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Review: Advances in Vascular Tissue Engineering Using Protein-Based Biomaterials

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Abstract

The clinical need for improved blood vessel substitutes, especially in small-diameter applications, drives the field of vascular tissue engineering. The blood vessel has a well-characterized structure and function, but it is a complex tissue, and it has proven difficult to create engineered tissues that are suitable for widespread clinical use. This review is focused on approaches to vascular tissue engineering that use proteins as the primary matrix or “scaffold” material for creating fully biological blood vessel replacements. In particular, this review covers four main approaches to vascular tissue engineering: 1) cell-populated protein hydrogels, 2) cross-linked protein scaffolds, 3) decellularized native tissues, and 4) self-assembled scaffolds. Recent advances in each of these areas are discussed, along with advantages of and drawbacks to these approaches. The first fully biological engineered blood vessels have entered clinical trials, but important challenges remain before engineered vascular tissues will have a wide clinical effect. Cell sourcing and recapitulating the biological and mechanical function of the native blood vessel continue to be important outstanding hurdles. In addition, the path to commercialization for such tissues must be better defined. Continued progress in several complementary approaches to vascular tissue engineering is necessary before blood vessel substitutes can achieve their full potential in improving patient care.

Introduction

The driving force behind the many efforts in vascular tissue engineering is the clear need for better replacements for blood conduits, especially for small-diameter (< 6 mm) applications, in which synthetic materials are prone to clotting and failure. For larger-diameter applications, polymers such as polytetrafluoroethylene and polyethylene terephthalate have been used to create vascular grafts for peripheral applications. However, for indications such as coronary bypass or below-the-knee bypass grafting, synthetic materials do not function well in the longer term. This clinical need has fueled the search for better, more biologically compatible blood vessel replacements.

Many approaches have been used to recreate the structure and function of blood vessels. The archetypal tissue engineering approach is to use a degradable synthetic scaffold that is seeded with cells. The general concept is that the polymer provides initial mechanical support to the engineered tissue and that, as it degrades, it is replaced by a protein matrix secreted by the cells. This method has been used widely for many tissues, including blood vessels, and the first engineered tissues based on this technology have entered the clinic.^{1,2} However, it is not clear that this approach will be the most suitable for all applications, and the broader field of tissue engineering includes many complementary paths. In this review, we will focus on approaches to vascular tissue engineering that aim to create fully biological engineered tissues without the use of synthetic materials for support.

Blood Vessel Structure

Tissues consist of cells embedded in an extracellular matrix (ECM) composed largely of proteins, glycoproteins, glycosaminoglycans, and proteoglycans. The protein components are often fibrillar and provide strength in tension and shear, whereas the proteoglycan “ground substance” sequesters water and ions and provides compressive resistance. The most abundant proteins in most tissue types are forms of collagen and elastin, with lesser amount of fibronectin, laminin, vitronectin, and other ECM proteins. Tissues contain specific cell types, which can produce and secrete proteins, as well as carry out mechanical functions such as cell contraction and migration. Tissues also have characteristic ECM composition and arrangement. It is the interplay between tissue-specific cells and the structure of the ECM that gives different tissues their specialized functions.

Blood vessels have a concentric layered structure, with each layer being distinct in its cell and protein composition. Figure 1 shows a schematic of the structure of a medium-sized artery, with the main features, components, and environmental influences listed. The innermost layer consists of a monolayer of specialized endothelial cells (ECs) called the intima, which forms a tight nonthrombogenic barrier between the lumen of the vessel and the rest of the vessel wall. This layer is critical not only in preventing unwanted clot formation, but also in preventing infection and inflammation of the underlying tissue, as well as in signaling to the muscular component of the vessel wall. Beneath the intimal layer is a layer of basement membrane enriched in collagen IV and laminin, followed by a fenestrated but acellular layer of elastin called the internal elastic lamina.

