The Heart and Great Vessels

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Cardiovascular disease is the leading cause of mortality worldwide. We have made large strides over the past few decades in management, but definitive therapeutic options to address this health-care burden are still limited. Given the ever-increasing need, much effort has been spent creating engineered tissue to replace diseased tissue. This article gives a general overview of this work as it pertains to the development of great vessels, myocardium, and heart valves. In each area, we focus on currently studied methods, limitations, and areas for future study.

Cardiovascular disease (CVD) is the leading cause of mortality worldwide associated with more than 17.5 million deaths per year (World Health Organization, www.who.int/cardiovascular_diseases/en). In particular, congenital heart defects are diagnosed in approximately 1% of live births, making CVD the most common congenital malformation of newborns. Despite the prevalence of heart disease and therapeutic advances over the past 30 years, definitive therapeutic options to address this health-care burden are limited. Here we will discuss the application of tissue engineering to construct functional cardiovascular tissue from a combination of biomaterial and cell types. We will begin by detailing recognized anatomy and transition to the method by which tissue engineering is used to mimic form and function.

FUNCTION, STRUCTURE, AND HISTOLOGY

Anatomy

The heart functions as a highly organized physiological pump composed of three layers: the inner endocardium, the thick myocardium, and the outer epicardium. The muscular myocardium generates the force needed to move blood with each contraction. The endocardium is lined by endothelial cells (ECs) that maintain a nonthrombogenic surface, and the epicardium serves as an additional fatty and connective tissue layer between the heart and the serous visceral pericardium.

The four-chambered heart includes two atria and two ventricles. The right atrium receives deoxygenated blood from the body by way of the inferior vena cava (IVC) and superior vena cava (SVC) and delivers it to the right ventricle,

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which pumps it through the pulmonary artery to the lungs to be oxygenated. The left atrium then receives the oxygenated blood from the lungs by way of the pulmonary vein and delivers it to the left ventricle, which pumps it through the aorta to supply the metabolic needs of the body. Located at the junction of the right atrium and SVC is the sinoatrial node, which spontaneously generates action potentials that propagate throughout the heart, generating a synchronous contraction.

Unidirectional flow through the heart is maintained by the tricuspid valve between the right atrium and ventricle, the mitral valve between the left atrium and ventricle, and the pulmonary and aortic valves located at the right and left ventricular outflow tracts, respectively. Grossly, the semilunar pulmonary and aortic heart valves are composed of three thin cusps that open easily when exposed to the forward blood flow of ventricular systole, and then rapidly close under the minimal reverse flow of diastole (Schoen 2011). Despite the force applied to the leaflets during diastole, prolapse is prevented by substantial coaptation of the cusps in a crescent-shaped region of the cusp termed the lunula.

Histology

The myocardium is composed mostly of cardiomyocytes. Cardiomyocytes comprise 80%–90% of the heart volume and are aligned and electrically coupled to surrounding cardiomyocytes. Supporting cell types, including endothelial and smooth muscle cells, organize themselves into a vascular network supplying nutrients to the cardiomyocytes, whereas fibroblasts generate a collagen-dense matrix.

Microscopically, the semilunar heart valve is composed of three layers: the ventricularis, spongiosa, and fibrosa. The fibrosa, which is exposed to the aortic lumen, is composed of primarily collagen fibers that are densely packed parallel to the cuspal free edge and provide most of the mechanical strength of the valve (Cole et al. 1984). The ventricularis layer faces the ventricle and is composed of collagen and radially aligned elastin fibers. Elastin forms an

encompassing matrix that binds the collagen fibrous bundles throughout the heart valve, thereby creating an elastin–collagen hybrid network that provides greater mechanical strength (Scott and Vesely 1995). The centrally located spongiosa is composed of glycosaminoglycans (GAGs) and loose collagen fibers. The GAG side chains of proteoglycans make a gelatinous substance to which other supportive extracellular matrix (ECM) molecules can form covalent cross-links (Flanagan and Pandit 2003).

The two most prevalent cell types in heart valves are valvular endothelial cells (VECs) and valvular interstitial cells (VICs). VECs line the surface of valve cusps. They provide a non-thrombogenic blood–tissue interface, maintain a semipermeable membrane that regulates the transfer of large and small molecules through the vascular wall (Rabkin-Aikawa et al. 2005), and play a critical role in the control of inflammatory and immune reactions (Schoen 2008). VICs are primarily responsible for the synthesis and remodeling of valve ECM, maintaining the appropriate structural and functional relationship needed for long-term heart valve competence. This is essential given the constant, rigorous mechanical movement of valves that results in persistent low-level valvular damage necessitating repair.

The great vessels carry blood to and from the heart and include the aorta, IVC, SVC, and pulmonary artery and vein. Blood vessels have three concentric layers: the innermost intima, the media, and the adventitia. The intima is composed of a single layer of luminal squamous ECs attached to the basement membrane. The ECs are in direct contact with flowing blood and form a smooth, antithrombogenic surface. Underlying the endothelium is the subendothelial connective tissue, which is thrombogenic and critical to platelet adhesion, the coagulation cascade, and prevention of hemorrhage during injury (Sarkar et al. 2007). The media is muscular layers of the vessel and is thicker in arteries because of the higher levels of elastin and smooth muscle needed for the propulsion of blood to the body. Last, the adventitia is the outermost connective tissue covering of the vessel.

