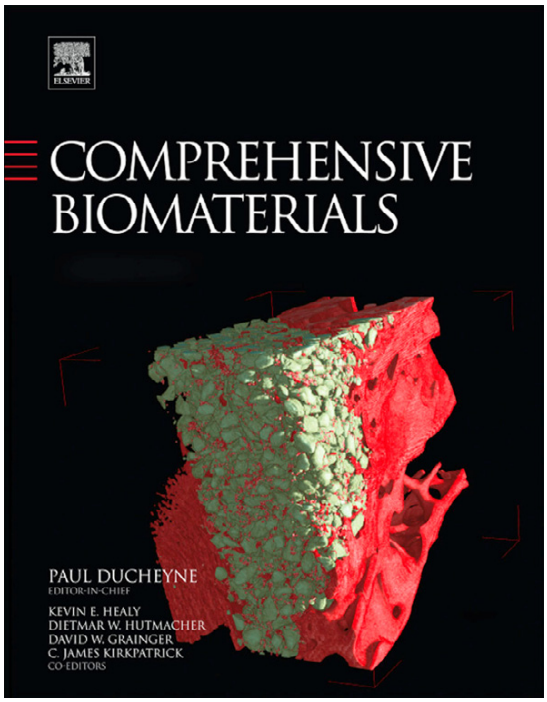

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5.527. Cardiovascular Tissue Engineering

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Glossary

Alginate An insoluble anionic polysaccharide found in the cell walls of brown algae.
Allograft Tissue graft generated from the same species as the recipient.
Elastin An elastic, fibrous glycoprotein in connective tissue, important role in blood vessels function.
Extracellular matrix (ECM) Multifunctional component of the extracellular portion of tissue providing structural support, growth factor sequestering, intracellular communication, and a key component of wound healing.
Fibrin An insoluble, nonglobular protein made from fibrinogen.
Hydrogels Derived from either synthetically or natural polymers are water-insoluble, and display flexibilities similar to natural tissue.
Polyglycolides (PGAs) Biodegradable, synthetic polymer that degrades through hydrolysis.
Polyhydroxyalkanoates (PHAs) Bacterial derived polyesters from fermentation of sugar or lipids.
Tissue engineering The *in vitro* construction of working biological systems utilizing a combination of cells and biomaterials to replace or repair either whole organ or portions of compromised tissue.
Vicryl Absorbable, synthetic polyglactin which becomes absorbed by hydrolysis.
Xenograft Tissue graft generated from a different species than the recipient.

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Abbreviations

BOOST trial	Bone-marrow-derived cell transfer after ST-elevation myocardial infarction trial
BPAECs	Bovine pulmonary artery endothelial cells
BPV	Bovine pericardial valves
CAD	Coronary artery disease
CaP	Calcium phosphate
ECM	Extracellular matrix
ECs	Endothelial cells
EHT	Engineered heart tissue
ePTFE	Expanded polytetrafluoroethylene
ESRD	End-stage kidney disease
GAGs	Glycosaminoglycans
H&E	Hematoxylin and eosin
hECM	Human extracellular matrix
IEL	Internal elastic lamina
IL-1	Interleukin-1
IL-6	Interleukin-6
IM	Internal membrane
MSCs	Mesenchymal stem cells
PAV	Porcine aortic valves
PCL	Poly-ε-caprolactone
PCU	Polycarbonate soft segment
PEG	Polyethylene glycol
PET	Polyethylene terephthalate
PEU	Polyether urethane
PGA	Polyglycolides
PHA	Polyhydroxyalkanoates
PIPAAm	Poly(*N*-isopropylacrylamide)
PLGA	Poly (D, L-lactic-*co*-glycolic acid)
POSS-PCU	Polyhedral ologomeric silsesquioxanes – polycarbonate soft segment
PTFE	Polytetrafluoroethylene
PUs	Polyurethane
PVA	Polyvinyl alcohol
PVD	Peripheral vascular disease
SBTE	Sheet-based tissue engineering
SIS	Small intestinal submucosa
SMCs	Smooth muscle cells
SPARC	Secreted protein acidic and rich in cysteine
TEBV	Tissue-engineered blood vessels
TEHVs	Tissue-engineered heart valves
TESA	Tissue engineering by self-assembly
TEVG	Tissue-engineered vessel grafts
TNF	Tumor necrosis factor

5.527.1. Introduction

The fields of cardiovascular tissue engineering and regenerative medicine have experienced tremendous expansion and progress over the past 20 years. Strategies have focused on the use of cells, tissues, scaffolds, and numerous combinations of these three components to address both scientific questions and clinical needs.

The most widely used definition of tissue engineering was proposed by Langer and Vacanti in a 1993 issue of *Science*, as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ."[1] This working definition is still relevant to the application of tissue engineering toward solving cardiovascular diseases. The subsequent chapter will focus on the following applications of tissue engineering in the cardiovascular field:

1. Cardiac patches
2. Cell delivery using biomaterials
3. Artificial blood vessels
4. Heart valves

While not an exhaustive list of topics within the field of cardiovascular tissue engineering, this list covers major areas of research advancement over the past few decades. Whether cardiac or peripherally focused, progress in these areas of research has the potential to impact tissue and organ function throughout the body. Because the cardiovascular system is dispersed throughout the human body, cardiovascular tissue engineering advancements can be seen as a rate-limiting factor for the development of thick tissues to repair or replace every critical physiological system (circulatory, endocrine, urinary, respiratory, musculoskeletal, digestive, lymphatic, integumentary, etc.). Without a well established cardiovascular system or microvascular perfusion, tissues would be confined by the limitations of the diffusion distance of oxygen in various tissues. This limitation would ultimately confine and restrict future advancements in tissue engineering. Therefore, long-term advancements in tissue engineering will depend, in part, on the ability to stimulate or facilitate the development of an appropriate vascular supply.

5.527.1.1. Bench Top to Bedside

The successful translation of discovery – when cardiovascular tissue engineering advancements find clinical or commercial homes – will depend on choices made during the development pathway from science to product. For example, the success of the translation from basic research findings into preclinical models depends, in part, on the appropriateness of the *in vitro* model. Likewise, the subsequent transition into the clinic depends, in part, on the relevance of the preclinical model to the clinical disease targeted. Finally, a balance of risks versus benefits must be considered when exploring the clinical translation of new discoveries. For certain technologies, a peripheral cardiovascular route to first-in-man clinical trials may have lower hurdles to overcome than a route going directly to cardiac applications. Initial clinical success in a lower risk procedure can help set the stage for future trials in higher risk applications.

To create a successful tissue-engineered construct, device, or technology, manufacturing as well as economic issues must be taken into account.[2] For example, scale-up considerations for tissue engineering products can present serious challenges. Researchers and scientists often view the reproducible creation

of small series of tissue-engineered constructs for benchtop or preclinical testing as a significant problem. This problem is only amplified when the production must be scaled up to meet the demands of a US population suffering in record numbers from vascular diseases. These diseases include the following:

1. heart failure that affects close to 6 million Americans (American Heart Association),
2. coronary heart disease that led to 448 000 bypass procedures in 2006,[3]
3. peripheral vascular disease that affects ~5 million adults,[4]
4. aortic valve stenosis that affects ~2% of people over the age of 65 and 3% of people over age 75.

If these manufacturing challenges can be overcome, mass production can transform a very expensive *in vitro* model into a commercially viable product. Thanks to economies of scale, the average cost per unit to create a product decreases as the volume is increased. This, in fact, may influence the choice of first target markets for tissue engineering companies looking for a hugely successful product launch. While this 'economies of scale' strategy has proven very successful in the device world, it has not been a lucrative approach for most tissue engineering companies. An alternative approach is to aim at niche markets with high-value products where economies of scales play a lesser role but where even a limited percentage margin results in significant profits. This can lead to sustainability and long-term growth. This model may be particularly appropriate for more complex technologies that still face significant development steps, and thus, more costly processes.

5.527.1.2. Successful Tissue Engineering Translations

Despite all of these challenges, many new tissue-engineering technologies are finding their way to successful clinical translation. In fact, economic activity in the combined fields of tissue engineering, regenerative medicine, and stem cell therapies has grown a remarkable fivefold from 2002 to 2007.[5] As of mid 2007, ~50 businesses offered commercial tissue-regenerative products or services with collective annual sales > $1.3 billion.[5] For continued success in cardiovascular tissue-engineered technology, scientists will need to overlay the future regulatory, clinical, manufacturing, and economic hurdles. The ability to do so will help secure the translation of future benchtop findings into the clinic.

