

The Development of Tissue-Engineered Grafts for Reconstructive Cardiothoracic Surgical Applications

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ABSTRACT: Surgical correction of congenital heart defects often requires the use of valves, patches, or conduits to establish anatomic continuity. Homografts, xenografts, or mechanical prosthetic devices are frequently implanted during these surgical procedures. These grafts however lack growth potential, are associated with increased risk of thrombosis and infection and have limited durability, thus increasing the morbidity and mortality of their application in pediatric cardiac surgery. These limitations are being addressed through the development of living, biologic tissue-engineered valves, patches, and conduits. Pilot studies and phase 1 clinical trials are currently underway to evaluate their feasibility, safety, and efficacy. The optimal scaffold, cell source, and conditioning parameters, however, still remain to be determined and are areas of active research. (*Pediatr Res* 63: 559–568, 2008)

Congenital heart defects (CHDs) occur in 0.8% of all live births in the United States (1). Although some cardiac defects are clinically irrelevant, others are associated with significant morbidity and mortality. In North America, CHDs remain the leading cause of death among infants (2). Although medical therapy alone successfully supports cardiac function in some patients with cardiac defects, many complicated CHDs require surgical correction to establish anatomic continuity and physiologic restoration of flow (3). It has been estimated that less than half of all CHDs (0.3–0.4% of all live births) are of surgical interest (4). Surgical correction of CHDs is generally reserved for cases in which other forms of therapy cannot maintain adequate circulation or the structural defects pose a threat to the development of the heart, lungs, or other organs. The form of surgical repair depends on the anatomic defects present, physiologic aberrances, and clinical presentation.

Following the first ligation of a patent ductus arteriosus in 1938 by Robert Gross, the field of pediatric cardiac surgery was born (5). The first open heart surgery was completed by Lewis in 1953 at which time he repaired an atrial septal defect under direct vision by inducing cardioplegia using hypothermia (6). John Gibbon was instrumental in further advancing this area of surgery with the introduction of a heart–lung machine that was described in 1954, which allowed for car-

diopulmonary bypass during open-heart surgery and was first used during the repair of a large atrial-septal defect (7). Using hypothermic cardioplegia to induce intraoperative cardiac arrest in conjunction with cardiopulmonary bypass allowed for improved visualization of cardiac defects, increased accuracy in diagnosis of such defects, and successful intracardiac repair (8). With the introduction of the Blalock–Taussig shunt in 1944 (9) and the Fontan procedure in 1971 (10), oxygen poor systemic blood could be redirected to the pulmonary system, allowing for repair of numerous complicated heart defects that result in cyanotic heart disease. Among these conditions are tetralogy of Fallot, hypoplastic left heart syndrome, pulmonary atresia, and tricuspid atresia. Although a relatively young field, pediatric cardiothoracic surgery, specifically repair of CHDs, has proven to be a rapidly advancing area of medicine. Implementing many techniques of cardiothoracic surgery developed over the last century, surgeons today are attempting to repair even the most complex congenital defects to improve patient survival, minimize morbidity, and improve quality of life.

Congenital structural anomalies of the heart may affect any parts of the atria, ventricles, outlets, and/or great vessels. Although primary repair of defects is sometimes possible, implementation of prosthetic replacement grafts has allowed for establishment of anatomic continuity and physiologic restoration in more complicated cases. The Fontan procedure often involves implantation of prosthetic conduits that connect the right atrium to the pulmonary artery directly when tricuspid atresia and/or pulmonary atresia are present (10). In addition, patients afflicted by coarctation of the aorta may require implantation of a prosthetic conduit or patch to connect the left ventricle with the aorta distal to the site of coarctation. Patients with a diagnosis of tetralogy of Fallot may require implantation of a prosthetic conduit to restore blood from the right heart to the pulmonary system and/or placement of a prosthetic valve to repair a stenosis. Patients with large atrial-septal defects or ventricular-septal defects may require implantation of prosthetic patches during repair. Although these grafts may be life saving, they have limited durability and are prone to infection, immunologic reactivity, and thrombosis, which often requires repeat operations in the

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Abbreviations: CHDs, congenital heart defects; IVC, inferior vena cava; P4HB, poly-4-hydroxybutyrate; P(CL/LA), ϵ -caprolactone and lactide acid copolymer; PGA, polyglycolic acid; PGLA, polyglycolic acid and polylactic acid copolymer; PHO, polyhydroxyoctanoate

future. Of particular concern in the pediatric population is the lack of growth potential of these currently available grafts.

Clinically significant aortic valve stenosis may be surgically repaired *via* a procedure described by Ross in 1967 (11). This procedure involves replacement of the stenosed aortic valve with a pulmonary autograft and subsequent replacement of the pulmonary valve with a prosthetic valve or homograft. Use of a pulmonary autograft into the aortic position provides a graft that grows as the patient grows, allows for favorable hemodynamics, frees a patient from the risk of anticoagulation, has limited infectious potential and does not contain foreign material thereby eliminating the risk of immunologic reactivity. The success of the Ross procedure is in part due to the success of the pulmonary autograft as it acts as an ideal valve replacement in the aortic position. A valvular graft placed into the pulmonary position does not develop dysfunction as readily as that in the aortic position. In addition, dysfunction of a valve in the pulmonary position is better tolerated than in the aortic position because of the lower pressures in the right heart and lower resistance in the pulmonary system. Many of the replacement valves placed into the pulmonary position, however, have limited durability and often require subsequent replacement. Depending on the type of replacement valve implanted, patients may be at an increased risk of endocarditis, thromboembolic events, bleeding effects of anticoagulation, calcification, and/or transmission of infections. It should also be noted that the Ross procedure potentially creates pulmonary valve disease in a previously healthy valve and treats a single valve disease with a two-valve procedure. Thus, failure of this procedure now places the patient at risk for repair of two valves.