DISEASE, TREATMENT, AND CURRENT LIMITATIONS

Pathophysiology

The most common cardiovascular pathology is ischemic heart disease, which occurs when a portion of the heart is not appropriately oxygenated, usually because of compromise of the coronary arteries that supply the heart. Coronary artery disease (CAD) is most often caused by atherosclerosis or an accumulation of fatty plaques within the vessel wall that narrows the blood vessel lumen and decreases the amount of blood received by the target. Decreasing blood flow, and therefore oxygen delivery, below a certain threshold leads to angina characterized by reversible discomfort or feeling of chest pressure. Complete occlusion of the artery leads to myocardial infarction (MI), which is associated with a typical pathological progression. Irreversible cell damage occurs within 20–40 min following occlusion (Kumar et al. 2010). Coagulative necrosis begins after 30 min, followed by a robust inflammatory response 24 h post-infarction that continues for 2 to 3 days. Macrophages ultimately dominate the infarcted zone by 5 to 7 days postinfarction and are responsible for removing dead cells and creating granulation tissue. Weeks to months after the infarction, collagen deposition dominates, and a fibrous scar is formed that reduces the contractile function of the heart and may lead to heart failure (Kumar et al. 2010).

The pathology of heart valve disease manifests itself in two ways. The first, stenosis, results in reduction of forward flow. The second, regurgitation, causes retrograde flow during diastole because of the inability of a valve to completely close. Aortic stenosis, when severe, presents as dyspnea on exertion, chest pain, and syncope. The most frequent cause of aortic stenosis is dystrophic calcification of the aortic valve cusps and annulus. Overall, prevalence of aortic stenosis is 2%, and the incidence of disease is progressively increasing as the American population ages. Mitral valve prolapse, a displacement of valve leaflets into the left atrium during systole, is the most common indication for surgical repair or replacement of this valve. It is

most commonly caused by myxomatous degeneration and is often seen in patients with Marfan syndrome, a connective tissue disorder, and can lead to valve incompetence and regurgitation (Kumar et al. 2010). Dysfunction of the tricuspid and pulmonary valves is usually a result of a congenital heart defect. Approximately 20,000 infants are born in the United States each year with a congenital heart malformation, many of which are associated with absence or malformation of the pulmonary valve or artery (Sales et al. 2010). Major congenital heart diseases of the right ventricular outflow tract include truncus arteriosus, pulmonary atresia with ventricular septal defect, Tetralogy of Fallot, transposition of the great vessels with ventricular septal defect and pulmonary atresia, and double-outlet right ventricle.

Current Treatment and Limitations

One of the key limitations to treating CVD is the lack of tissue regeneration after myocardial injury. Most therapies for acute disease are directed at immediate restoration of vascularization to decrease the amount of damage inflicted by the ischemic event. Coronary angioplasty involves reopening diseased vessels using a balloon or stent apparatus, whereas coronary artery bypass grafting (CABG) surgery involves rerouting blood past diseased segments of coronary arteries to revascularize the cardiac tissue supplied by the affected vessels. The saphenous vein and internal mammary artery are frequently used as conduits for CABGs, but these vessels may also be affected by atherosclerotic disease and are prone to stenosis because of a progressive thickening of the vessel wall known as neointimal hyperplasia. Once a significant amount of myocardium has sustained permanent damage, the majority of existing therapies aim to mitigate the progression to heart failure.

Standard treatment for end-stage valvular dysfunction is heart valve replacement. Prosthetic heart valves are either mechanical and composed entirely of synthetic material or bioprosthetic and fashioned from biological components. Approximately 50% of implanted

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valves in the United States are mechanical, whereas the remainder are bioprosthetic (Pibarot and Dumesnil 2009). Neither material possesses growth potential and are, therefore, not optimal for the pediatric population (Hammermeister et al. 2000). The mechanical heart valve has excellent durability but is associated with a substantial risk of thromboembolism and thrombotic occlusion because of the lack of an endothelial lining and the flow abnormalities that result from a rigid outflow structure (Rahimtoola 2003, 2010). To minimize this risk, chronic anticoagulation therapy is required for all mechanical valve recipients, but systemic anticoagulation renders patients vulnerable to hemorrhagic complications. The combined risk of thromboembolic complications and hemorrhage secondary to anticoagulation constitute the principal disadvantages of mechanical prosthetic valves (Cannegieter et al. 1994).

Bioprosthetic valve replacements, such as glutaraldehyde-fixed xenografts and allografts, are associated with a lower risk of thrombosis and hemolysis than mechanical heart valves (Hammermeister et al. 2000). Patients with glutaraldehyde-fixed xenograft valves do not require therapeutic anticoagulation, but, because they are composed of biologic material, they are prone to structural deterioration. Glutaraldehyde fixation of xenografts causes cross-linking of extracellular matrix collagen subunits that shields antigens and decreases immunogenicity. However, there is increasing evidence that these valves still elicit a significant immune response that leads to calcification and progressive damage (Manji et al. 2012). The structural deterioration eventually results in stenosis or regurgitation and is strongly age-dependent, affecting individuals under 35 years of age more commonly. Nearly uniform failure occurs after 5 years in patients of this age group, but 8%–27% fail after 20 years in those older than 65 years of age (Mykén and Bech-Hansen 2009). The higher rates of failure may be attributed to a more robust immune inflammatory response and metabolism observed in younger individuals (Manji et al. 2012).