5.527.2. Cardiac Patches

As the use of cell-based therapies in regenerative medicines has developed over the last decade, enthusiasm remains high that such treatments will provide a therapeutic option for patients with life-threatening cardiomyopathies. Work to date in this field has evaluated numerous cell types in both basic science and clinical settings for their ability to provide a source of cardiomyocytes (or promote their growth) and for their ability to provide tangible tissue regeneration *in vivo* (see **Chapter 5.507, Tissue Engineering and Selection of Cells**). A great deal of this work has utilized direct injection methods (i.e., syringe or catheter based), in which the desired cells are injected directly into the troubled heart tissue. Newer approaches rely on techniques where biologically active scaffolds or tissues are engineered *in vitro* and then implanted (see **Chapter 6.624, Cardiac Patch with Cells: Biological or Synthetic**). Most of these constructs utilize a biomaterial scaffold to support tissue assembly; however, other methods favor completely biological approaches.

5.527.2.1. Direct Injection Versus Tissues Constructs

To date, a number of cell-based therapies for treating cardiac injuries have been evaluated. These studies, both in the laboratory and in patients have primarily utilized direct injection as a delivery technique. While results from these clinical trials have demonstrated that these delivery techniques are safe and the cells well tolerated,[6–12] their limited success in regenerating lost heart muscle or regaining cardiac function leaves room for improvements. Evidence suggests that the major problem with direct cell injection into the heart is that few of the injected cells survive.[13,14]

In order to improve cell retention, many newer cell delivery methods have focused on bioengineered constructs, in which the desired cells are cultured and grown into biologically active tissue-like constructs,[15–17] which are currently being evaluated. They include (1) scaffold-based systems where cells are grown onto a degradable[18–20] material, (2) cell-containing hydrogels,[21,22] or (3) cellular sheets, free of any scaffold support.[17,23] Investigators have proposed that constructs for cardiac repair should be (1) contractile, (2) electrophysiologically stable, (3) mechanically robust, (4) quickly vascularized, and (5) non-immunogenic (see **Chapter 5.503, Biomaterials and the Microvasculature**).[16,21,22]

5.527.2.2. Biomaterials for Cardiac Tissue Engineering

5.527.2.2.1. Scaffold-based systems

Various biological and synthetic materials are currently being evaluated as the structural backbone for a number of *in vitro* engineered constructs. These include biological extracellular matrices such as collagens, elastin, fibrin, alginates, silk, etc., as well as biodegradable synthetic polymers such as polyglycolides (PGAs) (e.g., Vicryl™), polylactides, poly-ε-caprolactone (PCL), etc. Collagens, such as collagen type I sponge scaffolds, are flexible and porous allowing cellular integration. The placement of inert collagen type I scaffolds supplemented with adjunct cytokine administration has been shown to promote microvessel formation within injured rat myocardium.[24] Alginates have proven efficient in constructing tissue-engineered scaffolds. They can be efficiently seeded, retaining a high percentage of seeded cells within a brief time.[25,26] Alginate patches, however, appear to have poor integration into the myocardium because of limited microvessel formation. Integration may be improved through heterotopic transplantation, but questions about its clinical application exist.[15]

Extracellular matrices or various combinations of extracellular matrix (ECM) proteins have been explored as scaffold structures for tissue engineering purposes such as carriers for cell-based therapies. These materials can be readily casted, electrospun, or cultured using a variety of strategies to optimize surface area and void volume to create specifically designed scaffold carriers for cell therapies[27–29] (**Figure 1**).

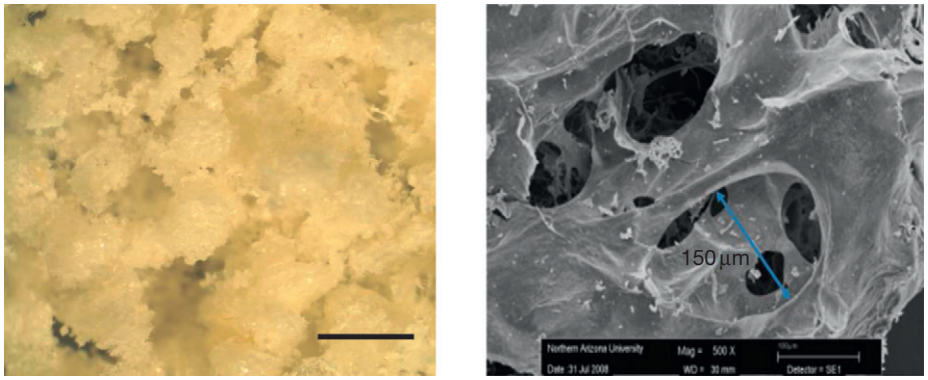

Figure 1 Left, lyophilized human extracellular matrix (ECM). Scale bar = 1 mm. Right, scanning electron micrograph of lyophilized human ECM. Arrow shown = 150 μm.

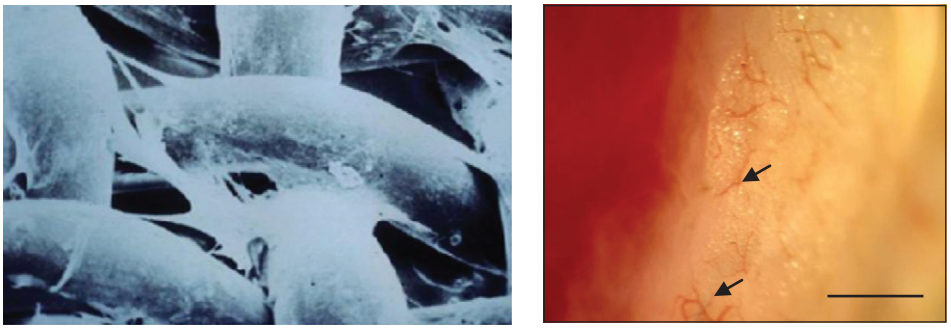

Figure 2 Left, scanning electron micrograph of a Vicryl™ scaffold seeded with human fibroblasts (cardiac patch). Scale bar = 50 μm. Right, cardiac patch placed onto the epicardial surface in a rodent infarct model. Arrows highlight new microvessels. Scale bar = 150 μm.

A number of laboratories have used Vicryl™ mesh or PGAs in construct formations. In addition, PGAs are employed in preseeded dermal repair constructs currently on the market (Theregen Inc.; Figure 2). PGAs are flexible and degradable and can be used to create woven or nonwoven scaffolds. Under standard culture conditions, PGAs degrade roughly 50% over 4 weeks. Such materials allow for spontaneous and synchronized contractions of seeded cardiomyocytes.[19,30,31]

5.527.2.2.2. Hydrogels

There has been work with liquid collagen I and Matrigel™ with additional growth supplements to create engineered heart tissue (EHT) *in vitro*.[21,22] These constructs were fabricated as circular bands and then fused together into 'multiloop' EHTs. These EHTs beat spontaneously and have been sutured onto the epicardial surface of the myocardium following acute myocardial infarction in rats. After 4 weeks, implanted EHTs remained on the epicardial surface of the myocardium with limited integration into the myocardium. Implanted EHTs contributed to improve cardiac function and even electrically coupled with the host myocardium.[21,22]

5.527.2.2.3. Cellular sheet structures

The creation of cells sheets that can be removed from their culture containers dates back to, at least, the mid-1970s and the work of Green and his associates. After painstakingly developing the appropriate culture media for the serial proliferation of human keratinocytes, a method was developed to produce, and enzymatically detach, cohesive sheets of human epithelium.[32,33] Soon after, this approach was used clinically and autologous sheets were produced and successfully implanted in seriously burned patients.[34] To our knowledge, this is the first cell-based therapy using cultured cells.

In the last decade, Okano and his group have popularized the use of poly(*N*-isopropylacrylamide)-coated cell culture plates to successfully create thin sheets of epithelial cells as well as cells of mesenchymal origin.[23,35,36] This coating is a temperature-sensitive polymer that allows for harvesting of fully intact cell sheets by lowering the ambient temperature, without the need for enzymatic digestion or mechanical manipulation (see **Chapter 5.530, Medical Applications of Cell Sheet Engineering**). Constructs grown and harvested in this manner can have multiple advantages. First, they do not include any foreign scaffolding that could create inflammatory responses and compromise the healing of a typically very fragile tissue, for example, cornea epithelium.[37] Second, the elimination of any enzymatic treatment preserves the integrity of the basement membrane of the cells sheets. As a result, these tissues can be directly applied to the desired tissue areas without the need for suture or other means of adhesion because of the preserved basal surface. Finally, the lack of enzymatic treatment also preserves the cellular organization of the sheet, and more particularly, relevant to cardiac applications, can help

maintain the cell–cell communication through gap junctions.[38] This is a critical advantage to create a contractile tissue as gap junctions are the structures that allow electrical transmission that results in the synchronous pulsation of the entire heart.[39] Culture containers coated with temperature responsive polymers are now commercially available under the brand name UpCell™ and are produced by Nunc™ (now part of ThermoFisher Scientific).