The muscular layer of the artery, called the media, is composed largely of collagens Type I and III, as well as lesser amounts of other proteins and proteoglycans. Smooth muscle cells (SMCs) that have a specialized contractile function populate this layer. The collagen matrix and the SMCs are generally aligned circumferentially or in a spiral pattern along the axis of the vessel. In larger vessels, elastic laminae further subdivide the media. Stimulation of SMCs by signals from the ECs of the lumen or directly by cytokines can cause the cells to contract or dilate in a coordinated fashion, which in turn leads to vessel contraction or dilation. In larger arteries, the medial layer is innervated, allowing the muscular layer to receive signals directly from the nervous system, and in some cases, microvessels, which supply the tissue with nutrients and oxygen, also infiltrate thick medial layers. Another elastin layer, called the external elastic lamina, which separates the medial layer from the adventitia, the outermost layer of the vessel wall, surrounds the medial layer. The adventitia consists mainly of a loose collagen matrix with embedded fibroblasts. This layer provides a substrate for a vascular supply to the artery wall and serves to anchor the blood vessel to the surrounding tissue, as well as to provide additional structural support.

Blood vessels can fail in a variety of ways. Injury, disease, or inflammation can weaken the vascular wall, which can lead to aneurysms and dissections, but the most common reason for vessel failure is occlusion due to atherosclerosis, an inflammatory disease that causes plaque build-up beneath the intimal layer of the vessel wall. The infiltration of monocytes into the intima, and a resulting increase in the migration, proliferation, and secretory activity of the vascular SMCs, forms this plaque. As the plaque grows and calcifies, it can cause a narrowing of the blood vessel lumen and can thereby decrease blood flow to the downstream tissues. Plaque rupture and subsequent clot formation also are potentially serious complications that can lead to infarction of the downstream tissue. The wide prevalence of atherosclerotic disease in western society has resulted in a strong need for better treatments for blocked vessels, including better options for blood vessel replacement. (For recent statistics on cardiovascular disease, see, e.g.,³.)

Scope of Review

This article is intended to be a focused review of the approaches to vascular tissue engineering that use proteins as the primary matrix or “scaffold” material for creating fully biological blood vessel replacements. There is a large body of work in this area, which dates back 20- to 30 years. In this review, we have concentrated on advances that have been made in the last decade. Our intent is not to neglect the important contributions of the pioneers in this field but rather to provide a concise update of recent work in this area.

To limit the scope of material, we have concentrated on methods to produce small- and medium-sized arteries and have not included the engineering of capillary networks or the microvasculature. Our emphasis is on the creation of the vascular wall (media and adventitia), rather than EC seeding, which is a complex field on its own. Again, this is not meant to play down the importance of the endothelium, because it is a critical component of any functional blood vessel. Instead, we recognize that EC-seeding will be a key enabling technology if engineered small- and medium-sized vessels are to be successful, and we refer the interested reader to recent reviews on this important topic.^{4,5} In addition, we have excluded approaches that use synthetic polymers (e.g., polyglycolic acid, polylactic acid, polycaprolactone, polyurethane) for creating tissue engineering constructs to focus on fully biological approaches. Several comprehensive reviews in the broader field of vascular tissue engineering have been published in recent years (see e.g.,^{6–8}), and the reader interested in other approaches is referred to these sources.

Finally, this review is not intended to give a detailed analysis of the many different materials and techniques that have been used to engineer fully biological, protein-based blood vessels. Rather, it is intended as an overview of the main approaches that have developed over the last 2 decades, with some commentary on the advantages and disadvantages of each method. It is hoped that this review will be a starting resource for those interested in this topic, and the reader is encouraged to delve into the cited primary literature for details. For readers who already are in the field of protein-based vascular tissue engineering, we hope this review will provide a concise compilation of recent advances and the context in which they were made.

Approaches to Vascular Tissue Engineering Using Protein-Based Biomaterials

Although their general structure is straightforward and well understood, native arteries are complex tissues with multiple cell types and ECM components. To understand these tissues, and therefore to engineer fully functional arterial replacements, requires expertise in cell biology, hemodynamics, cell—matrix interactions, biomechanics, and protein chemistry. At the stage of development at which most engineered vascular tissues are currently, the main functional parameters of interest are mechanical strength and differentiated cell function. Mechanical properties are usually expressed in terms of stiffness (or tensile modulus), as well as ultimate tensile strength and burst pressure. Biological function typically is expressed in terms of cell viability, morphology, and gene and protein expression, often with an emphasis on ECM deposition. Because of the critical importance of the EC layer in maintaining patency of vascular grafts, this is usually one of the most important endpoints of *in vivo* studies of such constructs. In the following subsections, we have outlined several approaches to producing blood vessel substitutes based on protein matrices and have commented on the key findings in terms of biological and mechanical function of these constructs.