THE APPLICATION OF TISSUE ENGINEERING TOWARD THE TREATMENT OF CARDIOVASCULAR DISEASE

Tissue-Engineering Theory

The central paradigm underlying tissue engineering involves combining cells with a platform matrix to create neotissue (Langer and Vacanti 1993). The matrix acts as a three-dimensional scaffold until proliferating cells produce sufficient ECM to support the organ's structure (Rabkin-Aikawa et al. 2005). Cells can either be cultured on the scaffold in vitro or seeded on the scaffold before implantation of the graft. If cells are to be cultured in vitro, this usually involves placement of the graft into a bioreactor that is used to mimic the physiologic conditions in which the target organ usually resides. Once implanted, the scaffold degrades as cellular proliferation and neotissue formation increases, leaving a living functional organ with growth capacity.

GREAT VESSELS

An ideal tissue-engineered vascular graft (TEVG) is an antithrombotic, nonimmunogenic, endothelialized structure with durable biomechanical properties akin to those of native vessels (Li and Henry 2011). Additionally, the grafts should have growth capacity, making them an ideal conduit for use in pediatric patients with congenital heart defects. The two recognized techniques for TEVG fabrication are scaffold-guided and self-assembled cell-sheet-based engineering. In this section, we give an overview of these two scaffold techniques as well as current limitations and future direction of study.

State of the Art

Scaffold-Guided Technique

The scaffold-guided approach uses a three-dimensional structure that serves as a supportive framework for TEVG integration into the native vasculature. Synthetic polymers such as poly(glycolic acid) (PGA) and its variants poly(lactic acid) (PLA) and poly(ϵ -caprolactone)

(PCL), which may be combined as copolymers, are used to fabricate scaffolds that degrade over time (Athanasίου et al. 1996; Patterson et al. 2012). The rate of degradation depends on various factors, including the properties of the materials used and the host's reaction to the materials. Longer degradation times maintain the mechanical properties of the scaffold for longer durations but also slow the remodeling process of the TEVG. Compared to natural material grafts, the construction of biodegradable synthetic TEVGs using various material ratios allows for more specific manipulation of biomechanical properties (Kim and Mooney 1998; Isenberg et al. 2006), but toxic byproducts from degradation and immunogenic response threaten graft incorporation (Higgins et al. 2003; Seifu et al. 2013).

Scaffolds derived from natural materials include those constructed with ECM-based gels, such as fibrin or collagen (Yao et al. 2005; Marelli et al. 2012), scaffolds constructed from decellularized allogenic or xenogenic tissue, or a combination of the two (Row et al. 2015). ECM-based grafts have an inherent bioactivity not seen in synthetic grafts (Hubbell 1999; Seifu et al. 2013) that promotes TEVG integration, but lack mechanical durability and consistency in comparison to synthetic grafts (Charulatha and Rajaram 2003; Badylak 2007). Decellularization is a process by which immunogenic cellular components are removed from tissues while maintaining the ECM framework for structure and biomechanical properties. Such a process enables endothelialization through seeding or ingrowth of host cells. Methods of tissue decellularization range from enzymatic digestion, detergent treatment, sonication, and hypo-/hypertonic immersion (Wilson et al. 1995). The degree of decellularization has not yet been optimized for TEVG fabrication, and xenografts may be associated with risk of pathogenic transmission from animal donor to human recipient (Leyh et al. 2006).

Self-Assembled Cell-Sheet-Based Technique

Sheet-based tissue engineering is a technique used to fabricate TEVGs with or without a scaffold

while avoiding the use of exogenous materials. With this method, fibroblast and smooth muscle cells (SMCs) are cultured in a manner that generates ECM deposition in a cohesive sheet that can be carefully detached, yielding a single sheet of living cells with a well-organized and intact matrix (L'Heureux et al. 2006). These sheets are then placed onto the lumen of a scaffold and extensively cultured in bioreactors to form TEVGs with high burst pressures (McAllister et al. 2009). An alternative approach is the application of the cells to an agarose rod, which avoids the problems posed by removal of this layer with specialized machinery (Seifu et al. 2013). Though this alternative method avoids the use of specialized equipment, the conduits produced using this technique lack native biomechanical strength.

Cell Sources

Cell sources for TEVG fabrication include mature somatic cells, adult progenitor cells, and induced pluripotent stem (iPS) cells (Sundaram and Niklason 2012). Mature somatic cells, such as fibroblasts, SMCs, and ECs, can be harvested from the recipient, seeded on a selected scaffold, and implanted with little risk of immunogenic response. Bioactive substances released from ECs, including plasminogen factor, interleukin (IL)-1, fibronectin, heparin sulphate, and nitric oxide maintain homeostasis, antithrombogenicity, and increase vascular patency. SMCs form the vessel media and augment biomechanical properties such as contractility (Neff et al. 2011). Biodegradable PGA scaffolds seeded by the Niklason group with SMCs and ECs cultured under pulsatile radial stress conditions for 8 weeks showed mechanical properties similar to native human arteries (Niklason et al. 1999). Such TEVGs are in preparation for clinical application (Peck et al. 2012).