The creation of cardiac tissue using this approach is encumbered with two limitations. While cultured sheets of stratified epitheliums are notoriously fragile, which is part of the attraction of the thermally responsive culture surfaces, even sheets made out of cardiomyocytes or mesenchymal stem cells (MSCs) appear to also be mechanically weak (Biomaterials 2005).[40] As the weakness of epithelial sheets is likely due to the lack of ECM in these constructs, one would expect cells of mesenchymal origin to be able to produce a more robust tissue. However, in some studies, cells were cultured in the absence of ascorbate compounds that are known to be necessary to stimulate maximal collagen production. Recently, cell sheets were produced using this same approach from cultures of smooth muscle cell (SMC) and fibroblasts.[41] These cultured cell sheets were supplemented with ascorbate 2-phosphate and 18.8 pM of copper (II) sulfate (believed to increase collagen cross-linking by lysyl oxidase) but were only cultured for a total of 7 days, which may be insufficient to generate sufficient mechanical strength. No sheets produced using this approach have been formally tested for their mechanical strength, and this appears to be a significant challenge for the design of cardiac patches that would play a significant mechanical role. Using much longer culture periods could provide sufficient time for the cell to assemble complex and relatively mature ECM structures leading to the production of stronger sheets. Another option could be to introduce a synthetic scaffold to provide the requisite mechanical strength, but some preclinical results suggest that a biological patch can lead to better tissue regeneration and function even in not cell seeded.[42]

Another limitation of this approach, and of most other tissue engineering approaches, is the limited thickness of tissues that can be produced as a result of diffusion limitations. This is particularly important for a clinically relevant heart patch as the wall of the human heart is over 1 cm thick. While a heart patch product can aim at having a purely parachrine effect, particularly if applied to a recently injured heart (myocardial infarct), it would be clearly beneficial to also be providing mechanical support as such support has been shown to be beneficial.[43] In addition, a robust patch that could be used to repair full- or near full-thickness defects could be used for more aggressive surgical intervention that would be needed to address many degenerative heart diseases. To address this limitation, Okano's group has proposed a number of strategies. One general method is to include endothelial cells in a stack of cells of mesenchymal origin (Biomaterials 2010).

This can be achieved by creating sheets from co-cultures, including endothelial cells or sheets made exclusively from endothelial cells as intercalated in the stack. Initial results *in vitro* were promising as the endothelial cells appear to reassemble into a network of elongated channels mimicking the native microvasculature. However, it remains to be seen if this network can be assembled and connected to the host circulation before cells in the middle part of the construct suffer from hypoxia. While preliminary studies in rats are promising,[44] scaling-up to a human size construct could be challenging. Another strategy proposes to serially implant sheets to allow each layer to be vascularized by the host[45] before the implantation of the next layer. This method has produced contractile and well perfused myocardial tissues of up to 1 mm in a rat model. Furthermore, these tissues could be successfully ectopically transplanted with their newly formed vasculature. This strategy of using the body as an *in vivo* bioreactor has certainly gained momentum in recent years.[46,47] While the multiple surgeries required would be a significant risk to the patient, the upside would clearly justify the risk in the case of a cardiomyopathy (**Figure 3**).

5.527.2.2.4. Limitations of cardiac constructs

While biomaterials such as polymer scaffolds provide structural support for culture of tissue constructs, there are a number of potential limitations surrounding biomaterial-based scaffolds,[17,21,22] including (1) the potential for inflammatory response, (2) possible toxic degradation products,[23,48] (3) variance of compliance between polymers and heart tissue,[21,22] and (4) the lack of interconnectedness between cells because of interference from the scaffold material.[21,22,48] The use of

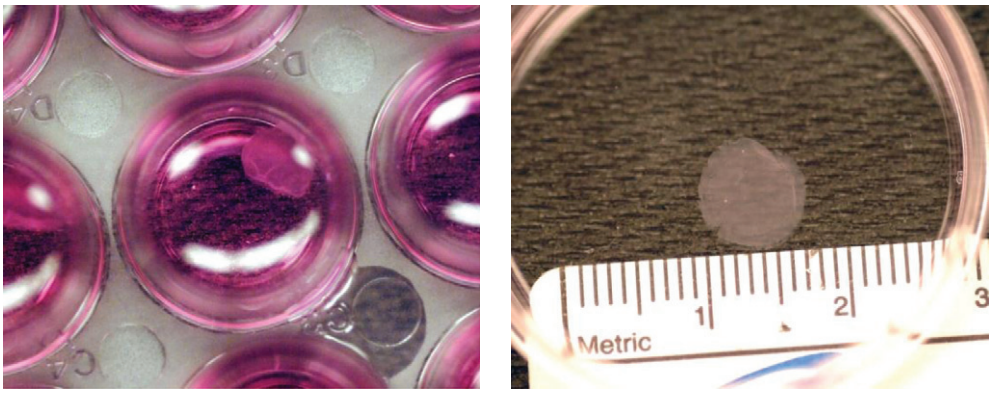

Figure 3 Bovine pulmonary artery endothelial cells (BPAEC) sheets harvested using 24-well PIPAAm temperature sensitive culture plates. Left, disassociated BPAEC cell sheet in culture well and media. Right, harvested BPAEC cell sheet harvested from original culture plate. Courtesy by J. Lancaster, 2009.

completely biological cell-sheets would likely avoid all the limitations mentioned above but may be restricted by their mechanical strength. Apart from the limitations described, a major obstacle of further developments, particularly enrollment into clinical applications, will be the procurement of suitable human stem or progenitor cells.

5.527.3. Cell Delivery Using Biomaterials

In recent years, many investigators have invested significant efforts into the use of living cells as a therapeutic agent (see **Chapter 5.507, Tissue Engineering and Selection of Cells**). These cells are intended to deliver certain products (e.g., growth factors) or to be a replacement cell type to replace lost tissues. These living cell therapies require that cells maintain their activity at the transplant location whether it is the bone marrow, skin, liver, or heart. This has presented a significant hurdle. Thus far, the best-published results of the beneficial effects of bone marrow cell transplantation after acute myocardial infarction come from the bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial.[49] In this study, significant improvements in left ventricular ejection fraction were seen at 6 months after treatment with bone marrow cells. However, these improvements were no longer significant at 18 months. A few investigators have reported cell retention as a major obstacle for these types of cell replacement therapies, indicating that only 1.3–2.6% of infused bone marrow cells are retained in the heart.[50] While these cell-based therapies continue to advance through the clinical trial gauntlet, many investigators are looking into a variety of delivery agents such as hydrogels, alginates, and polymer scaffolds to provide a hospitable environment for transplant cells that can subsequently be delivered into the target tissue.

Future cell therapy will depend on the delivery of a sufficient number of living functional cells, to the appropriate location for an ideal amount of time. While most therapies are initially combined with a traditional surgical intervention, these therapies can also take advantage of the wealth of minimally invasive strategies that have been developed to avoid traumatic procedures such as a thoracotomy. These minimally invasive techniques rely on injectable or collapsible biomaterials combined with a target cell population. The development of such interventional techniques has multiple advantages. For example, in cardiac applications, minimally invasive cell delivery can allow high-risk patient populations to be treated who may not be medically stable for large open surgical procedures. Additionally, recovery time for these patients can be minimized, which significantly benefits the patient's quality of life and decreases morbidity and mortality rates. A recent example of the clinical benefits of minimally invasive approaches for the treatment of cardiovascular disease is demonstrated by the rapid adoption of thoracic endografting instead of open surgical repair in patients with descending thoracic aortic aneurysms. The benefits of thoracic endografting are (1) less perioperative morbidity, (2) lower hospital costs, and (3) equal mid-term life expectancy when compared to open surgical repair.[51] Another clinical example where minimally invasive approaches have been leveraged in efforts to treat higher surgical risk patients is the use of minimally invasive robotic coronary bypass using the da Vinci S System. These procedures are performed under endoscopic vision with surgical assistance from the da Vinci S robotic system. These conditions allow for minimally invasive coronary artery bypass grafting with an enhanced ability to control precise and stable operative manipulations.[52]

Future cell-based therapies will likely focus significant efforts upon minimally invasive delivery. Additionally, the delivery vehicle or the avenue by which transplant cells are introduced into the target tissue will remain a key area of investigation. A variety of delivery formats are described below.