Cell-populated protein hydrogels

Because of the abundance and structural importance of fibrillar proteins in the blood vessel, one of the earliest approaches to vascular tissue engineering was to recreate protein matrices seeded with cells. The idea is to provide the appropriate ECM and cell type to create a tissue. Collagen type I is often used in this context because it is abundant in many tissues and can be isolated, solubilized, and reconstituted in a variety of ways. Use of a solubilized protein matrix allows living cells to be suspended in the protein solution, and this suspension then can be poured into a mold of the desired shape (e.g., an annular mold is used to create tubes for vascular tissue engineering). The protein matrix is then reconstituted by changing the environmental conditions (pH, temperature) such that, once the protein hydrogel has been formed, the cells are uniformly dispersed and immobilized in the matrix. This method is distinct from the common tissue-engineering approach of seeding cells onto a preformed scaffold, because the cells are directly embedded in the protein matrix at the time of gelation. Figure 2 shows an SMC-seeded collagen type 1 hydrogel that was molded into a tubular shape and cultured for 7 days.

In theory, protein hydrogel matrices have the advantage that a defined ECM surrounds the embedded cells in the same way that matrices surround many cells (e.g., SMCs) in native tissues. In addition, cells have receptors for the proteins used in such hydrogels and therefore can recognize and bind to them, as well as migrate through them and remodel or secrete them as needed. Indeed, the vigorous compaction that occurs over the first few days after preparation of a cell-seeded collagen gel is a hallmark of cell-mediated remodeling,⁹ and this process leads to the formation of a rudimentary tissue.¹⁰ In native tissues, ECM proteins are known to sequester and release growth factors and other biochemicals, and therefore there is potential that this mechanism could also be used in engineered tissues.

In practice, it has proven difficult to recapitulate the structure and function of even relatively simple tissues using only cell-seeded protein hydrogels. In the case of blood vessels, the seminal work in this area used cells embedded in tubular collagen type I hydrogels.¹¹ A multilayer structure was created with the intent of mimicking the structure of the native vessel, using SMCs and collagen as the medial layer and fibroblasts in collagen as the adventitial layer. A similar early model added dermatan sulfate proteoglycan to the hydrogel matrix.¹² In both these studies, a polyethylene terephthalate mesh was incorporated into the construct to provide mechanical reinforcement to the hydrogel matrices. These important early studies demonstrated the feasibility of using protein hydrogels in vascular applications but also pointed out the challenges of creating fully biological graft materials.

The lack of appropriate mechanical properties for use in the vascular system is a problem that has consistently hampered the development of protein hydrogel-based blood vessels. In the past decade, a variety of methods have been used to strengthen such constructs without the need for a synthetic reinforcement, based on the belief that a fully biological blood vessel substitute would provide the best solution over the long term. These approaches have targeted the organization and composition of the matrix proteins, as well as function of the cells, in an effort to enhance mechanical properties.

Supplementation of the culture medium used to grow the cells and constructs used for vascular tissue engineering is one approach to enhancing the properties of the engineered tissue. Ascorbic acid and retinoic acid (forms of vitamin C and vitamin A, respectively) have been shown to alter SMC metabolism and in particular the expression of ECM proteins.¹³ Stimulation of cells with growth factors also can alter their ability to compact and remodel protein lattices, which can result in altered mechanical properties. For example, stimulation of SMCs in collagen type 1 gels with exogenous platelet-derived growth factor or -transforming growth factor-beta increases gel compaction, whereas heparin decreases the degree of

compaction.¹⁴ Although a wide panel of small molecules, growth factors, cytokines, and other biologically active factors are available for modulating the function of SMCs, the effects and mechanisms of action of these supplements are not well understood. In particular, the use of these factors in 3-dimensional protein matrices presents challenges and opportunities in terms of dosing and delivery, and these issues need to be clarified to achieve consistent biological control of cell function in vascular tissue engineering.