Because ECs and SMCs are terminally differentiated, these cells have a lower proliferative capacity compared to stem cells and progenitor cells. Extensively studied cell lines include embryonic stem cells (ESCs), induced pluripotent stem (iPS) cells, endothelial progenitor cells (EPCs), mesenchymal cells, and bone mar-

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row-derived mononuclear stem cells (BM-MNCs). ESC use in TEVG fabrication has been impeded by ethical concerns for embryo destruction and overall technical difficulty of harvesting these cells. However, such concerns may be nullified with the use of iPS cells, which can be derived from autologous fibroblast.

Our group prefers the use of BM-MNCs in producing TEVGs because of relative ease of harvesting these cells and the previous success experienced by our group using this cell type (Shin'oka et al. 2001, 2005; Hibino et al. 2010). Interestingly, our work thus far has shown that seeded cells do not actually populate the graft but disappear soon after implantation (Harrington et al. 2011). The BM-MNCs attract host cells from the nearby vessel via paracrine signaling (Hibino et al. 2011a). These findings conflict with the classic tissue-engineering paradigm that cells must be seeded onto the graft and allowed to populate the scaffold before implantation. We instead uphold that grafts can be seeded and implanted the same day. The

transition to the use of the host as the bioreactor for the TEVG liberates cardiovascular tissue engineering from the time-consuming and expensive constraints imposed by ex vivo bioreactors (Hibino et al. 2005; Li and Henry 2011; Li et al. 2014).

Clinical Application

In 2001, 25 patients with an average age of 5 years underwent extracardiac total cavopulmonary conduit implantation using biodegradable TEVGs seeded with BM-MNCs (Fig. 1A–C) (Shin'oka et al. 2005). At 1 year, there was one patient diagnosed with partial mural thrombus who was treated successfully with anticoagulation. A late-term follow-up (average 5.8 years postimplantation) showed that four patients who developed TEVG stenosis were successfully treated with balloon angioplasty or stenting (Fig. 1D–G) (Hibino et al. 2010). The tissue-engineered vascular conduits had reduced incidence of calcification, with no risk of rejection

Figure 1. Late-term results of an extracardiac tissue-engineered vascular grafts implanted in humans during the modified Fontan procedure. (A) Three-dimensional computer tomography (CT) 1 year after implantation shows a patent graft with no aneurysmal dilation. (B) Tissue-engineered vascular graft (TEVG) angiography 4 years after implantation with no malformations. (C–F) Successful balloon angioplasty of four patients with TEVG stenosis. (From Hibino et al. 2010; adapted, with permission, from Elsevier © 2010.)

because of autologous cell seeding, minimal risk of infection, and potential for growth (Breuer 2011). Following the excellent results of Shinoka et al., our group is currently performing the first FDA-approved human clinical trial investigating use of TEVGs in children with congenital heart disease.

Current Limitations

Although successfully treated with angioplasty, the results of Shinoka et al. did show stenosis to be the most common graft-related complication and a major barrier to widespread clinical application. Small animal models were developed to study the mechanisms underlying TEVG neotissue development and stenosis (Fig. 2) (Hibino et al. 2011b; Lee et al. 2014) and have shown that macrophages are essential for neotissue formation (Roh et al. 2010; Hibino et al. 2011b), but extensive infiltration leads to stenosis. More recently, stenosis has been linked to a process called endothelial-to-mesenchymal transformation (Endo-MT), during which endothelial cells migrate deeper into the vessel wall and transition to a mesenchymal phenotype. If this occurs excessively, it can cause luminal narrowing. Endo-MT is driven by macrophage tissue transforming growth factor β (TGF- β) signaling, and experiments using TGF- β receptor blockers have shown decreased Endo-MT and increased graft patency (Duncan et al. 2015).

Despite successful use of TEVGs in high-flow low-pressure circulatory systems shown in clinical trials (Shinoka et al. 2001, 2005; Hibino et al. 2010), the application of tissue engineering to arterial revascularization has been limited (L'Heureux et al. 2006). The feasibility of such grafts for hemodialysis access has been established as the implanted Lifeline grafts (Cytograft Tissue Engineering, Novato, CA) in three patients showed vascular integrity over an 11-month course without signs of immunogenic responses (McAllister et al. 2009; Wystrychowski et al. 2014). In fact, multicenter trials are underway to evaluate the safety and efficacy of Cytograft and Humacyte graft use in this respect (Gui and Niklason 2014). Because our

inability to replicate arterial biomechanical properties, aneurysmal dilation of the graft and rupture are complications associated with conduits placed in high-pressure systems (Mirensky et al. 2009). Scaffolds with a longer degradation time can be used to allow continued biomechanical support while the neotissue develops, but this can lead to a prolonged foreign body response and calcification (de Valence et al. 2012; Tara et al. 2014).