5.527.3.1. Hydrogels

Polymers that form hydrogels are potentially useful for both tissue engineering and drug delivery therapeutic approaches. Hydrogels offer multiple advantages such as a good safety profile, a simple and rapid assembly process, and a large range of achievable geometries. They can also be injectable, and are a cost effective and cell-friendly substrate. However, because of their very high water content, hydrogels lack sufficient mechanical strength to be used as the main scaffold in many applications. Another drawback of hydrogels is that cell activity will often alter their shape. One classic example of this phenomenon is the so-called 'contraction' of collagen gels seeded with cells.[53] This compaction process is typically anisometric and can reduce the dimensions of these constructs by a large percentage. This can limit the usefulness of these scaffolds for the production of an intricate structure. Hydrogels can be classified as either naturally occurring or synthetically derived. Natural hydrogels include collagen (see **Chapter 2.215, Collagen: Materials Analysis and Implant Uses**), gelatin, fibrin (see **Chapter 2.217, Fibrin**), alginate, agarose, and hyaluronate (see **Chapter 2.214, Hyaluronic Acid**). Some synthetically derived polymer hydrogels include poly(acrylic acid), poly (vinyl alcohol), polyphosphazene, and poly(ethylene oxide).[54] Compatibility with the patient is an important consideration when using hydrogels because potential for mild or severe inflammatory response exists.[55] For example, alginates rich in mannuronic acid have been shown to stimulate an inflammatory response through a monocyte-activation pathway. These stimulated monocytes produced a variety of proinflammatory cytokines including TNF, IL-1, and IL-6.[56] It is also important to consider the rate of degradation once administered, as each hydrogel has its own specific characteristics and degradation profiles.[57] Ideally, the degradation behavior of the hydrogel should match or be closely compatible to the rate of new tissue formation.[58] The rate of new tissue formation is greatly tissue-dependant and the mechanical properties of these hydrogels are critical to their ability to create and maintain a space for new tissue formation *in vivo*.

5.527.3.2. Nanofibers

Another biomaterial approach for cell delivery for cardiovascular repair utilizes self-assembling peptide[59–61] or alginate[62] nanofibers (see **Chapter 2.205, Self-Assembling Biomaterials**). Both peptide and alginate nanofibers provide the heart with structural and physical support by replacing the lost or

compromised ECM; meanwhile, alginate derived versions appear to gradually dissolve[62] and therefore lack a long-term contribution of mechanical support. As reported by Davis *et al.*[59] and Padin-Iruegas *et al.*,[61] peptide nanofibers appear to help significantly with cellular survival, whether in conjunction with the exogenously administered cells or the endogenous cell populations of the target tissue, possibly through the establishment of microenvironments. In addition, incorporation of growth factor or cell signaling elements could greatly contribute to the therapeutic potential of nanofiber-based therapies.

5.527.3.3. ECM Proteins

ECM proteins have been evaluated as a potential scaffold structure to support various cell-based therapies (see **Chapter 2.207, Extracellular Matrix: Inspired Biomaterials** and **Chapter 2.220, Extracellular Matrix as Biomimetic Biomaterial: Biological Matrices for Tissue Regeneration**). For example, stem cell therapies have evaluated the use of a complex tumor matrix, Matrigel™. This product is marketed by BD Biosciences and is composed of Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. Matrigel™ has demonstrated the ability to facilitate the growth of various stem cell populations and maintain an undifferentiated state for a period of time.[63] The main ECM proteins within Matrigel™ are laminin and collagen. However, some of the challenges of Matrigel are that its origin is from a tumor-based etiology and the matrix itself is poorly defined.

Other investigators have looked at harnessing the cell-based ECM produced by tissue culture. Here, human ECM (hECM) can be produced to have embryonic-like characteristics and therefore may serve as a hospitable environment for culturing transplant cells (**Figure 4**), especially adult or embryonic stem cells.[64] Utilizing a hECM material as a delivery vehicle for cell-based therapies may facilitate improvement in engraftment and long-term outcomes of the therapy. In these studies, bioreactors were developed to expose neonatal fibroblasts to early embryonic conditions during the production of their ECM and conditioned culture media. The resulting hECM contained a protein matrix that includes collagen I (see **Chapter 2.215, Collagen: Materials Analysis and Implant Uses** and **Chapter 2.216, Collagen–GAG Materials**), tenacin, hyaluronic acid, and fibronectin, as well as proteins that have been found to be prevalent in embryonic ECM such as collagens III, V, and SPARC.

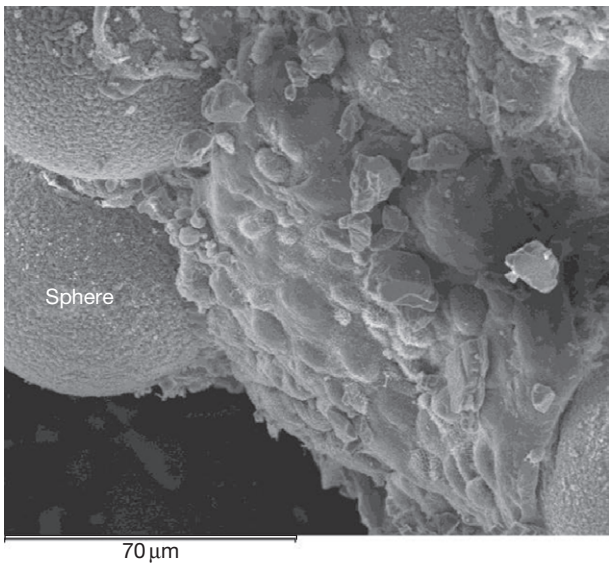

Figure 4 Embryonic stem cells were grown and expanded on the human extracellular matrix material.

5.527.3.4. Synthetic Polymer Scaffolds

Other cell delivery strategies have focused on the use of synthetically created polymer scaffolds. For orthopedic tissue engineering applications, biomimetic scaffolds provide a necessary framework for bone analogs. The osteogenic potential of PCL/calcium phosphate (CaP) matrices have been closely studied for their ability to facilitate growth and residence of a human MSC population for periods of up to 8 weeks. In these studies, MSCs were able to adhere, migrate, and differentiate along the osteogenic lineage within these scaffolds. The PCL/CaP scaffolds showed up to a 27-fold increased degradation compared to PCL scaffolds alone.[65]

Other synthetic polymer scaffolds that have been explored as cell delivery agents are resorbable or degradable poly(D,L-lactic-*co*-glycolic acid) (PLGA) microspheres. While these polymer scaffolds, to date, have not seen wide use within the cardiovascular research space, they are actively being studied for orthopedic applications.[66] In this application, initial cell viability studies have demonstrated the feasibility of using a PLGA/Polyethylene glycol (PEG) polymer matrix and a cell or drug technology.

Furthermore, a PLGA microsphere platform has been demonstrated to support bovine chondrocyte culture onto the PLGA microspheres. Positively charged PLGA microspheres showed the highest cell attachment, growth, and function compared to hydrophobic and negatively charged microspheres. It has been concluded that surface-modified PLGA microspheres can potentially be used as an injectable delivery system for cells into a tissue defect site.[67]

The resorbable PLGA microsphere platform has been widely evaluated as a drug delivery[68–71] platform but its use has been limited as a cell delivery vehicle, mostly into the orthopedic field.[67,72] This resorbable polymer technology may be limited by the relatively rapid degradation rate of PLGA *in vivo*, resulting in obliteration of its mechanical integrity prior to adequate tissue growth.

5.527.4. Tissue Engineering of Artificial Vesseles

Synthetic polymeric vascular grafts were developed to address three issues in vascular surgery: (1) the fact that there are no 'spare' large diameter vessels in the human body for autologous repair, (2) the unsatisfactory performance of nonautologous natural grafts (xeno- and homografts), and (3) the limited availability of small diameter autologous grafts (see **Chapter 6.628, Vascular Grafts**).[73] The development of synthetic vascular prosthesis is often credited to Voorhees, Jaretski, and Blakemore, who first published on synthetic

tubes.[74] In the following decades, two synthetic materials rapidly emerged as the most clinically successful; expanded polytetrafluoroethylene (ePTFE) and woven or knitted polyethylene terephthalate (PET) fibers (Dacron). While these materials performed well as large and even medium diameter conduits, synthetic vascular grafts have not proven successful in small diameter applications (<6 mm ID).[75,76]

Today, coronary artery disease (CAD) is the largest single cause of mortality in both men and women in the United States (American Heart Association). Despite the rapid adoption of percutaneous interventions for coronary procedures, there were still 448 000 bypass procedures performed in 2006. Peripheral vascular disease (PAD) is also a condition that can be treated by the use of a small diameter bypass graft. PAD affects ~8 million Americans and is associated with significant morbidity and mortality.[77] CAD and PAD have similar risk factors and, not surprisingly, are often found in the same patients.[78] In cardiac and peripheral bypass surgery, the best outcomes are achieved when diseased vessels are replaced with autologous veins or arteries. For example, the internal mammary artery has >90% patency over 7 years in cardiac applications, while the saphenous vein maintains ~50% patency after 7.5 years in peripheral sites.[79] However, many patients do not have adequate blood vessels for use as grafts because of either poor quality or insufficient quantity after a previous harvest. This is particularly true for PAD cases where good autologous vessels are often preferentially used to treat CAD. In these cases, the patients are restricted to modest treatment modalities often leading to myocardial infarction or limb amputation. Therefore, there is a substantial unmet clinical need for an alternative supply of vessels to replace small diameter diseased arteries.