Crosslinking of the protein matrix is another potential avenue to increasing the mechanical strength of engineered vascular tissues. The main challenge in crosslinking cell-seeded protein hydrogels is the need to avoid damage to the cellular component, because many crosslinking agents and methods are cytotoxic. One way to circumvent this problem has been to exploit the process of nonenzymatic glycation of proteins by reducing sugars to stiffen and strengthen collagen-based vascular constructs.¹⁵ Another approach has been to transfect SMCs to overexpress lysyl oxidase, the enzyme responsible for collagen crosslinking in most tissues, which produces stronger and stiffer collagen matrices.¹⁶ Although these crosslinking methods alone do not result in protein hydrogels of sufficient strength for implantation into the arterial system, they demonstrate the ingenuity that has been applied to increasing hydrogel mechanical properties, and it is likely that such techniques in combination with others described here will lead to mechanically suitable blood vessel substitutes.

The blood clotting protein fibrin also has been used to create 3-dimensional protein hydrogel matrices for vascular tissue engineering. This protein is of interest in tissue engineering because it is a main wound-healing protein, and it is known to stimulate regenerative and remodeling responses in a variety of cell types. Fibrinogen, which is the precursor to fibrin, is abundant in blood and can be isolated, purified, and solubilized in aqueous solution. Addition of the enzyme thrombin cleaves fibrinogen to yield insoluble fibrin, which aggregates to form protein fibers. This process has been harnessed to mold engineered vascular constructs that also undergo cell-mediated compaction in a manner similar to collagen, and fibrin has been used to create vascular conduits seeded with SMCs.^{17,18} As with collagen, a variety of parameters, including the concentration of fibrinogen and calcium¹⁹ and thrombin²⁰ and the presence of growth factors and medium supplements, influence the properties of these constructs.²¹ Collagen and fibrin also have been used in combination to form composite materials that have mechanical properties that are superior to those of the pure components alone.²² Varying the ratio of these proteins, as well as the total protein content, has been shown to change the mechanical properties of such constructs.²³ Although fibrin is not an ECM protein that is normally found in the blood vessel wall, it has the potential to augment the biochemical and mechanical properties of scaffolds for vascular tissue engineering. Figure 3 shows scanning electron micrographs of collagen, fibrin, and collagen—fibrin composite matrices.

Mechanical stimulation of protein hydrogel constructs has been used as a means of changing the structure of the matrix, as well as directing cell function. It has long been known that mechanical forces, in particular fluid shear, strongly affect ECs (for a recent review see, e.g., 24,25). It also is now clear that a variety of mechanical forces, including tension²⁶ and shear,^{27,28} affect vascular SMC function. Bioreactors designed to mimic these forces have been developed and used to examine the role of these forces in tissue remodeling. Figure 4 shows an example of a modular bioreactor system that provides flow through the lumen of the construct, as well as crossflow across the external surface. Cyclic circumferential strain often is applied in such systems in an effort to mimic the pulsatile stresses in the cardiovascular system. Such stimulation has been shown to induce remodeling of SMC-seeded collagen gels,²⁹ as well as to affect SMC phenotype³⁰ and induce elastin synthesis.³¹ The mechanisms of SMC-induced remodeling are not completely understood, although the embedded cells are presumably able to degrade the matrix around them, as well as synthesize new matrix in response to mechanical cues.³² In general, bioreactor culture has been shown to increase the

mechanical properties of protein hydrogel constructs, and newer bioreactor systems have begun to combine circumferential tensile strain with perfusion and shear,^{33,34} longitudinal strain,³⁵ and even electrical stimulation.³⁶ (For a review on this topic, see³⁷.)

The technology of creating and controlling ever more-complex protein-based hydrogels has advanced steadily over the past 2 decades. An attractive feature of using such reconstituted protein hydrogel matrices is that one has excellent control over the composition of the starting scaffold material. Living cells can be incorporated directly at the time of scaffold fabrication, and these cells will recognize and interact with the provided matrix. By controlling the composition of the protein matrix, it is potentially possible to direct cell differentiation, function, and remodeling in desired ways. However, the major challenge to this approach remains the difficulty in achieving appropriate mechanical properties for use in the arterial system. Longer-term culture (weeks to months) of such hydrogels in the presence of the appropriate medium supplements has been shown to increase their mechanical properties, and the first *in vivo* experiments using fully biological fibrin hydrogel scaffolds have recently been initiated.¹⁸

Cross-linked protein scaffolds

Efforts to increase the mechanical function of pure protein vascular constructs have focused mainly on crosslinking the protein matrix to increase its resistance to tension. As mentioned previously, most crosslinking methods are toxic to cells, and therefore the approach taken has been to create separate protein scaffolds that subsequently are seeded with cells. This method is more analogous to the traditional tissue-engineering approach of combining cells with a pre-formed scaffold, with the important distinction that the scaffold is made of protein instead of the more typically used synthetic polymers.