Alternative Strategies and Future Direction

Several characteristics of the scaffold play a large role in the eventual success or failure of the vascular graft. For example, porosity and fiber diameter have been shown to allow varying degrees of cellular infiltration as well as macrophage phenotypic differentiation (Wang et al. 2014). When considering the various scaffold properties that can be tested before arriving at an optimal design, it is easy to see that one can spend several lifetimes working with preclinical models before arriving at the perfect graft. A collaborative effort between our group and the laboratories of Jay Humphrey and Yadong Wang has therefore explored a more rational and systematic method of scaffold development using computational modeling. We developed an equation that successfully describes the biomechanical behavior of tissue-engineered venous grafts implanted in a murine model (Khosravi et al. 2015). The model accounts for several variables such as the rate of scaffold degradation and ECM turnover. Using the mechanical data obtained from grafts explanted at varying time points, the model can be calibrated in a way that allows it to accurately predict behavior over the entire life span of the vessel. Additionally, by feeding the model enough biomechanical data regarding the performance of grafts with varying porosity or fiber diameter, you can provide the model with enough information to run thousands of simulations *in silico* and provide a scaffold design that, once implanted, will most closely replicate native vessel performance. These experiments will hopefully provide a means of efficiently and rapidly designing vascular grafts and other tissue-engineered organs.

Figure 2. Tissue-engineered vascular graft (TEVG) remodeling in a mouse model. (A) Inflammation-mediated process of graft remodeling. Seeded bone marrow–derived mononuclear stem cells (BM-MNCs) attach to the scaffold and release cytokines. MCP-1 recruits host monocytes that infiltrate the scaffold and begin to direct neotissue formation, ultimately resulting in the formation of neovessels composed of concentric layers of smooth muscle cells recruited from the neighboring native vessel wall embedded in an extracellular matrix with a monolayer of endothelial cells lining the luminal surface. (B) TEVG gross and microscopic morphology changes over time and ultimately resembles the native inferior vena cava (IVC) with a smooth muscle cell layer lined by an endothelial cell layer as shown in gross images and hematoxylin and eosin-stained section slides. EC, Endothelial cell; SMC, smooth muscle cell; VEGF, vascular endothelial growth factor; BMC, bone marrow cell. (From Duncan and Breuer 2011; reprinted, with permission.)

MYOCARDIUM

Much of the effort for myocardial tissue engineering has gone toward the development of a patch that can be used to replace diseased myocardium. An ideal cardiac patch would not only propagate spontaneous action potentials but would also connect with the host vasculature; couple electrically with the surrounding myocardium; and generate force to improve the function of the failing heart. Two major methods of fabricating a cardiac patch exist, the first of which involves stacking sheets of cells grown in culture and the second on growing cells on scaffolds before implantation. We speak briefly on each of these as well as the use of cell-injection therapies in myocardial engineering.

State of the Art

Engineered Heart Tissue

Much progress has been made in engineered heart tissue (EHT) since early experiments showed contractility of embryonic chicken cardiomyocytes grown in culture (Eschenhagen et al. 1997). Efforts have been aided by the natural propensity of immature cardiomyocytes to organize three-dimensionally and generate spontaneous action potentials (Hirt et al. 2014)—an attribute that is essential for the development of cell sheets. In this method, myocytes are cultured on plastic dishes and then detach in a single intact monolayer. These sheets can be stacked to form thicker structures capable of performing work (please see Shimizu et al. 2002 for examples of cell sheets). Both mechanical and electrical stimulation during the culture period are important for the development of these structures. Mechanical strain stimulates proper alignment and maturation of cells (Hirt et al. 2014) and can be induced either by way of static tension using suspension (Eschenhagen et al. 1997; Baar et al. 2005) or exposure to cyclic stretching using motorized devices (Tulloch et al. 2011; Zhang et al. 2012). Electrical stimulation promotes coupling of cardiac constructs and improves contractile function and cellular organization (Radisic et al. 2004; Tandon et al. 2009, 2011; Chiu et

al. 2011; Vunjak-Novakovic et al. 2011; Maidhof et al. 2012).

Scaffolds

Synthetic or decellularized biologic scaffolds provide a three-dimensional structure on which cells can be cultured. The constituents and sizing specifications of the scaffold dictate the organization, maturation, and function of the forming tissue constructs. The scaffold materials used include, but are not limited to, the native heart matrix (Ott et al. 2008; Duan et al. 2011; Godier-Furnémont et al. 2011), natural hydrogels, such as collagen and Matrigel (Eschenhagen and Zimmermann 2005; Zimmermann et al. 2006; Song et al. 2010; Tiburcy et al. 2011), and synthetic polymers, such as poly(glycerol) sebacate (PGS) and polyacrylamide (Engelmayr et al. 2008; McCain et al. 2012; Zhang et al. 2012). Overall, the materials differ in the manner by which they are processed, their mechanical properties, their ultrastructure, and their biodegradability. Because of the great metabolic demand of cardiomyocytes, scaffold design must account for oxygen and nutrient delivery. Silvestri et al. provide a comprehensive review of scaffold formation for cardiac tissue engineering, including different scaffold types and fabrication methods (Silvestri et al. 2013).

Clinical Application

Successful preclinical animal models have led to several clinical trials and even clinical application of myocardial patches. Perhaps most notable is CorMatrix, which is constructed from decellularized porcine small intestinal submucosa (SIS). CorMatrix has been the subject of several preclinical (Mewhort et al. 2014) and clinical studies (Stelly and Stelly 2013; Yanagawa et al. 2014). Many of these studies report successful use, but there is some recent work documenting increased inflammatory response to the patch resulting in graft failure and necessitating removal (Rosario-Quinones et al. 2015; Woo et al. 2015). These studies also show poor integration of host cells into the scaffold. Long-

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term data is needed to determine whether this technology will be a feasible solution.

