6830360]
99. Rose AG, Forman R, Bowen RM. Calcification of glutaraldehyde-fixed porcine xenograft. *Thorax.* 1978; 33(1):111–4. [PubMed: 417417]
100. Jernigan TW, et al. Small intestinal submucosa for vascular reconstruction in the presence of gastrointestinal contamination. *Annals of Surgery.* 2004; 239(5):733–738. [PubMed: 15082978]
101. Lantz GC, et al. Small intestinal submucosa as a small-diameter arterial graft in the dog. *J Invest Surg.* 1990; 3(3):217–27. [PubMed: 2078544]
102. Kennealey PT, et al. A prospective, randomized comparison of bovine carotid artery and expanded polytetrafluoroethylene for permanent hemodialysis vascular access. *Journal of Vascular Surgery.* 2011; 53(6):1640–1648. [PubMed: 21609797]
103. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials.* 2011; 32(12):3233–3243. [PubMed: 21296410]
104. Cho SW, et al. Small-diameter blood vessels engineered with bone marrow-derived cells. *Annals of Surgery.* 2005; 241(3):506–515. [PubMed: 15729075]
105. Brown KE, et al. Arterial reconstruction with cryopreserved human allografts in the setting of infection: A single-center experience with midterm follow-up. *J Vasc Surg.* 2009; 49(3):660–6. [PubMed: 19268771]
106. Gui L, et al. Development of decellularized human umbilical arteries as small-diameter vascular grafts. *Tissue Eng Part A.* 2009; 15(9):2665–76. [PubMed: 19207043]
107. Schaner PJ, et al. Decellularized vein as a potential scaffold for vascular tissue engineering. *J Vasc Surg.* 2004; 40(1):146–53. [PubMed: 15218475]
108. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol.* 2003; 23(2):168–75. [PubMed: 12588755]
109. Li S, Henry JJ. Nonthrombogenic approaches to cardiovascular bioengineering. *Annu Rev Biomed Eng.* 2011; 13:451–75. [PubMed: 21639778]
110. Chien S. Effects of disturbed flow on endothelial cells. *Annals of Biomedical Engineering.* 2008; 36(4):554–562. [PubMed: 18172767]
111. Cicha I, et al. Endothelial dysfunction and monocyte recruitment in cells exposed to nonuniform shear stress. *Clinical Hemorheology and Microcirculation.* 2008; 39(1–4):113–119. [PubMed: 18503117]
112. Chiu JJ, Usami S, Chien S. Vascular endothelial responses to altered shear stress: Pathologic implications for atherosclerosis. *Annals of Medicine.* 2009; 41(1):19–28. [PubMed: 18608132]
113. Stanley JC, et al. Enhanced patency of small-diameter, externally supported Dacron iliofemoral grafts seeded with endothelial cells. *Surgery.* 1982; 92(6):994–1005. [PubMed: 6216623]
114. Schneider PA, et al. Preformed confluent endothelial cell monolayers prevent early platelet deposition on vascular prostheses in baboons. *J Vasc Surg.* 1988; 8(3):229–35. [PubMed: 3138436]
115. Williams SK, et al. Formation of a functional endothelium on vascular grafts. *J Electron Microscop Tech.* 1991; 19(4):439–51. [PubMed: 1797989]
116. Pasic M, et al. Long-term results in the dog carotid artery with small lumen vascular prostheses with microvascular endothelial cells. *Helv Chir Acta.* 1993; 60(3):381–5. [PubMed: 8119818]
117. Rosenman JE, et al. Kinetics of endothelial cell seeding. *J Vasc Surg.* 1985; 2(6):778–84. [PubMed: 4057435]
118. Miyata T, et al. Delayed exposure to pulsatile shear stress improves retention of human saphenous vein endothelial cells on seeded ePTFE grafts. *Journal of Surgical Research.* 1991; 50(5):485–493. [PubMed: 2038188]
119. Ott MJ, Ballermann BJ. Shear stress-conditioned, endothelial cell-seeded vascular grafts: improved cell adherence in response to in vitro shear stress. *Surgery.* 1995; 117(3):334–9. [PubMed: 7878541]