Proteins that are present in the vascular wall have been a main focus in attempting to make fully biological scaffolds. Reconstituted collagen type I has been used in this application. To obtain a mechanically robust protein scaffold from this material, collagen solutions typically are molded into a tube or sheet and are then dehydrated or lyophilized to remove the water and create a dense matrix.^{38,39} The resulting material can be further crosslinked to increase its mechanical properties before cells are added to the scaffold. Common agents used for this purpose include aldehydes and carbodiimides,^{40,41} although other methods also have been used.^{42,43} After the scaffold has been prepared, SMCs or other cell types can be seeded onto the scaffold, and because cells have receptors for these proteins, they will recognize the matrix and attach to it. Because native proteins are used, the cellular component also can remodel these materials during *in vitro* culture and after implantation, although it is not clear how the decellularization procedure and subsequent crosslinking affects this process.

Other proteins and blends of proteins also have been used in this approach. Because of elastin's importance in the recoil properties of blood vessels, elastin materials,⁴⁴ as well as collagen—elastin matrices,⁴⁵ in some cases also containing chondroitin sulfate (a glycosaminoglycan),⁴⁶ also have been examined for use as protein scaffolds for engineered blood vessels. In general, these materials also are cross-linked to increase their mechanical integrity before being seeded with cells. Hyaluronan, a glycosaminoglycan found in many tissues, including the blood vessel wall, has been used as a scaffold for vascular tissue engineering, but because it is not a protein matrix, it generally needs to be surface-modified to promote cell attachment.^{47,48} Other materials that have been investigated for use in creating protein scaffolds for blood vessels include fibronectin⁴⁹ and chitosan,⁵⁰ a polysaccharide with amine groups, which gives it some properties of proteins.

Other techniques aimed at creating pure protein scaffolds for vascular tissue engineering have emerged. The process of electrospinning involves drawing a jet of charged polymer solution

between a spinneret needle and a grounded target. The solvent evaporates as the polymer solution travels through the air, leaving a solid polymer fiber that gradually builds up into a non-woven fibrous scaffold. By controlling the flow rate of polymer through the needle, as well as the voltage and geometry of the spinning rig, one can vary the fiber size and density, as well as the overall shape of the scaffold that is created. For vascular tissue-engineering applications, a rotating mandrel is often used as the grounded target, and deposition of the fibers therefore creates a tubular mesh scaffold. A variety of synthetic polymers have been used, and the technology is advancing to create multi-component materials, as well as more-complex geometries. (For a recent review on the broader topic, see e.g.,^{51,52}). Electrospinning of protein fibers also has been demonstrated, including for vascular applications. As with other approaches, collagen and elastin are the main proteins of interest in these tissues, and tubular scaffolds have been created using electrospun versions of these proteins.^{53,54} Figure 5 shows scanning electron micrographs of electrospun collagen and elastin scaffolds. Electrospun fibrinogen also has been used in vascular applications,⁵⁵ and electrospun blends of proteins and synthetic polymers are being developed.^{56,57} The application of electrospinning to tissue engineering has focused mainly on synthetic polymers, and these systems are much better characterized than electrospun proteins. The process of electrospinning proteins remains less well understood and controlled, but there is considerable effort being applied to developing electrospinning for biomedical applications, and it is likely that protein spinning will be similarly developed in the coming years.

Decellularized native tissues

Another response to the poor mechanical properties of pure protein hydrogel constructs has been the use of decellularized tissues from a variety of sources as mechanically robust scaffold materials. It is the cellular component of transplanted allogeneic or xenogeneic tissues that is the most potent initiator of the immune response. For this reason, it has been reasoned that removal of foreign cells from a blood vessel will leave a suitable ECM from which to recreate a new tissue. In more recent efforts, the concept is to recellularize the “empty” tissue matrix with autologous cells to create an immune-acceptable engineered tissue before implantation. The potential advantage of this approach is that animal vessels, which are in relatively abundant supply, could be used as the protein scaffold and autologous cells could be expanded in culture to overcome cell supply limitations.