To address both the limitations of grafts currently used as well as donor organ tissue scarcity, tissue-engineering applications have emerged to provide alternate solutions (12). Tissue engineering is an interdisciplinary field that integrates work in the fields of medicine, engineering, and biologic sciences. One tissue engineering strategy for creating neo-tissue is based on seeding a scaffold with biologic tissue (Fig. 1) (13). Following the work of Weinberg and Bell (14) in which blood vessels were constructed from bovine collagen scaffolds seeded with autologous cells, biodegradable polymers were later analyzed for the construction of tissue-engineered grafts (Table 1). With the ability of seeded cells to remodel biodegrading scaffolds, tissue-engineered grafts have the potential to grow, self-repair, and develop into tissue similar in appearance and physiologic function to native tissue (15). Additionally, use of autologous cell sources may eliminate the risk of rejection and reduce the risk of thrombosis, thus increasing biocompatibility (3).

Surgical repair of CHDs using tissue-engineered grafts requires that these structures maintain certain properties. These grafts must integrate into the surrounding tissue, possess an acceptable healing response with limited inflammation and immunogenicity, remain nonthrombogenic and resist infection. An ideal graft should permit diffusion of nutrients and waste, enable cell adhesion, migration, proliferation and differentiation, and facilitate formation of the extracellular matrix and endothelial lining (16). Vascular grafts must also withstand

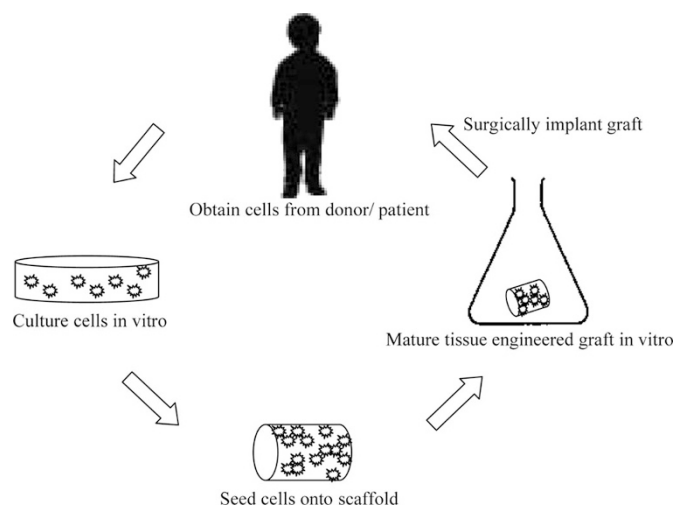


Figure 1. Approach to tissue engineering. Autologous cells are obtained from a patient and subsequently grown *in vitro*. Cells are then seeded onto a scaffold and matured before implantation into the patient.

Table 1. Various approaches to constructing tissue-engineering grafts

Seeding cells onto biodegradable scaffolds
Seeding cells onto acellular tissue scaffolds
Unseeded acellular tissue scaffolds which will repopulate in-vivo via circulating cells

hemodynamic stresses while maintaining elasticity to prevent aneurysm formation and respond appropriately to constrictive or relaxation signals from hormones and pharmacologic agents in the circulatory system. Finally, clinical application of these grafts mandates that they be easily manipulated by surgeons and stable enough to hold suture material.

TISSUE-ENGINEERED CARDIAC VALVES

CHDs involving the valves of the heart can lead to the devastating effects of stenosis with outflow obstruction or regurgitation with loss of unidirectional blood flow. Stenosis leads to hypertrophy of the myocardium whereas regurgitation leads to volume overload. Mixing of oxygenated and deoxygenated blood causes chronic hypoxemia and cyanosis. Inefficiency of the cardiovascular system causes potentially lethal conditions if left untreated.

Since Hufnagel and Harvey first implanted a prosthetic valve in 1953, numerous types of prosthetic valves have been used to replace diseased valves (17). Currently, treatment for patients with valvular disease is heart valve replacement surgery using a mechanical prosthetic valve, glutaraldehyde-fixed xenograft, homograft, or autograft *via* the Ross procedure. Replacement valves can be life saving and often lead to improved quality of life; however, they are not without significant risks (18,19). The relative strengths and weaknesses of these replacement valves have been identified (Table 2), thus allowing clinicians to best tailor selection of the graft to the individual. Caldarone *et al.* found that while younger age correlated with reduced time to valve replacement, diagnosis, type of prosthesis, and size of prosthetic valve also effect valve longevity (20). Mechanical valves are durable but are