5.527.4.1. The Evolution of Tissue-Engineered Blood Vessels

The clinical complications of small diameter synthetic prostheses commonly result from an inflammatory response to and pathologic remodeling of the prosthetic material. These failure modes have led to enhanced interest in new approaches for designing vascular grafts. A tissue engineering approach may overcome certain obstacles faced by synthetic grafts in small-diameter vascular applications.[80] For example, a tissue-engineered blood vessel (TEBV) would presumably be more responsive to physiological signals and avoid the pathological remodeling seen in synthetic grafts. To be successful, a tissue-engineered vessel grafts (TEVG) must have sufficient strength to withstand cyclic loading and changes in blood pressure. It should also have an antithrombotic luminal surface, and ideally, should match the compliance of the host vessels at the anastomotic sites.[80] While one would hope that tissue-engineered constructs would enhance their functionality over time via tissue remodeling *in vivo*, it should be noted that a TEVG must fulfill its mechanical role immediately upon implantation. Similarly, unlike tissue-engineered skin, cornea or other nonload bearing tissues, the remodeling of the blood vessel must not lower its mechanical strength below a certain threshold to avoid dilation and rupture. Consequently, inflammation, rejection, or other biodegradation processes are of particular importance for the successful outcome of a TEBV.

Often cited as the first TEBV, a construct produced by casting a tube of collagen gel containing bovine vascular cell lines (SMCs, fibroblasts, and endothelial cells) was reported by Weinberg *et al.*[81] in 1986. This first model did not have any synthetic material with the aim of avoiding the detrimental effect of introducing foreign materials into the body. This was in line with the developing concept, which would later be called 'tissue engineering,' of creating 'biological substitutes that restore, maintain, or improve tissue function.' However, without additional mechanical support, these vessels were highly distensible and failed during burst strength testing at low intraluminal pressures.[81] This approach was widely repeated in the following decade with collagen or other biological gels, and while valuable as *in vitro* models, these completely biological constructs still displayed limited burst strengths.[53,82–84] The next generation TEBVs were hybrids incorporating synthetic and biological materials.[85] In the ensuing years, the field of vascular tissue engineering became dominated by biomaterial design and the original vision of a biological construct was relegated to the realm of 'desirable but unachievable.' However, it eventually became evident that the presence of a permanent synthetic scaffold would negate the potential benefits of including biological or living components.

5.527.4.2. Biodegradable Scaffolds

In the late 1980s and early 1990s, numerous investigators used various resorbable synthetic polymers to produce fully biodegradable vascular grafts.[86–89] The hope was to produce an 'off-the-shelf' template that would guide the body's natural repair process toward vascular regeneration. However, to this day, finding the right balance between scaffold degradation and extracellular regeneration in order to maintain sufficient mechanical strength has proven to be very challenging. In 1999, Niklason *et al*. produced TEBVs by culturing bovine and porcine cells under pulsatile conditions on poly(glycolic acid) (PGA) felt for 8 weeks. These small diameter vessels had rupture strengths of up to 2150 mmHg.[90] However, it was found that PGA degradation may have led to dedifferentiation of the SMCs, which could possibly lead to hyperplasia and graft occlusion.[91] Over the last decade, this approach did not yield mid- or long-term results in an animal model and attempts to develop a construct with similar mechanical strength using human cells were unsuccessful.[92–94] Around the same period, Shum-Tim *et al.*[95] produced medium diameter graft using a PGA:polyhydroxyalkanoate (PHA) copolymer and ovine vascular cells. Short segments implanted in the abdominal aorta of lambs showed promising results up to 3 months with favorable patency. In parallel, Shin'oka *et al.*[96] developed a similar approach, using PCL and PGA, which led to the first implantation of a TEVG in a human. These TEBVs were implanted in the low-pressure pulmonary circulation of infants with severe congenital defects. This method has evolved toward the use of bone marrow cells and a PGA/ε-caprolactone/L-lactide copolymer.[97] While this tremendous success does not address the need for arterial bypass conduits, it finally established the feasibility and potential of vascular autologous cell-based therapy.

Other researchers have examined the use of various processed animal tissues as another type of biodegradable scaffold

for tissue engineering. While homografts and xenografts have been shown to be poor conduits, over a decade of efforts in developing decellularization technologies has brought the promise of natural scaffolds that would not trigger immune response. This approach is based on the idea that the removal of all cellular components could leave behind exclusively the ECM. One of the first and most widely used decellularized ECM is the porcine small intestinal submucosa (SIS), which has been used clinically for many years. As a vascular graft, SIS has been used in animal models since 1990 with relatively positive results[98,99] but has yet to be tested in humans for that application. Recently, a modified porcine SIS was used as a vascular prosthesis.[100] After implantation in a rabbit, these grafts required native endothelial cell infiltration and aggressive anticoagulation therapy in order to prevent thrombosis. These results underline the importance of endothelial cell coverage in TEBV for small diameter arterial bypass. This was also exemplified in studies using ePTFE grafts endothelialized with autologous EC isolated from vein, fat, or blood.[101–103] Using this technique, dramatic improvements have been seen in some clinical cases, particularly for below-the-knee procedures.[104,105] While decellularized tissues offer an attractive option for many regenerative approaches, these tissues appear to, at least partly, trigger some immune recognition.[106,107] This suggests that they may not be appropriate for vascular tissue engineering where even a minor inflammatory reaction could lead to a negative outcome.

5.527.4.3. A Cell-Synthesized Scaffold

In the mid-1990s, L'Heureux *et al*. came to the conclusion that reconstituted ECM proteins such as collagen were unlikely to provide the requisite mechanical strength needed for creating an implantable TEBV. Even if these reconstituted matrix proteins did provide the requisite mechanical strength after various physicochemical treatments, these scaffolds would be aggressively degraded by the immune system because of their unnatural chemical or tridimensional organization. Faced with this realization, L'Heureux turned to cultured cell as a source of unprocessed and naturally organized ECM. The general approach, which was later termed 'tissue engineering by self-assembly' (TESA), takes advantage of the ability of cells of mesenchymal lineage to assemble complex and abundant ECM when cultured for extended periods in the presence of ascorbate. While TESA can be used to direct cells to deposit natural ECM in various geometries, the initial developmental work was aimed at producing sheets of living cells and the ECM they produced. This particular version of TESA was labeled 'sheet-based tissue engineering' (SBTE).

For building blood vessels, L'Heureux *et al.*[108] created sheets from human adult dermal skin fibroblasts and from umbilical vein SMCs. After a month of culture, the sheets were detached from their culture substrate and were wrapped around a temporary tubular support. After an additional culture period, referred to as 'maturation,' of 8 weeks in a bioreactor providing active perfusion, all the individual layers fused together to form a cohesive living cylinder. In this initial model, three concentric layers were formed to mimic the native architecture of a human blood vessel. The outermost layer was formed by a rolled fibroblast sheet and played the role of the adventitia. The median layer was formed by a rolled sheet of SMCs and played the role of the tunica media. The innermost layer was called the 'internal membrane' (IM) and was made of a rolled fibroblast sheet that was matured and then devitalized by dehydration. The IM was included in the design to mimic the antimigratory role of the internal elastic lamina (IEL). The IEL is an effective physical barrier that separates the SMCs of the media from the plasma and the more fragile and less proliferative endothelial cells. Damage to the IEL has been identified as an important factor in the development of atherosclerosis but also of intimal hyperplasia observed after balloon angioplasty.[109,110] One would also expect it to be a key factor in the development of intimal hyperplasia at the anastomosis with a vascular graft. The IEL is largely made of elastin, which is known to be resistant to most enzymes produced by SMCs. However, the IM is largely made of collagen, which is sensitive to many matrix metalloproteinases. Accordingly, the IM was designed to be much thicker than the native IEL; the strategy being that cell migration toward the lumen would be significantly inhibited during the inflammatory phase following implantation. In fact, future long-term *in vivo* studies would confirm that the IM is not readily degraded and remains acellular for months.[111] These constructs could then be removed from around the mandrel and its lumen that is seeded with endothelial cells (**Figure 5**).

These TEBVs had supra-physiological burst pressures in excess of 2500 mmHg at 3 mm internal diameter. They were implanted in a canine arterial bypass model and, as such, were the first completely TEBVs with clinically relevant mechanical properties.