Current Limitations

The inability to vascularize thick functional grafts has been a significant barrier of EHT fabrication. The thick cell-sheet myocardial constructs are not inherently vascularized and oxygen diffusion is limited. Therefore, the metabolic demand of cardiomyocytes is a trying impediment to the viability of such constructs. Sequentially stacking sheets onto the host myocardium allows vascularization of one layer after another (Shimizu et al. 2006), but this method has limited clinical application. Altering the cell types seeded onto the graft may allow formation of prevascularized patches, as shown in one study using cardiac patches created from human embryonic stem-cell-derived cardiomyocytes, human umbilical vein endothelial cells, and fibroblasts (Stevens et al. 2009). These grafts had microvessels that attached to the coronary circulation of rats on implantation and resulted in patches that were 10-fold larger than the unvascularized counterparts when implanted in a skeletal muscle model (Stevens et al. 2009). Much work remains to be done, but this study serves as proof of concept that EHT can be vascularized, and vascularization results in thicker grafts similar to native myocardial tissue.

Alternative Strategies and Future Endeavors

An alternative approach to cardiac patches is the direct injection of either cells or biologic materials, such as collagen or alginate, into the diseased myocardium. Animal studies have shown that injection of fetal or neonatal cardiomyocytes improved left ventricular function and thickness, thus attenuating pathological remodeling after MI (Li et al. 1996; Reinecke et al. 1999; Müller-Ehmsen et al. 2002; Huwer et al. 2003). However, these findings have limited clinical relevance because human fetal and neonatal cardiomyocytes cannot be readily obtained for transplantation because of ethical issues.

The search for a more clinically relevant cell source has led to the transplantation of skeletal myoblasts (Dorfman et al. 1998), embryonic stem-cell-derived cardiomyocytes (ESC-CMs) (Klug et al. 1996; Etzion et al. 2001; Kehat et al. 2001), bone marrow-derived mesenchymal stem cells (BM-MSCs) (Shake et al. 2002; Toma et al. 2002) and hematopoietic stem (HS) cells (Orlic et al. 2001; Balsam et al. 2004; Murry et al. 2004) into animal models of MI. The use of skeletal myoblasts and MSCs in this way has been translated to clinical trials. A meta-analysis of recent clinical trials with injection of bone marrow and peripheral blood mononuclear cells showed a significant, albeit low (3%), increase in left ventricular ejection fraction (LVEF) in a dose-dependent manner as well as significant reductions in infarct size and end systolic volume in patients treated by intracoronary cell injection after acute MI (Lipinski et al. 2007). Injection of resident cardiac stem cells (SCPIO, CADUCEUS) has also shown promising functional improvements in phase I clinical studies and restoration of viable tissue per magnetic resonance imaging (MRI), presumably because of the new CMs in addition to vascular cells (Bolli et al. 2011; Makkar et al. 2012).

Because of improving technologies, it is becoming more feasible to differentiate pluripotent stem cells, such as ESCs or induced pluripotent stem cells (iPSCs) into cardiomyocytes and to expand them to sufficient numbers to support the diseased myocardium. The discovery of human iPSCs (Takahashi et al. 2007) and the ability to generate cardiomyocytes from them (Zhang et al. 2009) could provide unlimited numbers of autologous cardiomyocytes for cell therapy without the ethical concerns raised by the use of human epithelial stem cells (hESCs). Studies from a number of groups have shown that it is possible to generate cardiomyocytes from mouse (Kattman et al. 2006) and human ESCs (Yang et al. 2008) and iPSCs (Zhang et al. 2009).

HEART VALVES

An ideal graft for heart valve replacement would be biocompatible, readily available, durable,

and have the potential for growth and repair. Tissue engineering offers the potential to create such a nonthrombogenic, biomimetic, and immunologically compatible tissue (Rabkin-Aikawa et al. 2005). This section focuses on the various methods of developing tissue-engineered heart valve (TEHV) with some insight provided on current limitations and new methodologies.

State of the Art

Similar to great vessel and myocardial tissue engineering, TEHV scaffolds can be manufactured from either synthetic or natural materials such as ECM components like collagen, fibrin, elastin, and glycosaminoglycans, or decellularized native tissues (Hodde 2002). Commonly used synthetic polymers in tissue engineering include PGA, poly(L-lactic acid) (PLLA), copolymer poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), and polyhydroxyalkanoate (PHA).

Biologic Scaffolds

Given the complex structure of heart valves and the ability to maintain structure using decellularization methods, the most common biologic TEHV scaffolds have been decellularized allogenic or xenogenic tissues. Several preclinical studies using decellularized heart valve grafts have been documented in the literature (Iop and Gerosa 2015; James et al. 2015; Sierad et al. 2015; Syedain et al. 2015). The decellularized porcine SIS, CorMatrix, has also been used as a pulmonary valve replacement in porcine models (Matheny et al. 2000), with explanted constructs revealing resorption of the submucosal matrix, fibrous connective tissue growth, and formation of neovasculature. In a more recent study, Fallon et al. replaced the tricuspid valves in four sheep with the porcine SIS ECM sheet (Fallon et al. 2014). Explanted valves at 3, 5, 8, and 12 months showed evidence of progressive tissue remodeling, host-cell infiltration, and structural reorganization of the ECM bioscaffold over the course of the study with no evidence of rejection.