Table 2. *Advantages and disadvantages of valvular grafts*

Valvular graft	Advantages	Disadvantages
Mechanical prosthesis	Durable	Thromboembolic events
	Readily available Easy to handle	Hemolysis Hemorrhage secondary to anticoagulation No growth/remodeling
Decellularized xenograft	Readily available	Possibly immunogenic
	Nonthrombogenic Decreased risk of calcification	Structural deterioration Infectious complications No growth/remodeling Decellularization may alter physical properties Must attract cells <i>in vivo</i> Mechanism of cellular attraction/adhesion remains uncertain
Homograft	Nonthrombogenic Biocompatible	Donor scarcity Calcification Structural deterioration Infectious complications No growth/remodeling
Tissue-engineered graft	Readily available	Labor intensive processing
	Nonthrombogenic	Function relies on deposition of neotissue
	Growth potential	Inflammatory response
	Remodel <i>in vivo</i>	Difficult to control cell adhesion and tissue reorganization
	Low infectious risk Control structure Control properties	Limited perfusion to deep cells Scar tissue replaces graft

associated with an increased risk of thrombosis and hemolysis (21). Patients receiving mechanical valves must be maintained on anticoagulation (22,23) with a risk of hemorrhagic complications (0.5–3.3 events/100 patient-years) (24) that poses a great danger in the pediatric population. Duran and Gunning described the first implantation of a heterologous valve into a dog in 1965 (25). This was subsequently followed by implantation into humans (26). Patients receiving such xenograft valves are at increased risk of infectious complications such as endocarditis and will require additional valve replacement surgeries because of graft degeneration and limited durability (27,28). Pediatric patients have a higher incidence of homograft calcification (29) and accelerated rates of graft degeneration, which may be attributable to age-related immune mechanisms (30,31). Although cryopreserved homografts are the most biocompatible replacement heart valve available, donor scarcity, transmission of infection and transplant-associated problems limit their use (32,33).

Properties of native valves have been described in Table 3. Ideal replacement heart valves should be durable, biocompatible, nonimmunogenic, nonthrombogenic, readily available, easy to handle, and have the potential to grow and remodel *in vivo* (Table 4) (34). These valves should not require anticoagulation and should be associated with a low risk of infectious complications (24). Tissue engineering principles have

Table 3. *Properties of native valves*

Unidirectional blood flow
Mechanical strength
Cellular viability
Growth
Repair/remodeling

Table 4. *Properties of ideal replacement valves*

Durable
Biocompatible
Nonimmunogenic/noninflammatory
Nonthrombogenic
Readily available
Easy to handle
Easy to implant
High suture retention strength
Ability to withstand high pressures
Growth potential
Remodel <i>in vivo</i>
Adequate mechanical function
Adequate hemodynamic performance
Flexible
Support cellular viability/proliferation
Support cellular attachment
Porous macrostructure (support cellular migration and waste removal)
Resorbable
Low infectious risk

been applied in the construction of an ideal replacement valve. Tissue-engineered valves may eliminate the need for additional operations and the morbidity and mortality associated with these procedures.

Tissue-engineered valves have been constructed by seeding biodegradable synthetic valve matrices, decellularized allografts or xenografts, or by implanting unseeded decellularized allografts (35). Scaffolds coated with an endothelial cell layer have better long-term patency rates than their unseeded counterparts (36). Scaffolds have been composed of various types of polymers or polymer combinations (Table 5). Cells seeded onto a scaffold also support neo-tissue formation. Cell sources have included vascular-wall derived cells, peripheral blood-derived cells, and bone marrow-derived cells (Table 6) (37). Use of autologous tissue may eliminate the risks of infection, rejection, hemolysis, and thrombosis (38). This would also eliminate the need for a tissue donor (39).

Tissue-engineered valves constructed from varying combinations of scaffolds and cell sources matured under differing growth conditions have been studied in both animal models and humans (40). Early work in the development of tissue-engineered cardiac valves was completed by Breuer *et al.* (41). Tissue-engineered lamb heart valve leaflets were made by seeding a degradable synthetic polymer scaffold made from polyglycolic acid (PGA) and a copolymer of PGA and polylactic acid (PGLA) with myofibroblasts and large-vessel endothelial cells obtained from either the femoral or carotid arteries of 20-d-old lambs. These leaflets were then implanted into the donor lambs in the position of the native right posterior pulmonary valve leaflet. Although the polymeric scaffolds degrade by hydrolysis (over 6–8 wk), these scaffolds initially provide biomechanical strength and work together with the seeded cells to develop neo-tissue. Evaluation of the

Table 5. Advantages and disadvantages of various biodegradable polymers used in tissue engineering

Biodegradable polymer	Advantages	Disadvantages
PGA	Rapid degradation (6–8 wk) Strength Facilitates neotissue formation FDA approved	Lose mechanical strength at 2–4 wk Limited pliability Increased graft thickness Does not maintain predefined structure
PLA	Limits hydrolysis/degradation FDA approved	Poor substrate for cell growth <i>in vitro</i>
PHA	Flexible	Prolonged degradation time
PHO	Strength	Prolonged degradation time
P4HB	Rapid degradation (8 wk) Thermoplasticity	Limited cellular/collagen uptake
Decellularized allografts	Limited immunologic reactivity Growth potential	Donor scarcity Infectious complications
Decellularized glutaraldehyde	Readily available	Infectious complications
Fixed xenografts	Strength Durability Limited immunologic reactivity	Early structural deterioration Limited pliability Lacks growth potential

cardiac leaflet by two-dimensional echocardiography and color Doppler ultrasonography on postoperative days 1, 7, 14, and 21 demonstrated that although the tissue-engineered valves were thicker than the native leaflets and did not move as freely as native tissue, there was no evidence of stenosis or clinically significant regurgitation. Upon histologic evaluation of the leaflets, the seeded grafts demonstrated an extracellular matrix similar to that of native leaflets. On the other hand, lambs receiving unseeded grafts showed evidence of leaflet degradation with worsening regurgitation, eventually rendering the leaflets dysfunctional.