The idea of using decellularized vessels in this manner has been around for at least 2 decades,⁵⁸ but attempts at recellularizing this material are more recent and are now one of the more common tissue-engineering technologies. Decellularization is usually achieved using solvent extraction of the lipid component and partial digestion using proteases to remove the cells,⁵⁹ although there are a number of protocols for achieving this end. The goal is to retain the protein scaffold structure of the tissue while removing all potentially immunogenic cells. (For a recent review on this topic, see, e.g.,⁶⁰.) In some cases, the isolated scaffolds are subsequently crosslinked before being re-seeded with the desired cells. Because of the variability in tissue across different donors, more-standardized and -automated processing techniques are being developed.⁶¹ Modification of the extraction protocol can be used to preferentially remove some components of the matrix, such that the remaining scaffold is enriched in a desired protein, (e.g., collagen or elastin).⁶² Figure 6 shows a scanning electron micrograph of a decellularized and purified elastin matrix. Such purified elastin scaffolds have been used as support sleeves for collagen-based hydrogel constructs. Figure 7 shows histological samples of a native artery, a collagen hydrogel construct, and a collagen hybrid construct that is reinforced with an elastin sleeve.

For vascular applications, arteries (carotid, aorta) and veins (jugular) of porcine and canine origin are the most widely used,^{63–65} presumably because of their similarity in size to

important small-diameter human vessels (e.g., the main coronary vessels). Depending on the method used to process and decellularize the matrix, the mechanical and biochemical properties can vary. In general, the decellularization process produces weaker tissues, but subsequent crosslinking makes the materials stronger and stiffer. In some cases, the decellularized vessels are similar in mechanical behavior to native vessels after processing. Creating engineered vessels using allogeneic or xenogeneic cells and then decellularizing these to create a scaffold for autologous cells has also been proposed.⁶⁶ The potential advantage of this approach is that custom scaffolds could be created before they are needed using foreign cells and then could be seeded and implanted when needed using host cells.

Another material that has been used in this context for more than 2 decades is small intestinal submucosa (SIS).^{67,68} This matrix is usually of porcine origin and consists of the fibrous protein-rich layer immediately below the mucosal layer of the small intestine. Its main components are collagens, as well as some remaining proteoglycans, and it has been suggested that SIS also retains various growth factors that are important in its regenerative function.⁶⁹ Already successful as a clinical biomaterial in a variety of soft tissue applications, SIS continues to be investigated for vascular applications (see, e.g.,^{70–72}). As with other protein-based matrices, it has been shown that SIS matrix can be remodeled using cellular action after transplantation.^{73,74} The initial material tends to be stiffer than native vascular tissue,⁷⁵ and attempts have been made to combine SIS with elastin sleeves to more closely mimic native compliance.⁷⁶

Self-assembled scaffolds

The idea of combining cells with a scaffold is central to most tissue-engineering approaches, and in most cases, the scaffold is “exogenously” supplied. Indeed, the concept behind the approaches described above is that supplying a suitable protein scaffold will provide the mechanical robustness and biochemical cues required for engineered constructs to become functional blood vessels. An alternate approach is to allow the cellular component to synthesize and assemble its own scaffold. Culturing monolayers of fibroblasts and SMCs in medium enriched in ascorbic acid causes greater production of collagen type I, which over time in culture leads to the creation of collagen-rich cell “sheets” that can be lifted off the culture surface intact. Such sheets have been used to create vascular constructs by wrapping them around a mandrel to produce a tubular structure, and multilayered constructs that mimic the cellular composition of the artery can be produced in this way.⁷⁷ These fully biological engineered vessels have displayed burst pressures similar to those of native vessels, and there is evidence of biological functions such as response to vascular agonists that control vasodilation and constriction.^{78,79} Animal studies have shown that these constructs can be lined with an endothelium and remain patent over weeks to months in an autologous nude rat model, as well as in immunosuppressed canine and primate models using xenogeneic (human) cells.⁸⁰ Initial clinical trials to test the safety of these constructs as vascular access grafts recently have begun,⁸¹ and this model is the first fully biological engineered blood vessel to be implanted in the arterial system of humans.