Synthetic Scaffolds

Initial work using synthetic scaffolds focused on polymers composed of PGA and PLA (Breuer et al. 1996). However, the biomechanical profile of the construct is substantially different from that of a native heart valve. TEHVs fabricated using PGA and PLA copolymer-based matrices are thicker and less pliable than native valves. Hoerstrup and colleagues developed a novel composite scaffold material consisting of PGA mesh coated with a thin layer of poly-4-hydroxybutyrate (P4HB), which is a flexible, rapidly degradable, and thermoplastic polymer (Martin and Williams 2003). Autologous myofibroblasts and ECs from ovine carotid artery were seeded onto the scaffolds, cultured for 14 days in a bioreactor that gradually increased pressure and flow across the valve, and subsequently implanted in an ovine model (Hoerstrup et al. 2000). When explanted, they showed increased ECM synthesis, remodeling, and organization, as well as mechanical properties at 20 weeks that were almost indistinguishable from those of native valves.

The Search for Appropriate Cell Sources

An ideal cell source for TEHVs would show phenotypic plasticity and be able to adapt biomechanical properties in response to dynamic alterations in flow. A potential cell source for a TEHV would be autologous VICs and VECs. The use of these cells would eliminate the risk of rejection while maintaining the requisite phenotypic profile (Flanagan and Pandit 2003). However, to isolate and culture an adequate number of cells for clinical use from a small biopsy would be challenging, and the risks of such a procedure are formidable.

Autologous myofibroblasts derived from saphenous vein have similar phenotypic properties to VICs and represent a more feasible cell source for TEHV fabrication (Schnell et al. 2001). In a long-term TEHV study, the first of its kind, conducted by Dohmen et al. (2002), investigators seeded a decellularized pulmonary valve allograft with cells isolated from saphenous

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nous vein and cultured in a bioreactor for 2 weeks. Results showed 100% survival with adequate valvular pressure gradients and no incidence of calcification at 10-year follow-up (Dohmen et al. 2002).

Likewise, mesenchymal stem cells show promise, although many of the details remain to be elucidated. Biodegradable polymeric scaffolds cultured with mesenchymal stem cells in vitro showed an organized internal structure and mature tissue development. Despite the encouraging results, it is not clear whether bone marrow stromal cells reliably differentiate into appropriate cell types in the scaffold or whether they continue to remain differentiated in vitro, ensuring long-term function and durability of the replacement heart valve (Perry et al. 2003). Investigations utilizing circulating endothelial and smooth muscle progenitor cells are at a similar stage (Simper et al. 2002). Given their remarkable differentiation potential, embryonic and adult stem cells may become valuable resources for heart valve tissue engineering, however, the former imposes ethical barriers.

Cell-Seeding Techniques

Traditional methods of seeding polymer scaffolds used static cell culture techniques in which a concentrated cell suspension is pipetted onto polymer scaffolds and left to incubate for various periods of time. In dynamic cell seeding, either the medium or the medium and scaffold are in constant motion during the incubation period. Dynamic cell seeding is often used in combination with a bioreactor and offers improved cellular attachment, infiltration, and alignment in the direction of flow in comparison to static cell seeding (Sutherland et al. 2002; Nasser et al. 2003).

A bioreactor is a biomimetic system used to optimize in vitro neotissue development as it provides an environment for the graft that mimics in situ the physiologic or pathophysiologic conditions that are to be corrected with the engineered tissue. Factors such as shear stress, flow rate, flow profile, pressure, and media composition can be easily manipulated to change

experimental conditions (Rippel et al. 2012). An ideal bioreactor for TEHV should consist of pulsatile flow and cyclic flex to mimic the complex environment that implanted valves must withstand (Sacks and Yoganathan 2007). Exposure to pulsatile flow modulates the biomechanical properties of the neotissue, which is important for developing TEHVs that are resistant to premature valve deterioration (Hildebrand et al. 2004).

Clinical Applications

Dohmen et al. (2002) reported the first successful use of a TEHV using a decellularized cryopreserved pulmonary allograft. Since then, Dohmen and colleagues have published long-term follow-up data on a series of 11 patients, who underwent the Ross procedure with a TEHV to surgically reconstruct the right ventricular outflow tract. In each case, the allograft was seeded in a bioreactor with autologous vascular endothelial cells that had been isolated from a segment of forearm vein 2–4 weeks before the operation. At 10-year follow-up, the tissue-engineered heart valves showed excellent hemodynamic function. All patients remained in New York Heart Association class I heart failure and computed tomography showed no evidence of calcification or valve degeneration (Dohmen et al. 2011).