Using a similar degradable PGA and copolymer PGLA mesh, human dermal fibroblasts with bovine aortic endothelial cells or sheep myofibroblasts with sheep endothelial cells were seeded onto scaffolds (42). These grafts were implanted into sheep with histologic and immunohistologic evaluations conducted 14 d later. The endothelial cells formed a monolayer on the vessel lumen. Analysis revealed that the engineered valve

leaflets were morphologically and histologically similar to native tissue, therefore supporting seeding of the valvular grafts.

Construction of trileaflet heart valves using PGA with PGLA was limited by cusp stiffness, thickness, and nonpliability (43). In response, Sodian *et al.* constructed a trileaflet prosthetic heart valve from a polyhydroxyalkanoate biodegradable polymer scaffold seeded with autologous ovine cells obtained from carotid arteries (44,45). Following cell seeding, the valves matured in a bioreactor that provided pulsatile flow for 1, 4, and 8 d. The seeded cells proliferated and formed a confluent layer oriented in the direction of flow. An extracellular matrix containing collagen also developed in the seeded grafts. On the other hand, grafts that had not been seeded failed to show appropriate tissue formation or collagen content comparable with that of native tissue. Previous work by Shinoka *et al.* confirmed that animals receiving tissue-engineered pulmonary valve leaflets seeded with autologous cells developed less infectious complications and failed to show shrinkage or deterioration of the valves when compared with valves seeded with allogenic cells (43), therefore further supporting the use of autologous cells in the construction of tissue-engineered valves.

Additional studies using different copolymer combinations were conducted by Stock *et al.* in which valves and associated conduits were made from composite scaffolds of PGA and polyhydroxyoctanoates (PHO) (46). The constructs were seeded with autologous ovine cells, incubated, and implanted in sheep to replace both the native pulmonary valve and main pulmonary artery. The sheep did not receive postimplantation anticoagulation. These valves were evaluated by echocardiography, histology and biochemical analysis at time points ranging from 1 to 24 wk. The seeded grafts remained patent throughout the study and demonstrated viable cells, which formed an organized structure containing an endothelial cell lining. The unseeded control valve, however, developed thrombus at 4 wk postimplantation. Neither group of valves showed growth over the study period. Although PGA was completely degraded by 24 wk, the conduits continued to demonstrate PHO. Residual PHO likely contributes to lack of growth of the conduit during the 24-wk period, thus limiting clinical applicability in pediatric patients.

To address the limiting factor of prolonged scaffold degradation time, Hoerstrup *et al.* studied a composite pulmonary valve scaffold made of PGA coated with a rapidly bioabsorb-

Table 6. Advantages and disadvantage of various cell sources used in tissue engineering

Cell source	Advantages	Disadvantages
Bone marrow stem cells	Easy to obtain Readily available Extensive <i>in vitro</i> proliferation Differentiate into various tissues May be autologous cells	Difficult to control cell differentiation Mechanisms of differentiation Processes are poorly understood
Vascular derived cells	Autologous cells Often available in pediatric patients and patients without vascular disease	Invasive procedures to obtain cells Not available in patients with vascular disease, atherosclerosis, surgically absent vessels
Peripheral blood progenitor cells	Easy to obtain Readily available Autologous cells	Slower proliferation than bone marrow cells

able polymer, poly-4-hydroxybutyrate (P4HB) (47). The scaffolds were seeded with autologous lamb cells and conditioned in a bioreactor, which provided both pressure and flow to promote mechanical strength and modulate cellular function (48,49). These valves were implanted into the donor lambs and evaluated histologically and mechanically at day 1 and at 4, 6, 8, 16, and 20 wk. By 8 wk postimplantation, complete degradation of the polymer scaffold was seen. Explanted valves evaluated at later time points demonstrated layered neo-tissue, a partial endothelial cell lining and an extracellular matrix similar to that of native valves. Mechanical properties of the explanted valves resembled those of native tissue at the corresponding time courses. Additionally, there was no evidence of thrombus formation or stenosis of the prosthetic valves. Comparison studies between P4HB and PGA valvular scaffolds showed that PGA took up more cells and formed greater amounts of collagen than P4HB, thereby limiting the use of P4HB (50).