In vivo self-assembly of vascular conduits also has been developed recently using a modification of the Sparks mandril method for creating fibrous collagen conduits. In this model, the body's fibrotic response to foreign materials is harnessed. Silicone tubing is implanted in the peritoneal cavity of the intended graft recipient through a minimally invasive procedure. Over several weeks of implantation, the silicone material becomes encapsulated in granulation tissue consisting largely of collagen type I and fibroblasts and eventually is covered by a mesothelial monolayer.⁸² The silicone tubing and encasing tissue then can be harvested via a small incision. Removal of the inner silicone material and eversion (i.e., turning inside-out) of the granulation tissue sleeve creates a fully biological tube with mesothelial cells in the

lumen and a collagen-fibroblast wall. Figure 8 shows a histological section of the wall of such a tube created in the rat. Subsequent transplantation of these constructs into the vascular system of the host animal has shown that they can remain patent for periods of months and that extensive tissue remodeling and cell differentiation takes place such that the construct changes to resemble a native vessel over time. The initial studies on this approach to vascular tissue engineering were performed in the rat and rabbit, although canine large-animal studies have also been completed.⁸³

Current and Future Challenges

The science and technology needed to produce fully biological blood vessel substitutes has progressed steadily over the last 25 years. The field has benefited from incremental advances in our understanding of vascular biology and tissue engineering and from important and innovative solutions to key challenges. It is encouraging that the first clinical trials using fully biological blood vessel substitutes have been initiated. The first trial has targeted the vascular access indication, rather than the more complex and risky by-pass application, but this is an important first step in establishing tissue engineering as a therapy in the vasculature. In addition, most of the other complementary approaches described in the preceding sections are progressing into more-advanced animal studies.

In spite of these advances, there are serious challenges that prevent the widespread clinical application of fully biological vascular grafts in small-diameter applications. Overcoming the immune response in any vascular application is particularly difficult because of the direct exposure of the material to the bloodstream. In this respect, the endothelial lining of any graft will be crucial to its success, and at this stage, it appears likely that only living, autologous ECs will be able to meet the joint challenges of being nonthrombogenic and immune-tolerated. This topic has not been covered in detail in this review, but there is an abundance of research being conducted in the area of EC sourcing and functional reconstruction of the endothelium (reviewed in e.g.,^{4,5}). An advantage of protein-based biomaterials in this arena is that it is likely that EC function will be easier to achieve and maintain on such a matrix than on a synthetic biomaterial. In addition, the protein matrix itself is likely to be better tolerated and more easily remodeled and replaced than hydrocarbon polymer scaffolds.

Sourcing of ECs, SMCs, and other cell types needed for construction of a blood vessel substitute remains a challenge. Availability of cells for creating autologous replacement tissues is a primary concern, especially because the patients who require bypass surgeries are often elderly, with systemic cardiovascular disease. Stem and progenitor cells offer a potential solution to this sourcing problem. Embryonic stem cells have been shown to differentiate toward EC^{84, 85} and SMC^{86,87} lineages, but the science of control of embryonic stem cell differentiation is still in its infancy, and the problems of immune tolerance are not solved with this cell type. Bone marrow—derived stem cells also have been shown to differentiate toward vascular phenotypes^{88–90} and represent an autologous cell source. The broader topic of stem cells in vascular tissue engineering is addressed in several recent reviews,^{91–93} and this is a topic that is of importance across the field of tissue engineering.

As fully biological vascular constructs have been developed, functional concerns beyond simple burst strength and cell viability have become more of a focus. More physiological cell function in terms of vasoactivity has been achieved,^{94,95} which may lead to better graft performance *in vivo*. Using such biologically functional blood vessel substitutes as models to study vascular biology *in vitro*, as well as testbeds for drug screening or evaluation, is another potential application of vascular tissue engineering. Similarly, the mechanical characterization of protein-based vascular grafts has progressed. In recognition of the complex behavior of native tissues, more emphasis is being placed on characterizing the viscoelastic nature of the

constructs that are produced.⁹⁶ In particular, the incorporation of elastin or elastin-mimetic peptides into protein matrices has become a main goal,^{97,98} in order to enhance construct recoil and prevent aneurysm formation. The movement of the field toward mimicking more-specialized features of vascular tissue is a testament to the progress that has been made, although the recapitulation of native tissue mechanics and contractility is an ambitious endpoint. It is not clear how close to native properties a blood vessel construct must be to be used as a therapy.