120. Inoguchi H, et al. The effect of gradually graded shear stress on the morphological integrity of a huvec-seeded compliant small-diameter vascular graft. *Biomaterials*. 2007; 28(3):486–95. [PubMed: 17034847]
121. Ott MJ, Olson JL, Ballermann BJ. Chronic In Vitro Flow Promotes Ultrastructural Differentiation of Endothelial Cells. *Endothelium*. 1995; 3(1):21–30.
122. Ballermann BJ. Adding Endothelium to Artificial Vascular Grafts. *News Physiol Sci*. 1998; 13:154. [PubMed: 11390780]
123. Kim BS, et al. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nature Biotechnology*. 1999; 17(10):979–983.
124. Niklason LE, et al. Functional arteries grown in vitro. *Science*. 1999; 284(5413):489–93. [PubMed: 10205057]
125. Cross KS, et al. Long-term human vein graft contractility and morphology: a functional and histopathological study of retrieved coronary vein grafts. *Br J Surg*. 1994; 81(5):699–705. [PubMed: 8044552]
126. Niklason LE, et al. Morphologic and mechanical characteristics of engineered bovine arteries. *Journal of Vascular Surgery*. 2001; 33(3):628–638. [PubMed: 11241137]
127. L'Heureux N, et al. A completely biological tissue-engineered human blood vessel. *Faseb Journal*. 1998; 12(1):47–56. [PubMed: 9438410]
128. Mironov V, et al. Perfusion bioreactor for vascular tissue engineering with capacities for longitudinal stretch. *J Craniofac Surg*. 2003; 14(3):340–7. [PubMed: 12826805]
129. Zaucha MT, et al. A novel cylindrical biaxial computer-controlled bioreactor and biomechanical testing device for vascular tissue engineering. *Tissue Eng Part A*. 2009; 15(11):3331–40. [PubMed: 19385725]
130. Syedain ZH, Weinberg JS, Tranquillo RT. Cyclic distension of fibrin-based tissue constructs: evidence of adaptation during growth of engineered connective tissue. *Proc Natl Acad Sci U S A*. 2008; 105(18):6537–42. [PubMed: 18436647]
131. Syedain ZH, et al. Implantable arterial grafts from human fibroblasts and fibrin using a multi-graft pulsed flow-stretch bioreactor with noninvasive strength monitoring. *Biomaterials*. 2011; 32(3):714–22. [PubMed: 20934214]
132. Gui L, et al. Construction of tissue-engineered small-diameter vascular grafts in fibrin scaffolds in 30 days. *Tissue Eng Part A*. 2013; 10:10.
133. Tower TT, Neidert MR, Tranquillo RT. Fiber alignment imaging during mechanical testing of soft tissues. *Ann Biomed Eng*. 2002; 30(10):1221–33. [PubMed: 12540198]
134. Driessen NJ, et al. Computational analyses of mechanically induced collagen fiber remodeling in the aortic heart valve. *J Biomech Eng*. 2003; 125(4):549–57. [PubMed: 12968580]
135. Shadwick RE. Mechanical design in arteries. *J Exp Biol*. 1999; 202(Pt 23):3305–13. [PubMed: 10562513]
136. Cox RH. Differences in mechanics of arterial smooth muscle from hindlimb arteries. *Am J Physiol*. 1978; 235(6):H649–56. [PubMed: 736152]
137. Niklason LE, et al. Enabling tools for engineering collagenous tissues integrating bioreactors, intravital imaging, and biomechanical modeling. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(8):3335–3339. [PubMed: 19955446]
138. Zoumi A, et al. Imaging coronary artery microstructure using second-harmonic and two-photon fluorescence microscopy. *Biophysical Journal*. 2004; 87(4):2778–2786. [PubMed: 15454469]
139. Ferruzzi J, et al. Mechanical assessment of elastin integrity in fibrillin-1-deficient carotid arteries: implications for Marfan syndrome. *Cardiovasc Res*. 2011; 92(2):287–95. [PubMed: 21730037]
140. Timmins LH, et al. Structural inhomogeneity and fiber orientation in the inner arterial media. *Am J Physiol Heart Circ Physiol*. 2010; 298(5):19.
141. Steelman SM, et al. Perivascular tethering modulates the geometry and biomechanics of cerebral arterioles. *J Biomech*. 2010; 43(14):2717–21. [PubMed: 20655047]
142. Hu JJ, Humphrey JD, Yeh AT. Characterization of engineered tissue development under biaxial stretch using nonlinear optical microscopy. *Tissue Eng Part A*. 2009; 15(7):1553–64. [PubMed: 19063662]