To overcome the limitations associated with polymeric scaffolds, decellularized xenograft scaffolds were studied. Removing the cellular components from native valves leaves behind the extracellular matrix of the natural valves. It is unclear, however as to the effects of the decellularization process on the biomechanical properties of the tissue. Initially, these valves were fixed in formaldehyde; however, these did not have long-term success secondary to graft instability (51,52). As a result, the glutaraldehyde-fixed valve was developed and first implanted in 1971 (53,54). Glutaraldehyde fixation leads to cross-linking of the extracellular matrix proteins, which provides a sterile scaffold with reduced antigenic potential. Although glutaraldehyde-fixed xenografts are associated with decreased immunologic recognition by human recipients and are more stable than their unfixed counterparts, these grafts are associated with significant drawbacks. Cytotoxicity of the fixatives (*i.e.*, glutaraldehyde) often precludes adequate endothelialization of the grafts (55). Glutaraldehyde cross-linked valves lack cellular viability or the ability for growth or remodeling. These grafts are also stiffer than native tissue (41,45). Early work using these valves led to clinical cardiovascular deterioration as a result of prosthetic thrombotic stenosis, valve degeneration, and endocarditis which developed at late postoperative time points (56). In several cases of failed valves, the xenografts demonstrated stenosis secondary to fibrin deposition (57,58). Despite this, the risk of thrombosis remains lower than in those patients receiving mechanical valves. Therefore, recipients of these valves do not typically require anticoagulation therapy. Additionally, improved antibiotics have reduced the infectious complications associated with xenograft implantation. The risk of early structural deterioration of the fixed xenografts in younger patients remains significant. Early work using these replacement glutaraldehyde-fixed porcine valves showed a significant number of pediatric recipients with accelerated calcification of the valve cusps that led to severe stenosis and regurgitation (59,60). Progressive deterioration of the valves leads to collagen disruption and calcification that ultimately results in graft failure and necessitates eventual replacement of the grafts. Although studies comparing porcine valve replacements with mechanical valve replacement in the pediatric

population demonstrated superior outcomes when receiving xenografts, these valves are plagued by limitations that leave us searching for an ideal replacement valve (61).

Goldstein *et al.* devised tissue-engineered heart valves (SynerGraft porcine heart valves, CryoLife Inc., Kennesaw, GA) from acellular composite porcine aorta, noncoronary aortic leaflet and anterior mitral leaflet with the goal of addressing the problems of graft stiffness, limited growth potential, and limited durability of glutaraldehyde-fixed xenograft heart valves (62). Goldstein's valves were designed to provide optimal performance with decreased immunogenic potential while maintaining the ability to grow, repair, and remodel. Three tissue-engineered valve leaflets were attached to a single root and subsequently implanted into sheep in the pulmonary valve position and into humans in the aortic or pulmonary valve position. These porcine leaflets demonstrated recellularization with the development of fibroblasts and myofibroblasts similar to those found in native leaflets. Although previous animal studies of implanted allogenic acellular matrix scaffolds seeded with autologous cells demonstrated subvalvular muscular calcification extending to the valve leaflet base in one animal (63), evaluation of the SynerGraft valves failed to show stenosis or significant calcification in any of the animal models. When implanted into four male children with CHDs, however, the results were devastating (64). Of the four children who received these valves, three died within 1 y of receiving the graft and the fourth child underwent prophylactic removal of the valve. Histologic examination of the failed valves demonstrated fibrous hyperplasia without cell repopulation or endothelialization. The recipients underwent strong inflammatory responses to the xenogenic collagen matrix leading to graft degeneration and structural valve failure.

Dohmen *et al.* described the first human implantation of a tissue-engineered heart valve (Fig. 2) made from decellularized pulmonary allograft seeded with autologous vascular endothelial cells obtained from a forearm vein 4 wk before implantation (65). This valve was implanted in a human in the pulmonary position during the Ross procedure. Postoperatively, the recipient had slight regurgitation (which remained stable at 1 y follow-up) with a normal functioning valve that

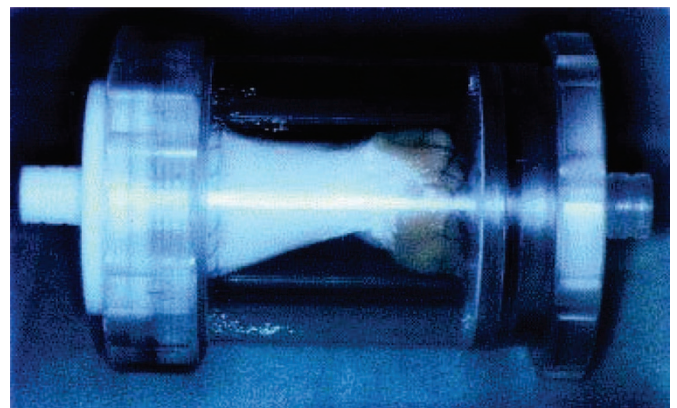


Figure 2. A bioreactor containing a tissue-engineered valve, which is attached at both ends to facilitate cell seeding. Reprinted from Dohmen *et al.* *Ann Thorac Surg* 74:1438–1442, Copyright © 2002 The Society of Thoracic Surgeons Published by Elsevier Inc., with permission.

lacked calcification. Similar results were found when the same group implanted their valve into the pulmonary position of another patient during the same procedure (66). Although both patients seemed to have undergone clinical improvement following their operations, the amount of time necessary to construct such a valve makes it less likely to be useful in the perinatal period or in the case of life-threatening CHDs for which immediate repair is necessary.

Constructing a graft with a readily available cell source is advantageous for cases in which an extended graft preparation period is not possible. Such a source includes cells obtained from peripheral blood. Implantation of decellularized human pulmonary allograft heart valves seeded with autologous endothelial progenitor cells obtained from peripheral blood was first reported by Cebotari *et al.* (38). Following conditioning of the seeded allografts in a bioreactor for 21 d, the valves were implanted into two pediatric patients with tetralogy of Fallot and associated pulmonary valvular disease. At 3.5 y postimplantation, there was no evidence of valve degeneration. Both patients initially had pulmonary regurgitation following their surgeries; however, the diameter of the pulmonary valve annulus increased proportionately as the child grew leading to resolution of regurgitation. At follow-up, both children continued to show normal somatic growth without limitations of physical activity or cardiac arrhythmias.