Beyond the scientific and technical challenges lie a range of other hurdles that engineered blood vessel substitutes must overcome to have an important clinical impact. For such therapies to be adopted and used widely, they must also be commercially viable. A recent review on the topic of translating vascular tissue-engineering technologies to the clinic does an excellent job of summarizing some of the key obstacles that need to be addressed.⁸¹ These include not only the scientific and technical challenges described above, but also such things as manufacturing ease, regulatory affairs, and product reimbursement. A key point is that the most important parameter in evaluating a new therapy should be clinical efficacy, and over the longer term, tissue-engineering approaches have the potential to improve efficacy dramatically, although perhaps at a higher initial cost. These points are critical to the field of tissue engineering as a whole, and as these technologies near the clinic, there has been an increased focus on them (see, e.g.,^{99–101}).

The protein-based biomaterials described in this review offer some clear advantages as scaffolds for vascular tissue engineering over alternate approaches based on synthetic polymers. They also bring their own distinct challenges, and several decades of focused research in the field has recently resulted in only limited clinical testing. We would like to reiterate the fact that this review has focused on one branch of the field of vascular tissue engineering and that information and insight from all the complementary approaches will be needed to bring about the clinical effect that this field promises. With continued progress, we are confident that the approaches described above will lead to new ways to treat cardiovascular disease and to improve patient care.

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Cells

- Endothelial Cells
- Smooth Muscle Cells
- Fibroblasts

Extracellular Matrix

- Collagen, ECM proteins and ground substance
- Elastin

Environment

- Soluble Factors
- Vasa Vasorum
- Nerve Cable
- Mechanical Forces
 - Tension
 - Compression
 - Shear
- pH, pO_2
- Cell-cell contacts
- Cell-matrix contacts

FIG. 1.

Schematic of structure of a medium-sized blood vessels, showing the main cellular, extracellular matrix, and environmental components (not to scale). Color images available online at www.liebertpub.com/ten.

FIG. 2.
Image of a collagen type I hydrogel construct containing vascular smooth muscle cells after 7 days in static culture. Color images available online at www.liebertpub.com/ten.

FIG. 3. Scanning electron microscope images of protein hydrogel matrices: (A) collagen type I, (B) fibrin, (C) collagen-fibrin composite (scale bar 300 nm).

FIG. 4.

Example of a bioreactor system used to mechanically stimulate engineered vascular tissues *in vitro*: (A) three-module bioreactor showing lumen and external flow direction (arrows), (B) schematic of bioreactor flow circuit during construct culture. (Used by permission from Williams, C., and Wick, T.M. Perfusion bioreactor for small diameter tissue-engineered arteries. *Tissue Eng* **10**, 930, 2004.)

FIG. 5. Scanning electron microscope images of (A) electrospun type I collagen, (B) electrospun elastin (scale bar = 1 micron in both panels). (Used by permission from Boland, E.D., Matthews, J.A., Pawlowski, K.J., Simpson, D.G., Wnek, G.E., and Bowlin, G.L. Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front Biosci* **9**, 1422, 2004.)

FIG. 6. Scanning electron microscope image of decellularized elastin scaffold (scale bar 100 nm). (Used by permission from Lu, Q., Ganesan, K., Simionescu, D.T., and Vyavahare, N.R. Novel porous aortic elastin and collagen scaffolds for tissue engineering. *Biomaterials* **25**, 5227, 2004.)

FIG. 7. Hematoxylin and eosin staining of (A) native porcine carotid artery, (B) collagen hydrogel construct, (C) hybrid construct with elastin sleeve and collagen hydrogel. (Used by permission from Berglund, J.D., Nerem, R.M., and Sambanis, A. Viscoelastic testing methodologies for tissue engineered blood vessels. *Tissue Eng* **10**, 1526, 2004.)

FIG. 8.

Hematoxylin and eosin–stained longitudinal section of tube of granulation tissue after 2 weeks of growth in a rat. Arrow indicates side from which silicone tubing was removed (magnification $\times 300$). (Used by permission from Campbell, J.H., Efendy, J.L., and Campbell, G.R. Novel vascular graft grown within recipient's own peritoneal cavity. *Circ Res* **85**, 1173, 1999.) Color images available online at www.liebertpub.com/ten.