143. Bourget JM, et al. Human fibroblast-derived ECM as a scaffold for vascular tissue engineering. *Biomaterials*. 2012; 33(36):9205–13. [PubMed: 23031531]
144. Hasan A, et al. Electrospun scaffolds for tissue engineering of vascular grafts. *Acta Biomaterialia*.
145. Thomas V, et al. Electrospinning of Biosyn((R))-based tubular conduits: structural, morphological, and mechanical characterizations. *Acta Biomater*. 2011; 7(5):2070–9. [PubMed: 21232639]
146. Soletti L, et al. A bilayered elastomeric scaffold for tissue engineering of small diameter vascular grafts. *Acta Biomater*. 2010; 6(1):110–22. [PubMed: 19540370]
147. Subramanian A, Krishnan UM, Sethuraman S. Fabrication of uniaxially aligned 3D electrospun scaffolds for neural regeneration. *Biomed Mater*. 2011; 6(2):1748–6041.
148. Zhao Y, et al. The development of a tissue-engineered artery using decellularized scaffold and autologous ovine mesenchymal stem cells. *Biomaterials*. 2010; 31(2):296–307. [PubMed: 19819544]
149. Dahl SL, et al. Readily available tissue-engineered vascular grafts. *Sci Transl Med*. 2011; 3(68):3001426.
150. Quint C, et al. Decellularized tissue-engineered blood vessel as an arterial conduit. *Proc Natl Acad Sci U S A*. 2011; 108(22):9214–9. [PubMed: 21571635]
151. Dahl SL, et al. Mechanical properties and compositions of tissue engineered and native arteries. *Ann Biomed Eng*. 2007; 35(3):348–55. [PubMed: 17206488]

Fig. 1. Schematic diagrams of TEV ECM created from different non-mechanically conditioned methods. **A** Labels each ECM component of different TEVs. **B** The ECM of collagen gel-based TEVs with sparse *de novo* collagen fibers. **C** The ECM of fibrin gel-based TEVs, where cross-linked tropoelastin (elastin) can be seen. **D** The ECM of cell-sheet engineered TEVs. **E** The ECM of electrospun polymer scaffolds. **F** Degradable polymer scaffold seeded with cells on the day of implantation. **G** The ECM of decellularized arteries. **H** The ECM of native muscular arteries represented by undulated collagen fibers and mature elastic fibers as well as SMCs aligned in parallel with collagen fibers.

Fig. 2.

Shows different vascular bioreactors that apply mechanical conditioning to generate TEVs.

A Describes Niklason pulsatile bioreactor in a schematic diagram. A peristaltic pump creates cyclic circumferential stretching by flowing PBS through the system. A silicone stopper is used to cap the bioreactor and air filters allow gas exchange between the incubator and the bioreactor. A pressure transducer measures and monitors the pressure at the upper stream of the flow system. ECM of the pulsed TEVs is mainly consisted of collagen fibers and SMCs as illustrated in a zoom in view. Small fragments of remaining PGA can still be seen in the ECM. **B** Schematic diagram of Tranquillo bioreactor. Syringe is mounted on reciprocating pump that causes the pulsed medium to flow transmurally through the tissue and axially through the lumens. TEVs in the front are shown to retract axially from the unfixed-ends.

Table 1

Summarizes the advantages and disadvantages of TEVs created from different vascular engineering methods (non-mechanically conditioned). Cited references and the mechanical properties of these TEVs are also and listed in this table.