Although preliminary short-term data for the development of tissue-engineered valves are encouraging, much work remains to be completed in the construction of an ideal graft. Obtaining autologous cells for seeding from recipient patients requires invasive procedures to access these cells, additional hospitalizations and increased risks of complications. Additionally, cells that are cultured in xeroserum during preparation present risk of immunogenic reaction upon implantation. With the recent use of autologous bone marrow cells and mononuclear cells from peripheral blood, some of these concerns have been addressed. Additionally, the tissue-engineered valves that have been studied thus far have been implanted into low-pressure systems. Application of these valves in a high-pressure system is still pending. Although great strides in the field of valvular tissue engineering have been made, continued efforts will enable development of viable, self-remodeling valvular substitutes with growth potential for further application in repair of CHDs.

TISSUE-ENGINEERED CARDIOVASCULAR PATCHES

Many cardiac defects require complex reconstruction of the heart and associated pulmonary and systemic connections. Repair of complete atrial-ventricular septal defects, augmentation of a hypoplastic, stenotic right ventricular outflow tract, or repair of tetralogy of Fallot often use nonautologous constructs, such as patches. Available patches include autologous pericardium, knitted polyester, Gore Tex (polytetrafluoroethylene), and bovine pericardium. Shortcomings of these patches include the inability to grow, remodel, and regenerate as well as increased risk of infection and aneurysm. These constructs are also subject to obstructive tissue ingrowth and

fibrotic responses with shrinkage and calcification leading to graft failure (67). Autologous pericardium is difficult to handle (68,69) while synthetic patches are associated with thromboembolic complications, hemolysis, and infective endocarditis (70,71). Although flexible, strong, and easy to handle, bovine pericardium may be associated with graft calcification and contraction (72,73).

Upon repair of CHDs using Dacron patches, presence of the foreign body induced a scar tissue healing response with contracture of the implant subsequently necessitating reoperation (74). Clinical studies using tissue-engineered vascular patches, however, have failed to show evidence of such an inflammatory response (39). This is likely the result of degradation of the polymer scaffold, leaving behind only autogenous neo-tissue. One group also observed that Dacron grafts were associated with prosthetic thickening and stiffness (75). In addition, the same study analyzing Dacron patches in the repair of CHDs, demonstrated tissue growth originating from the edges of the implanted graft with the center region of patches larger than 2.5 cm in width lacking tissue (74). Absence of an endothelial layer in this region of repair may place a patient at increased risk of thrombus or vegetation formation (70). This is of particular concern, as we know that thrombus formation often occurs in the presence of an unendothelialized foreign body. A tissue engineered patch designed *in vitro*, however, can be seeded with autologous endothelial cells so as to reduce the risk of thrombus formation.

One concern with tissue engineered cardiovascular patches is premature degradation of the polymer scaffold, leaving behind a weakened region of repair susceptible to aneurysmal dilatation. This is of particular concern for patch repairs exposed to high pressures. An advantage of tissue-engineered patches is the ability to control various properties of the graft, including scaffold degradation time. Utilization of different combinations of polymers and various scaffold preparation techniques enable researchers to develop patches with varying biomechanical and degradation profiles for numerous applications.

Tissue-engineered principles have been applied to the development of cardiac patches for repair of CHDs. Schmidt *et al.* used autologous human umbilical cord-derived fibroblasts and endothelial progenitor cells seeded on PGA/P4HB scaffolds to construct these patches (76). Endothelial progenitor cells obtained from human umbilical cord are readily available and can be harvested during the prenatal period (*via* ultrasound guided chordocentesis) making them an attractive cell source for tissue-engineered patches that require implantation in the immediate postnatal period. This would allow for treatment of CHDs diagnosed *in utero*. Following cell seeding, polymer scaffolds used in this model were conditioned in a bioreactor system for 27 d before implantation. All samples demonstrated evidence of layering of viable tissue. Collagen and proteoglycans were found in the extracellular matrix, with a higher content found in patches that had been conditioned with cyclic strain. The lumen contained endothelial cells and the newly formed tissue contained myofibroblasts. Mechanical properties of the tissue-engineered patches were similar to those of native analogous tissue; however, the patches failed to reach

physiologic strength. These patches therefore are restricted to low-pressure systems such as the right ventricular outflow tract.

Bone marrow provides another cell source that is readily available and less invasive to obtain than cells from the walls of autologous blood vessels. These cells are also useful in patients who lack adequate blood vessels for harvest because of preexisting vascular disease or use in previous procedures or surgeries. Bone marrow cells differentiate into smooth muscle cells and endothelial cells (77–79). Cells found in the bone marrow can also differentiate into several cell types *in vivo*, which contribute to angiogenesis and vasculogenesis (80). Cho *et al.* constructed vascular patches by seeding autologous bone marrow-derived cells onto decellularized matrices obtained from the inferior vena cava (IVC) of dogs (81). Following differentiation of bone marrow mononuclear cells, they were seeded onto autologous matrices and implanted into canine models. Patch recipients did not receive postoperative anticoagulation or antiplatelet agents. Three weeks postimplantation, the grafts were explanted and analyzed histologically, immunohistochemically, and by electron microscope. Presence of endothelial cells, smooth muscle cells, collagen, and elastin were reported in the explanted constructs. All vascular patches remained patent without thrombus formation, thus supporting continued investigation of bone marrow as a cell source in the construction of tissue-engineered grafts.