In the next decade, L'Heureux and his team focused on translating these promising early results into clinical reality. For that purpose, the assembly strategy was streamlined and SMCs were eliminated from the process without affecting the mechanical strength of the vessels. In addition, the TESA method was adapted to cells (fibroblasts and venous endothelial cells) isolated from age- and risk-matched individuals, that is, older individuals suffering from cardiovascular disease. Finally, the bioreactor system was simplified to be compatible with a high-throughput, reliable, and economically viable production process. The resulting vessels displayed an average burst pressure of 3468 ± 500 mmHg at a diameter of 4.2 mm.[111] This new human TEBV design was tested in immunosuppressed canines and primates as well as in nude rats for up to 9 months without signs of mechanical failure. On the basis of the promising preclinical results, vessels were built for a series of 10 patients (age 29–89) suffering from end-stage renal disease (ESRD) and in need of a vascular graft for hemodialysis access. These TEBVs displayed burst pressures as high as 5763 mmHg (average 3340 ± 849) at a diameter of 4.8 mm and were implanted as an arteriovenous shunt. The clinical protocol called for a 3-month observation period to demonstrate mechanical stability followed by the use of the graft for hemodialysis access, that is, the graft would be punctured with two 16-gauge needles 3 times per week. In the initial report, one graft had a primary patency (patency without intervention) of 11.5 months despite the very unfavorable hemodynamics and mechanical injuries associated with this clinical application.[112] In a later report, these TEBVs showed primary

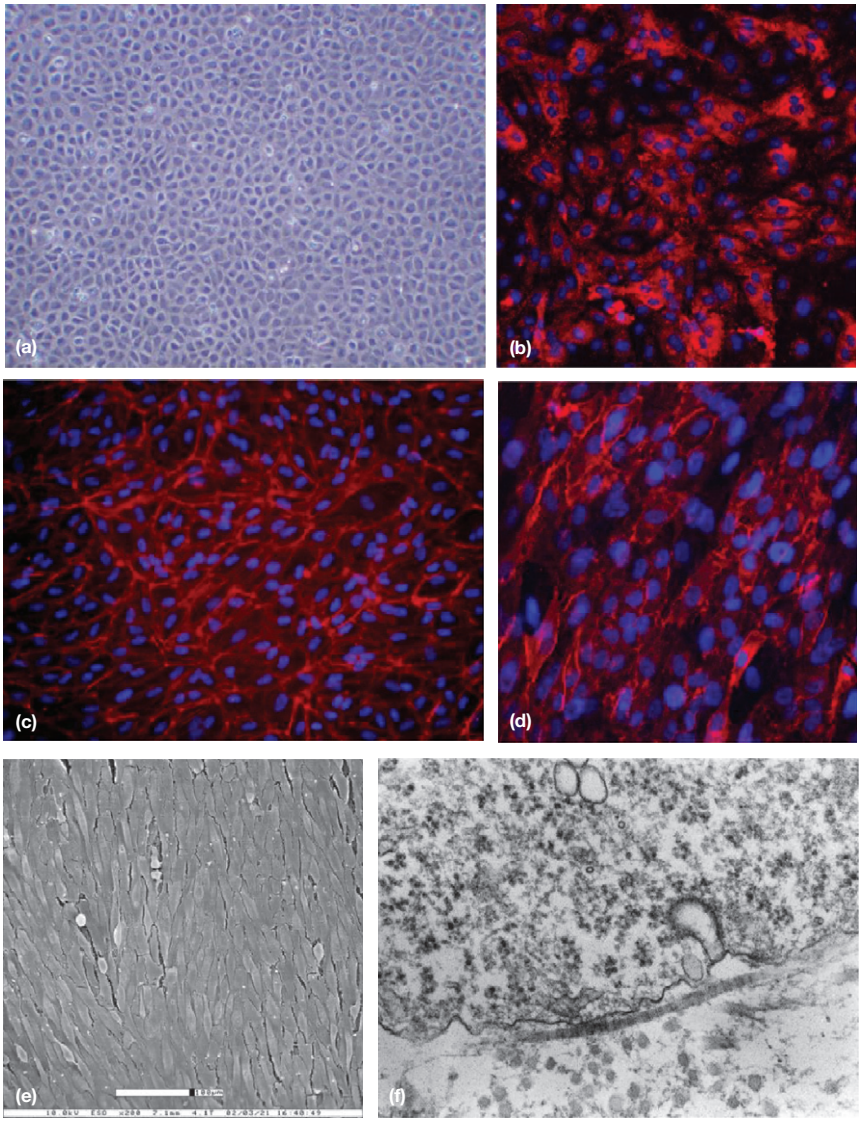

Figure 5 Endothelialization of tissue-engineered blood vessel produced by tissue engineering by self-assembly. (a) A phase contrast micrograph shows the typical 'cobble stone' appearance of a confluent culture of human venous endothelial cells on polystyrene tissue culture flask. (b) Von Willebrand factor (red) is a marker specific to endothelial cells and is commonly used to identify and characterize the purity of culture *in vitro* using immunolabeling techniques (nuclei are blue stained by Hoechst 33258). ECs can also be labeled on the surface of tissue-engineered blood vessel to determine the degree of confluence of the cells as well as their phenotype. (c) *En face* view of a confluent endothelium on the luminal surface. The actin microfilaments have been stained with phalloidin (red) to demonstrate a pericytoplasmic arrangement suggestive of a mature endothelium (nuclei in blue). The endothelium of the tissue-engineered blood vessel can also be stained for specific markers to confirm the nature of the monolayer. (d) PECAM/CD31 (red) specifically labels the cell–cell contacts between endothelial cells in confluent endothelium. (e) Scanning electron microscopy can also be used to ascertain the coverage of the luminal surface of a tissue-engineered blood vessel but this method can often cause cell losses due to the harsh treatment. (f) Transmission electronic microscopy can be used to study biomaterial/EC interaction. In this micrograph, the cytoplasmic membrane of an EC can be seen in close apposition with a collagen fiber of the luminal surface. The characteristic 67 nm periodicity of the striation of a native collagen fiber can be observed.

patency rates of 78% at 1 month, and 60% at 3 and 6 months after implantation.[113] These rates approached the objectives of the Dialysis Outcomes Quality Initiative (a program run by the National Kidney Foundation Disease) across all patient populations for native vein fistulas.[114] Considering that native veins perform practically twice as well as ePTFE grafts and that this study included only patients with a high-risk of graft failure, these results suggest that this new self-assembled 'biomaterial' could be particularly well-suited for vascular applications (**Figure 6**) (see **Chapter 2.205, Self-Assembling Biomaterials**).

5.527.4.4. Elastin as a Natural Scaffold

Natural polymers such as elastin are potential alternatives to synthetic materials. Unlike collagen, this ECM has the potential to be assembled in mechanically strong tissues that are capable of resisting the body's degradative responses.[115]

Elastin, derived from its water-soluble precursor tropoelastin, consists of repetitive glycine-rich hydrophobic domains of variable length interspaced with alanine-rich regions containing crosslinkable lysine residues. In its native form, elastin is a network of elastic fibers that are cross-linked between lysine residues. Cross-linking enhances elastin's biostability and controls its mechanical properties. Cross-linking occurs after cellular secretion of tropoelastin with local fiber deposition. Because elastin cannot be recovered from tissue in its native form, simpler proteins comprising repetitive polypeptide sequences have been assembled in an effort to duplicate the desirable properties of elastin.

McMillan and coworkers have produced large elastin-like proteins by assembling gene segments encoding the peptides VPGVG and VPGKG that represent the mobile domains in elastin. The genes isolated from this assembly process are up to 3000 base pairs long, contain 39 repeats of the sequence $(VPGVG)_4$ (VPVKG), and produce an 81 kDa protein after expression in *E. coli*.[116] This protein can be spun into fibers, filaments, and ribbons with a tensile strength of about 35 MPa and the material modulus 1.8 GPa.[117] Elastin-like polymers made by these methods have been used as carriers for antitumor drug delivery.[118]

Polymeric scaffolds of varying sizes based on the repeating elastin sequence VPGVG have also been constructed by sequential polymerization of the pentapeptide after solid-state chemical synthesis. These elastin-like proteins will self assemble to form fibers.[119] Materials with similar properties comprising the pentapeptides VPGKG and VPGIG have also been made using recombinant approaches,[120] and elastin-mimetic proteins can be crosslinked into flexible films and filaments by a variety of methods.[121–123] In addition, the solid polymer blocks that form from these proteins have also been used as a substrate to assemble functional biomotors.[124]

Further modifications to these polypeptides, providing additional complexity, are necessary for the attachment or adhesion of human endothelial cells, an important milestone for the *in vivo* use of these polymers as substrates.[29] A sequence derived from the cell binding domain of fibronectin[125] has been incorporated during assembly of polypeptides as an essential component of the polymer substrate[126–130] to support endothelial cell adhesion, function, and activity.