Early studies using decellularized xenografts have not been as successful. In 2003, Simon et al. reported failure of SynerGraft decellularized porcine valves implanted in four children as pulmonary valves for right ventricular outflow tract reconstruction. Two valves evaluated at 6 weeks and 1 year were severely degenerated after implantation, and one valve ruptured 7 days after implantation, ultimately resulting in three deaths. The fourth child's valve was explanted because of cases of unfortunate mortality and morbidity (Simon et al. 2003). A more recent review conducted from 2006 to 2010 of 93 patients undergoing implantation of another decellularized xenograft type, Matrix P/Matrix P Plus, for right outflow tract reconstruction also showed high incidence of graft failure (Perri et al. 2012).

There have been more promising results with decellularized allografts, shown by pulmonary valves decellularized using the same SynerGraft technology of the xenograft fabrication process. Studies published to date show decreased short-term stenosis or regurgitation, decreased clinically significant insufficiency, lower peak valve gradients, and fewer interventions in SynerGraft decellularized allogenic grafts when compared to standard cryopreserved valves (Tavakkol et al. 2005). Brown and colleagues performed a multicenter retrospective cohort study of 342 patients undergoing right ventricular outflow tract reconstruction with SynerGraft or standard cryopreserved valves and showed decreased regurgitation in the SynerGraft group and overall safety and efficacy at 4 years postimplantation (Brown et al. 2010).

Current Limitations

Despite having cellular components removed, decellularized grafts still have the propensity to elicit an immune response. One reason for their continued immunogenicity may be incomplete valve decellularization (Paniagua Gutierrez et al. 2015). Incomplete penetrance during the process can lead to cellular remnants that cause inflammatory response days after implantation (Simon et al. 2003). This immune reaction may also be against the extracellular matrix itself. A review of patients receiving Matrix P decellularized pulmonary grafts also noted giant-type inflammatory cells on explanted samples, supporting an immune reaction similar to a foreign body response (Perri et al. 2012). There was also limited repopulation of the graft with host cells, which is another limitation of this technology noted by other studies as well (Cicha et al. 2011).

Alternative Strategies and Future Endeavors

An alternative method to scaffold formation introduced by the Hoerstrup group combines the use of synthetic biomaterials with decellularization to create “off-the-shelf” homologous valves for implantation (Dijkman et al. 2012).

Scaffolds created from a nonwoven polyglycolic acid mesh and coated with poly-4-hydroxybutyrate (PGA/P4HB) are seeded with human vascular-derived fibroblasts and incubated in a bioreactor for 4 weeks, during which time the seeded cells produce layered collagen along the graft. The scaffold is then decellularized. After implantation in nonhuman primates as pulmonary valve replacements, these valves showed layered collagen formation and substantial, homogenous cellular repopulation by host cells after 8 weeks, allowing the grafts to reach a cellularity comparable to that of native valves (Weber et al. 2013). Syedain et al. (2015) had comparable results using a similar method, but with a fibrin-based scaffold in an ovine model. The grafts showed graft endothelialization and host-cell repopulation after 6 months. By growing homologous cells on biodegradable scaffolds, the immunologic challenge imposed by xenogenic transplants is avoided. This technology can potentially solve the problem of demand associated with use of homografts.

Another important advancement in TEHV research is the development of catheter-based delivery systems for valve implantation (Schmidt et al. 2010; Moreira et al. 2015). Tissue-engineered valves can be loaded onto stents and inserted transapically for deployment into the pulmonary (Weber et al. 2013; Driessen-Mol et al. 2014; Schlegel et al. 2015) or aortic (Emmert et al. 2012) position. Though this technique requires a mini-sternotomy to expose the apex of the heart, it shows the use of catheter-based delivery methods for tissue-engineered technology. This application of minimally invasive medicine may open the door to fetal intervention with the novel therapies offered by tissue engineering. Correction of congenital malformations before birth may prevent the secondary sequelae of cardiovascular defects and prevent fetal demise (Weber et al. 2012).

WHOLE HEART

Much of the research regarding whole heart tissue engineering are proof-of-concept studies testing the effectiveness of decellularization methods. Decellularization of native heart ma-

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terial is a powerful approach to easily recapitulate the in vivo architecture and extracellular composition of the heart. In 2008, Ott et al. decellularized whole rat hearts using a perfusion system of 1% sodium dodecyl sulfate (SDS) in deionized water (Ott et al. 2008). When reperfused, the decellularized heart maintained its vascular channels, demonstrating the preservation of overall native morphology. When reseeded with neonatal rat cells, the heart regained a cellularized appearance, and sections cut from the reseeded heart were able to propagate action potentials initiated by an external source. The whole heart graft also showed pharmacological responsiveness to phenylephrine. Overall, this study provided a basis for whole heart decellularization with subsequent repopulation, but much work is left to be performed as the model only provided a cardiac pump function at 2% of the adult rat heart output (see Ott et al. 2008 for images of decellularized rat hearts).

SUMMARY

The increasing incidence of CVD and the limited availability of transplant organs have more than validated the field of cardiovascular tissue engineering. Various scaffolds and biologic tissue have been used to recapitulate the native architecture of the heart and great vessels. Significant advances have been made in scaffold development, manipulation of progenitor cells for seeding, bioreactor development for culturing, and, more recently, minimally invasive delivery of tissue-engineered organs to target sites. However, several questions remain unanswered, and the complexity behind such questions necessitates collaborative efforts across disciplinary fields. Future clinical application of this novel approach depends on it.

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