TEV Fabrication Techniques	Advantages	Disadvantages	Literatures/References
Collagen-gel based	<ul style="list-style-type: none">• Was the first biocompatible and biologically-based vascular grafts	<ul style="list-style-type: none">• Consists of denatured collagen protein with disorganized structure	[65–67, 70–75]
	<ul style="list-style-type: none">• Can be constructed into multiple-layer structure to resemble the concentric structure of native arteries	<ul style="list-style-type: none">• Attenuates <i>de novo</i> collagen synthesis	
	<ul style="list-style-type: none">• Can be free of synthetic materials	<ul style="list-style-type: none">• Does not support elastogenesis	
		<ul style="list-style-type: none">• Low mechanical strength; UTS: 58kPa, elastic modulus: 142kPa (with mechanical conditioning) [72]	
Fibrin-gel based	<ul style="list-style-type: none">• Manipulation of gel properties (polymerization rate, compliance, and so forth)	<ul style="list-style-type: none">• Low suture retention strength: 5.941 g/g (in comparison to 24,300g/g of native lam carotid artery) [82]	[78, 79, 81, 82], [130]
	<ul style="list-style-type: none">• Promotes collagen and tropoelastin synthesis		
	<ul style="list-style-type: none">• Good mechanical strength; UTS: 1.1MPa, elastic modulus: 4.7MP [130]	<ul style="list-style-type: none">• No arterial implantation reported	
	<ul style="list-style-type: none">• Implantation in venous location (jugular vein, lamb)		
Cell-Sheets Engineering	<ul style="list-style-type: none">• Was the first implantable biological-based TEVs		[83–86], [127], [143]
	<ul style="list-style-type: none">• High burst pressure: 3490 mmHg [84], UTS: >3MPa, elastic modulus >20MPa [143]	<ul style="list-style-type: none">• Demands a high cost and long production time	
	<ul style="list-style-type: none">• Has a concentric-layer structure, characteristic of that of native arteries		
	<ul style="list-style-type: none">• Clinical trials of implanting autologous TEVs as arterio-venous shunts		
Electrospinning of Polymeric Scaffolds	<ul style="list-style-type: none">• Adjust polymer scaffolds via changes of electrospinning settings	<ul style="list-style-type: none">• Commonly leads to dense layers, small pore size, and low porosity of the scaffolds	[88–93], [144–147]
	<ul style="list-style-type: none">• Tailored for desired mechanical properties. Burst pressure up to 3000mmHg [144, 145], UTS up to 17.5MPa [144, 146], and elastic modulus up to 40.4MPa [144, 147]	<ul style="list-style-type: none">• Results in poor cell infiltration and impedes cell migration	
		<ul style="list-style-type: none">• Slow degradation of the scaffold impairs remodeling and integration with the host environments	
Biodegradable Polymeric Scaffolds	<ul style="list-style-type: none">• Can be tailored to achieve high porosity and large pore size to facilitate cellular infiltration and migration	<ul style="list-style-type: none">• Weak mechanical strength. Suture retention strength < 50g. Elastic modulus <50kPa [Wei's Nature Medicine]	[94–97]
	<ul style="list-style-type: none">• Degradation of the scaffolds <i>in vivo</i> during host remodeling		

TEV Fabrication Techniques	Advantages	Disadvantages	Literatures/References
Decellularized Tissue Scaffolds	<ul style="list-style-type: none">• Clinical trials TEVs of TEVs as extracardiac total cavopulmonary connection	<ul style="list-style-type: none">• Might not be applicable for arterial implantation	
	<ul style="list-style-type: none">• Reduces immunogenic reactions via removing of cellular contents		
	<ul style="list-style-type: none">• Preserves the mechanical strength; Burst pressure: 2400mmHg [148], UTS: 1.618Mpa, and Elastic Modulus: 7.41MPa [106]	<ul style="list-style-type: none">• Decellularized xenografts still have potential to trigger immunogenicity	[100–107], [148]
	<ul style="list-style-type: none">• Haven been used as arterial grafts in clinical trials	<ul style="list-style-type: none">• Limited by availability of native human vessels, and vessel dimensions	

Table 2

Summarizes the advantages and disadvantages of different vascular bioreactors used in studying collagen growth and remodeling of TEVs.

Bioreactor	Types of TEVs supported	Conditions	Advantages	Disadvantages
Niklason et al. 1999 [124] Niklason et. 2009 [137]	PGA scaffold-based TEVs	Cyclic circumferential stretch	Applies circumferential stretch to TEVs for up to 8 weeks Results in robust and implantable TEVs. Burst pressure: 3337mmHg, suture retention strength: 260g [149], UTS: 1.44MPa [150], elastic modulus: 11.8MPa [151] Supports assessment of collagen remodeling and growth noninvasively	Does not support axial loading
Mironov et al. 2003 [128]	Native arteries & silicone tubing	Cyclic circumferential & axial stretch	Applies multi-directional stretching, up to 200% of axial stretch	Does not support growth of biological TEVs
Zauchra et al. 2009 [129]	Collagen-based TEVs	Cyclic circumferential & axial stretch	Applies multi-directional stretching to assess collagen remodeling and mechanical properties noninvasively	Is not suitable for long term simulation of multi-axial stretch Is not shown to sustain growth of biological TEVs
Syedain et al. 2011 [131]	Fibrin-based TEVs	Cyclic circumferential stretch and incremental axial shortening	Applies cyclic circumferential stretch via pulsatile flow of medium Allows for axial shortening of TEVs Good mechanical strength; UTS: 1.76MPa, elastic modulus: 2.5MPa	Neither apply cyclic axial load nor have control over axial shortening
Bose® ElectroForce® BioDynamic® Test Instruments	Native and engineered tissues	Cyclic & constant circumferential, axial, and torsion loads	Apply multi-directional stretching with accurate control of parameters Serve as an excellent device to test multi-axial mechanics properties of biomaterials and engineered tissues	Are not designed to sustain <i>in vitro</i> growth of biological TEVs