Elastin-like materials with as many as four different domains have been synthesized and assembled to provide additional functionality to the synthetic elastin-like molecules.[131,132] These structures may potentially be useful in tissue engineering applications such as vascular grafts, stem cell matrices, and organs such as bladders.[117] However, assemblies of repeating peptides cannot match the complexity or biological activity of native elastin. With the exception of some early work by Urry *et al.*,[133] no *in vivo* data on elastin-like proteins have been published, and their performance as engineered structures is unknown. In contrast, electrospun tropoelastin fibers support attachment, migration, and extensive proliferation of human mesenchymal cells.[134] What is already known about tropoelastin suggests that this material may serve as a novel biomaterial with complex structure and superior performance for vascular scaffolds.[27]

The challenge of engineering blood vessels that have the mechanical properties of native vessels has been and remains significant. The native human artery structure is ~30–50% elastin (Figure 7). In a variety of rat strains, the percent dry weight of elastin has been reported to be between 30 and 40%.[135] The aging process has been shown to produce a number of vascular changes including a progressive rise in arterial stiffness.[136,137] Studies show that elastin's characteristics are critical to vascular grafts and that 'to ensure appropriate mechanical function of the vessel and to prevent vessel stenosis, successful tissue-engineered vascular replacements must incorporate an elastic component.'[138] According to leading researchers, "successful tissue-engineered blood vessels incorporating elastin are, therefore, the Holy Grail of future vascular interventions."[139]

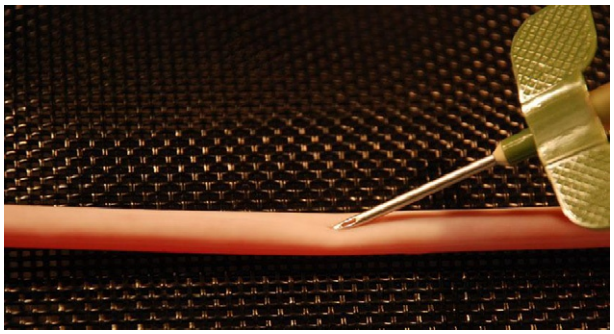

Figure 6 A completely biological human tissue-engineered blood vessel. This small diameter blood vessel (4.8 mm internal diameter) was produced with cells from a female patient suffering from end-stage renal disease and on dialysis. Vessels produced for this patient had an average burst pressure of 5204 ± 424 mmHg, which is higher than the burst pressure of the saphenous vein commonly used for bypass surgery, and higher than most human arteries of a similar diameter. The vessel is shown as it is being tested for its ability to resist a puncture from a 16 Ga hemodialysis needle. While burst pressure may be a good indicator for the suitability of a graft to perform as a typical bypass graft, resilience to injury is a key property for a good arteriovenous shunt. This *in vitro* test is performed under hydrostatic pressure to observe leaking during puncture, which will last up to 4 h in the clinical setting, and after removal of the needle. Synthetic grafts are notorious for their propensity to bleed once the needles are removed from the graft. This natural tissue, because it can be easily punctured at an angle, easily seals after removal of the needle even *in vitro* where only aqueous solutions are used.

5.527.4.5. Mechanical Testing

The mechanical properties of native vessels are well understood scientifically and held as the primary reason for their outstanding performance as bypass grafts. However, the field has not yet been able to produce a biomaterial that ideally matches the mechanical characteristics of native arteries and that has a low biological impact. Native arteries such as the canine carotid artery are not only composed of a variety of cell types (e.g., endothelial cell lining and SMC media layer), but also have an important basement membrane and ECM composed of elastin (Figure 8). These specific cell types have important biological and mechanical properties for a functioning blood vessel. Additionally, the biomechanical properties offered by elastin have not yet been replicated synthetically in the field by any of a variety of polymer scaffolds.

For example, Figure 9 shows the stress–strain curves of a canine carotid artery versus a polyurethane scaffold tube. The dramatic differences can be seen where the native carotid artery exhibits the typical viscoelastic behavior in contrast to the linear behavior of the polyurethane scaffold tube.

5.527.4.6. Size and Scope of the Peripheral Market

As many as 12 million Americans over the age of 50 are affected by peripheral artery disease (biotechnologyireland.com (a)). Approximately 600 000 grafts are implanted each year in the United States alone and this is growing annually because of an aging population (gore.com). The estimated $2.0 billion market for vascular grafts (biotechnologyireland.com (a)) is a subset of the worldwide interventional cardiology market, which was estimated at $11 billion in 2008 (biotechnologyireland.com (b)). The market for vascular grafts is global in reach, but the vast majority of vascular grafting procedures occur in the United States and other developed nations. Only the most remote third world medical providers do not have regular access to these grafts. In determining the ideal initial market, the regulatory environments are key factors. With recent changes in European regulatory practices, it is expected that the time required for obtaining approvals will become substantially similar to those in the United States.

5.527.4.6.1. Market size and growth

A clearly defined market size for vascular grafts is somewhat difficult to obtain as each existing player in this market defines it differently. A general study of this market tells us that today this market is in the $20–30 billion range.

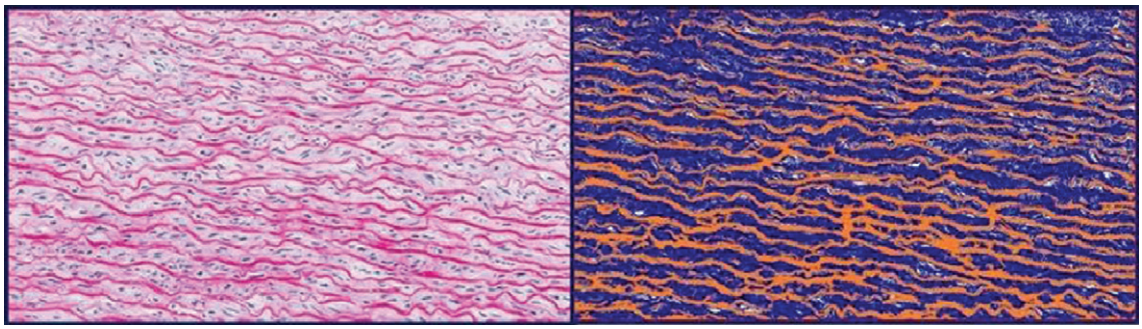

Figure 7 Left: H&E staining of a porcine aorta. Right: Area quantification of elastic fibers in a porcine aorta. Orange markup shows elastic fibers at 39.1% of total area.

Figure 8 Immunohistochemical staining of smooth muscle cell differentiation markers and basement membrane protein (elastin) in canine carotid arteries.

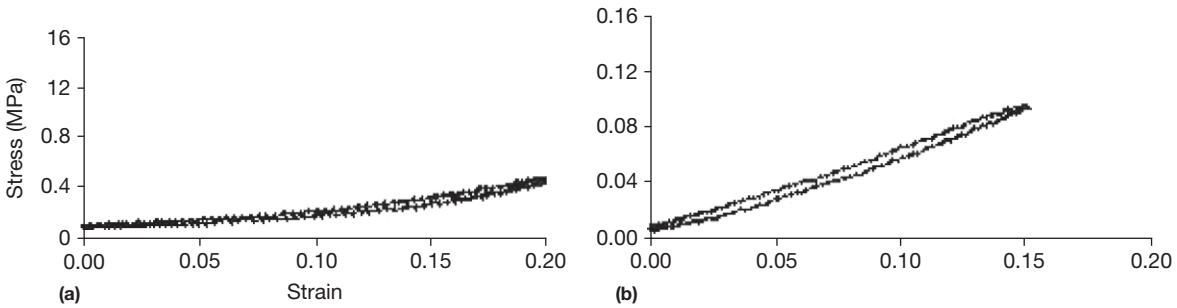

Figure 9 Stress-strain curves for (a) native carotid artery, (b) unseeded polyurethane vascular scaffold.

The vascular graft market has been consistently showing an average growth of 10–20% for last several decades (data from annual reports). As population grows and ages, the need for such devices increases. The market for these devices is directly dependent on aging population and is somewhat resilient to economic recessions and fluctuations.

An example of a peripheral target for first-in-man clinical application is a TEBV for vascular access in hemodialysis patients. Dialysis vascular access is the lifeline of the ESRD patient undergoing hemodialysis. A typical ESRD patient receives 1.5–2 interventions per year to maintain vascular access for hemodialysis. The grafts implanted to provide this access typically fail because of compromised patency. A tissue engineering solution to this problem has been the Holy Grail for many investigators who aim to improve patency rates and long-term survival of the vascular access graft. Once a TEBV technology demonstrates clinical success in the dialysis vascular access market, numerous additional clinical markets will likely follow (e.g., peripheral bypass).

In 2004, the total annual cost of vascular access complications was estimated to be nearly $8000 per patient risk year, with a total of $1–1.5 billion, or ~10% of the total ESRD budget. More than 20% of hospitalizations of ESRD patients are attributed to complications with vascular access. The growth of ESRD patients is expected to double by the year 2010. Thus, dialysis access care will grow into a multibillion dollar cost.

5.527.5. Tissue Engineering of Heart Valves

The first replacement heart valves were made from autologous or homologous sources of human tissue (Figure 10).[140] Since the 1960s, allograft replacement valves have been used to repair compromised or nonfunctioning heart valves in patients. These replacement valves are classified as either mechanical or biologically derived (see Chapter 6.626, Cardiac Valves: Biologic and Synthetic). To date, efforts to create tissue-engineered heart valves (TEHVs) have shown limited effectiveness. In this section, we will briefly discuss the field as a whole, how biomaterials will contribute to future developments, and the place tissue engineering may serve for this clinical application (see Chapter 5.528, Tissue Engineering of Heart Valves).