Tissue-engineered vascular patches constructed from autologous bone marrow mononuclear cells were first implanted into human patients in 2001 (82). Before using bone marrow cells, this group had implanted patches made from biodegradable scaffolds seeded with autologous cells that had been harvested from peripheral veins (83). Because harvesting peripheral veins is an invasive and lengthy procedure requiring additional hospitalization, this group sought aspiration of bone marrow to obtain a readily available cell source for seeding the tissue engineered polymer scaffolds. Bone marrow was aspirated on the day of surgery, which eliminated both the need for a donor and exposure to xeroserum during the cell culture process, thereby reducing the risks of rejection and infection and significantly decreasing the time for graft preparation from 4 wk to less than 4 h. The scaffolds prepared were comprised of either a PGA nonwoven fabric sheet or poly-L-lactide (PLLA) mesh coated with a copolymer of lactide and ϵ -caprolactone (P(CL/LA)). The tissue-engineered patches were initially implanted into patients with defects of the pulmonary artery, however, were later extended to include a patient with a common atrium, which was separated by means of a tissue-engineered sheet. Since 2001, 19 tissue-engineered patches made of polymer scaffolds seeded with bone marrow-derived cells have been implanted for repair of CHDs (39). Although histologic evaluation of implanted grafts in living human recipients is not possible, there has been no evidence of graft rupture, dilatation, calcification, thrombosis, stenosis, or obstruction as assessed by postoperative echocardiography, catheterization, computerized tomography, and/or magnetic resonance imaging. Lack of thrombus formation or stenosis, suggest that the remodeling process that occurs after patch implantation is well tolerated clinically. In addition, cellular

proliferation and regeneration, and deposition of neo-tissue do not appear to adversely affect implantation of the tissue-engineered patches. Unlike in procedures in which infective endocarditis follows implantation of synthetic patches, patient receiving tissue-engineered patches have not been afflicted by infective complications. These early results are promising in the application of tissue-engineered patches for repair of CHDs and warrant continued evaluation.

TISSUE-ENGINEERED VASCULAR CONDUITS

Conduit implantation for restoration of anatomic continuity between the ventricle and pulmonary artery was first described in 1964 by Rastelli *et al.* (84). In 1988, de Leval introduced the total cavopulmonary connection procedure which involved implantation of a conduit to join the systemic circulation to the pulmonary circulation (85). Since that time, reconstruction of complex CHDs involving abnormal pulmonary arteries and/or right ventricular outflow tracts have used conduits to reestablish anatomic continuity and physiologic blood flow (86). Conduits have been used in repair of pulmonary atresia, tetralogy of Fallot, transposition of the great arteries, truncus arteriosus, single ventricle physiology, and other complex anatomic defects (87). Conduits used for surgical repair of these abnormalities have included those made of Dacron (polyethylene terephthalate) or Gore-Tex (polytetrafluoroethylene), homografts and more recently, tissue-engineered conduits (88). Although in years past, prosthetic or bioprosthetic conduits had been used in the pediatric population, patient growth and progressive deterioration, and calcification of the replacement conduits led to stenosis and subsequent conduit failure (30,89). Obstructed Dacron conduits have a fibrinous peel that develops in the lumen while homografts are often calcified at time of graft stenosis (89). The limited conduit lifespan necessitates repeat operations in pediatric recipients thereby making tissue-engineered conduits that grow, remodel, and regenerate an attractive alternative (90).

It has been hypothesized that low blood flow states increase susceptibility to thrombus formation in previously used prosthetic and bioprosthetic conduits (91). Others have attributed the increased risk of thrombus formation and stenosis/obstruction of these conduits to deficiencies of thrombomodulin, increased fibrosis, neo-intimal hyperplasia, or calcification (87,92). Tissue-engineered conduits that remodel in response to varying physiologic conditions would prove advantageous in that they may decrease the risk of thrombus formation. One major consideration, however, when developing tissue-engineered conduits is that the rate of scaffold degradation must be balanced with that of neo-tissue formation so to minimize the risk of wall thinning or weakening and subsequent aneurysm formation. This is particularly true for conduits exposed to arterial pressures.

Tissue-engineered pulmonary arterial conduits made of acellularized ovine pulmonary arteries seeded with autologous endothelial cells from carotid arteries were implanted into immature, growing sheep by Leyh *et al.* (93). Unseeded acellular pulmonary conduits were implanted into control sheep. At 6 mo postimplantation, the pulmonary artery con-

duits were retrieved from the study subjects. At this time period, neither group demonstrated evidence of thrombus formation or calcification. Histologic analysis revealed that the control specimens underwent degeneration of the extracellular matrix with reduced amounts of collagen and proteoglycans. The seeded conduits illustrated an increased cellularity within the extracellular matrix, resembling that of native ovine pulmonary artery. Increased cellularity may be the result of greater cell turnover; however, this needs to be further evaluated to ensure that fibrosis does not develop at a later time. Loss of conduit wall integrity seen in the control specimens placed these animals at increased risk of developing aneurysmal dilatation of the tissue-engineered conduits. Conduits that had been seeded with endothelial cells failed to show aneurysmal dilatation and instead demonstrated increases in diameter proportional to that of the native pulmonary arteries in the growing animals. Keeping in mind that currently used grafts lack growth potential, results of this study provide encouragement for the application of tissue-engineered conduits in repair of CHDs in growing children.