5.527.5.1. Classic Mechanical and Biological Heart Valves

Currently, a number of mechanically and biologically derived heart valves are available. Mechanical valves are derived mainly from nonbiological materials such as polymers or other biomaterials. Biological valves or bioprostheses are derived from human or animal tissues.[141] Bioprostheses are derived mainly from porcine aortic valves (PAV) or bovine pericardial valves (BPV) and are glutaraldehyde preserved.[142] These valves are defined by predictable rates of deterioration, with >50% of the replacement valves failing within 15 years.[143]

5.527.5.2. Device Challenges

Heart valve replacement surgery represents the only therapy for end-stage aortic valve disease. However, prosthetic valves have a number of limitations. Both the classic mechanical and biological heart valves are limited by deterioration that results from either calcification or noncalcification.[144] These problems ultimately affect the overall function and implant life of the valves. Calcification deterioration arises when calcium accumulates within the valve as a result of the inability of the glutaraldehyde treated tissue to maintain low calcium levels. Noncalcification degradation generally refers to the natural deterioration of replacement valves.

Additionally, mechanical valves are typically recognized by the body as a foreign substance and therefore facilitate concerns with thromboembolic complications, requiring lifelong anticoagulation therapy.[145] Because of the well described leaflet deterioration and calcification with biological or bioprosthetic valves, reoperation and replacement are common future clinical outcomes.[145,146]

Finally, both mechanical and biological valves lack the fundamental ability to grow, repair, or remodel in the patient.[147] This is especially important when considering the treatment of congenital heart valve disease in pediatric patient populations.[148]

5.527.5.3. Tissue-Engineered Heart Valves

Tissue engineering is currently being evaluated to determine if it can offer an alternative to mechanical or biological heart valves.

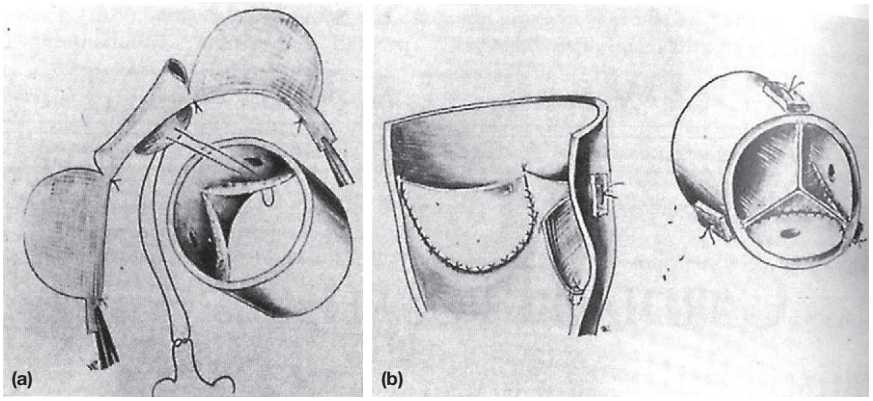

Figure 10 (a) Fascia lata strip cut to form three cusps. Then it is sutured to the aortic annulus. (b) The commissures are anchored with sutures placed through the aortic wall and tied over Ivalon sponge pledgets. Reproduced from Senning, Å. *J. Thorac. Cardiovasc. Surg.* **1967**, *54*, 465–470.

Tissue engineering may offer solutions to overcome current limitations of mechanical or biological heart valves while offering unique advantages. These advantages may include a living autologous structure, improved biocompatibility, and the ability to grow, repair, and remodel.[149–151] Classically the 'Holy Grail' of replacement valves would be neither obstructive nor thrombogenic, capable of lasting the lifetime of the patient, and would possess cellular regenerative and homeostatic properties.[144] In theory, TEHVs may allow for creation of such a replacement valve. Recent works for developing TEHVs have evaluated varying endothelial cell types cells for population of decellularized[152,153] or synthetic valves.[154]

Focus on decellularized scaffolds has presented promising opportunities for the development of a TEHV (see **Chapter 2.221, Decellularized Scaffolds**). Once the field has progressed to the point of wide-utility of a decellularized scaffold, the next major decision that needs to be addressed is whether it should be an allograft only (e.g., human origin), or if it could be a xenograft (see **Chapter 5.507, Tissue Engineering and Selection of Cells**). If decellularized xenograft scaffolds are to be explored, they present source and acquisition advantages while presenting certain key complications such as disinfection criteria and suspected antigenicity of xenograft (ECM) proteins.[155] This idea has previously been suggested by *in vitro* and *in vivo* studies.[156–158]

A significant concern exists with the concept that a decelluarized xenograft scaffold may actually be more inflammatory than current iterations of cryopreserved homografts. An unfortunate clinical example of a decelluarized xenograft heart valve (Synergraft) highlights the importance of truly understanding the ability of a decellularized ECM to provoke the immune system as well as the innate nonspecific inflammatory pathway.[159] In other studies, decellularized porcine leaflets were reported to be more attractive (stimulated macrophage response) than extracts of human native pulmonary cusps that had not been decellularized.[155] These studies suggest that future decisions on an appropriate decellularized ECM point to the human allograft over a xenograft source.

5.527.5.3.1. Regeneration versus repopulation

Attempts at producing TEHVs are classified as either regeneration or repopulation based. Regeneration methods involve an implantable biologically active matrix comprising both cellular and connective elements. Repopulation methods utilize harvested valves rinsed and voided of cellular elements and repopulated *in vivo* by the recipients own cells.[147]

5.527.5.3.2. Biomaterials used for heart valves

The use of biomaterial scaffolds for the development of replacement heart valves will continue to face the complex offering provided in the native biological ECM of heart values. Specifically, this ECM consists of collagen, elastin, and GAGs (see **Chapter 2.215, Collagen: Materials Analysis and Implant Uses** and **Chapter 2.216, Collagen–GAG Materials**). Collagen is primarily responsible for the structural integrity and biomechanical strength of native heart valves.[160] Elastin provides significant tissue resilience over time and over repetitive heart cycles. glycosa minoglycans (GAGs) play a pivotal

Table 1 Summary of biomaterials evaluated for use in the development of heart valves

Material	*Advantages*	*Disadvantages*	*Comments*	*References*
Silicone	Favorable flexibility and biocompatibility	Short durability; distorted and thickened leaflets; tearing; thrombosis formation	Structural failures and impaired hemodynamic performance and durability	[163,164]
Polytetrafluoroethylene (PTFE)	Favorable hemodynamic properties	Low resistance to thromboembolism and calcification; free edge inversion and stiffening of the leaflet	PTFE was found to be unsuitable because of major complications	[165–168]
PUs: Polyester	Good viscoelasticity	Susceptible to hydrolysis	Biodegradation and calcification have been reported as the main problems	[168,169,170]
PUs: Urethane	Good resistance to hydrolysis	Low resistance to oxidation (PEU) and prone to calcification (PCU)	Biodegradation and calcification have been reported as the main problems	[171–173]
PVA	Good mechanical properties	Not suitable for dipcasting	Mechanical properties were satisfactory but PVA needs to be further studied	[174]
SIBS	Enhanced resistance to hydrolysis, oxidation	Causes platelet activation and thrombogenicity	Resistance to oxidation and hydrolysis along with its biostability makes it a leading material for development of heart valves	[170,175]
POSS-PCU nanocomposite	Excellent resistance to oxidation, hydrolysis and calcification; excellent biocompatibility; antithrombogenicity	No reports yet	Positive material characteristics have led to the current evaluation of POSS-PCU as a heart valve	[162,176]

role in enabling valve tissue to withstand compressive forces.[148] In recent years, a number of biomaterials have been evaluated during the development of mechanical heart valves. These include polymers such as polylactic acid (PLA)/PGA and copolymers, which were used in the mid 1990s and more recently, PHAs. PHAs are naturally occurring bacteria derived polymers and a possible alternative to petroleum-based plastics with many potential uses.[147,161]

Efforts in the development of synthetic heart valves leaflets have included strategies that use nanocomposite polymers. For example, polycarbonate soft segment (PCU) and polyhedral oligomeric silsesquioxanes (POSS) nanoparticle (POSS-PCU) has been evaluated as a synthetic material for heart valve leaflets because of its favorable biocompatibility and biostability.[163] Tensile strength of POSS-PCU has been shown to be significantly higher than PCU alone materials, 55.9 ± 3.9 versus 28.8 ± 3.4 N mm^{-2} at 37 °C, respectively.[163]

Numerous other biomaterials have been explored for use in polymer heart value development. A summary in **Table 1** helps to describe some of the key biomaterials that have been evaluated to date.

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