Pulmonary artery segments were also replaced with tissue-engineered conduits in growing lambs by Shinoka *et al.* (94). Similar to earlier work completed by this group in the application of tissue-engineered valves (41), the conduits were made of autologous myofibroblasts and endothelial cells that were seeded *in vitro* onto a PGA scaffold. Echocardiography and angiography confirmed that seeded grafts remained patent with nonaneurysmal increases in diameter, while control unseeded grafts developed thrombosis with subsequent occlusion as early as 2 wk postimplantation. Complete degradation of the polymer scaffold was seen by 11 wk. The remaining tissue resembled native pulmonary artery in collagen content and demonstrated elastic fibers and a luminal endothelial lining similar to those seen in native tissue. At the study conclusion, there was evidence of continued tissue remodeling of the grafts with a slight elevation of microscopic calcium content, though this was not found macroscopically.

Although not as critical in low-pressure systems, concerns have been raised over the rapid degradation of PGA scaffolds. It has been hypothesized that when placed into a high-pressure system, a rapidly degrading scaffold may place a recipient at risk of aneurysm formation. Therefore, tissue-engineered conduits made of a copolymer scaffold comprised of PGA and P(CL/LA) were studied. These conduits were used to replace segments of the IVC in dogs (12). Characterization of the conduits in a low-pressure system enabled researchers to make necessary alterations for later application into a high-pressure system. The scaffolds were seeded with cells obtained from femoral veins of dogs that had been incubated for 7 d before implantation. There was no evidence of residual polymer scaffold at 3 mo postimplantation and none of the IVC replacement conduits showed occlusion or aneurysmal dilatation. One dog was observed for 13 mo at which time the IVC graft was without stenosis or occlusion. The grafts demonstrated endothelial cells that lined the luminal surfaces and extracellular matrix components that were comparable with native tissue.

Shinoka *et al.* later described the first implantation of vascular conduits into pediatric patients during repair of



Figure 3. Yellow arrow highlights the tissue-engineered conduit connecting the inferior vena cava to the right pulmonary artery in a total cavopulmonary connection placed during repair of Tetralogy of Fallot. Reprinted from Shinoka *et al.* *J Thorac Cardiovasc Surg* 129:1330–1338, Copyright © 2005 The American Association for Thoracic Surgery Published by Mosby, Inc., with permission.

CHDs (39). A PLLA with P(CL/LA) scaffold was seeded with cells harvested from the patient's own bone marrow on the day of surgery. The seeded conduits were placed in culture media for 2–4 h before implantation. The conduits were used to form extracardiac total cavopulmonary connections during repair of right ventricle outflow defects or single ventricle anatomy (Fig. 3) (39,95). Doing this provided the pulmonary system with deoxygenated blood from the systemic system. As of June 2005, 23 tissue-engineered conduits had been implanted for repair of CHDs (39). Unlike the cases of tissue-engineered patches implanted by this group and previously described in this review, histologic evaluation was conducted on one conduit that had been implanted into a patient that later died for a reason unrelated to the graft placement. This conduit had remained patent and demonstrated endothelium-like cells on the graft lumen. All patients receiving tissue-engineered conduits were evaluated by echocardiography, angiography, computerized tomography, and/or magnetic resonance imaging. Patients who had received these conduits did not show evidence of graft thrombosis, stenosis, dilatation, rupture, or failure. Additionally, graft diameter was dependent on flow through the graft, with grafts placed in low flow systems showing decreases in diameter and those in high flow systems showing increases in diameter. This portrays one of the major advantages of tissue-engineered grafts in that growth and remodeling occur in response to the surrounding environment. Although we lack long-term follow-up for patients who have received tissue-engineered conduits, the results obtained thus far are encouraging. The preliminary data obtained to date supports continued research in this area and continued efforts in

the development of tissue-engineered conduits for reconstructive cardi thoracic surgical applications.

CONCLUSION

The field of tissue-engineering cardiovascular structures for application in the repair of CHDs is young, yet rapidly progressive. We continue to search for optimal cells sources, scaffold matrices, and growth conditions when constructing grafts. Much data supports cell seeding in the assembly of tissue-engineered constructs; however, the fate and contribution of the seeded cells remain unknown. Immunofluorescent evaluation of tissue-engineered patches seeded with retrovirally labeled cells revealed presence of the seeded cells at 2 wk postimplantation (96); however, the role of cells from adjacent native tissue and the role of circulating cells on scaffold-cell interactions and remodeling remain to be determined (97).

Currently, we lack long-term studies of tissue-engineered grafts that would allow for additional characterization of scaffolds used. The effects of combining cells with varying scaffold matrices under different growth conditions have not yet been identified. Many questions still remain unanswered in the field of tissue engineering of cardiovascular structures used in the repair of CHDs. Despite this, it is clear that the care of patients afflicted with CHD has been and will continue to be greatly influenced by further developments in tissue engineering.

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