

Tissue Engineering Therapy for Cardiovascular Disease

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Abstract—The present treatments for the loss or failure of cardiovascular function include organ transplantation, surgical reconstruction, mechanical or synthetic devices, or the administration of metabolic products. Although routinely used, these treatments are not without constraints and complications. The emerging and interdisciplinary field of tissue engineering has evolved to provide solutions to tissue creation and repair. Tissue engineering applies the principles of engineering, material science, and biology toward the development of biological substitutes that restore, maintain, or improve tissue function. Progress has been made in engineering the various components of the cardiovascular system, including blood vessels, heart valves, and cardiac muscle. Many pivotal studies have been performed in recent years that may support the move toward the widespread application of tissue-engineered therapy for cardiovascular diseases. The studies discussed include endothelial cell seeding of vascular grafts, tissue-engineered vascular conduits, generation of heart valve leaflets, cardiomyoplasty, genetic manipulation, and in vitro conditions for optimizing tissue-engineered cardiovascular constructs. (*Circ Res.* 2003;92:1068-1078.)

Key Words: tissue engineering ■ cardiovascular system ■ heart valves ■ vascular grafts ■ cardiomyoplasty

New drugs and innovative devices have improved the quality of life for patients with cardiovascular disease but have not necessarily decreased morbidity or mortality. End-stage cardiovascular disease eventually becomes refractory to therapy. Organ replacement is eminently successful but sparingly used.¹ Successful treatment of cardiovascular disease is limited in many situations by the lack of suitable autologous tissue to restore injured cardiac muscle or to serve as vascular conduits to replace or bypass diseased or occluded vessels. In cases in which autologous material is lacking, synthetic graft material may be used. However, compared with native tissue, the performance of synthetic material often pales as a tissue replacement. Synthetic materials are more prone to thrombosis and calcium deposition, and they lack the ability to grow. Optimal replacement tissue for the cardiovascular system would be biocompatible while also exhibiting growth potential. Tissue engineering is proposed as a solution to these problems by replacing tissue or organ function with constructs that contain specific populations of living cells. The present review will address the current status in the development of tissue engineering in the cardiovascular system, including a general discussion of vascular cell characterization and culture as well as specific issues relating to arterial conduits, heart valves, cardiomyocyte restoration after infarction, and gene therapy.

Arterial Replacements

Vascular bypass can be accomplished with autologous veins or arteries or with synthetic grafts composed of materials

such as Dacron or expanded polytetrafluoroethylene. Although both native and synthetic grafts can be used in the high-flow environments of large-diameter grafts, only the former is acceptable in the smaller-diameter low-flow vessels.² Unlike the 85% to 95% long-term patency in large-diameter vessels, small-diameter (<5-mm) synthetic grafts have met with early thrombotic complications and late intimal hyperplasia, often leading to total graft occlusion.³ Less than 50% of the small-diameter femoropopliteal grafts remain patent 5 years after implantation.⁴ The lack of satisfactory long-term patency of small-diameter grafts has been attributed to the inherent thrombogenicity of their luminal interface. Because the availability of autologous material is often limited, there has been considerable effort toward increasing the success rates of prosthetic grafts.

Endothelial Cell Seeding of Vascular Grafts

An ideal artificial vascular graft closely mimics the natural vessel. It is resistant to thrombosis, inflammation, and neointimal proliferation, and for all intents and purposes, it looks like a native vessel. As such, the ideal graft possesses the structural integrity of a native vessel and resists degradation and adverse remodeling under a variety of pressure conditions. Moreover, the ideal graft functions metabolically and biochemically like a native vessel, presenting a luminal substrate that optimizes healing and circulating cell adhesion. Although mechanical strength is a property that can be reproduced with passive materials, metabolic function requires cellular machinery. The lack of viable endothelial cells

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TABLE 1. Polymer Surfaces Investigated for Endothelial Attachment, Proliferation, and Function

Polymer Surface	References
Polytetrafluoroethylene (PTFE)	12, 22
ePTFE/denucleated ePTFE	10, 20
Dacron	11, 12, 24
pHEMA/MMA copolymers	13–15
Polyurethane	22
Polyethyleneterephthalate (PET)	16, 25
Poly(ether urethane urea) (PEUU)	21
Perfluorosulfonic acid (Nafion)	18, 19
Polyvinyl chloride	23

(ECs) on the luminal surface of artificial grafts contributes to synthetic graft thrombogenicity and promotes intimal proliferation within the graft. EC seeding of synthetic grafts has been attempted to mitigate these limitations. Herring et al⁵ were the first to report the successful isolation of ECs and their subsequent transplantation onto synthetic vascular grafts. Suspensions of isolated canine venous ECs were used to precoat 6-mm Dacron grafts before implantation in a canine model.⁶ Four weeks after implantation, they observed 76% patency rates for seeded grafts versus 22% rates for unseeded grafts.^{6,7} Explanted grafts possessed an intact EC lining supported by smooth muscle cells (SMCs) along with penetrating vasa vasorum. Work by other groups has confirmed that seeding Dacron grafts with ECs before implantation into animals results in the recovery of ECs lining the graft lumen after explantation.^{8–12} Since these studies, numerous laboratories have focused on the preparation of polymer surfaces to enhance EC attachment (Table 1).^{13–26} This approach has been limited in some cases by poor retention of ECs on biomaterial surfaces once exposed to blood flow. Thus, significant effort has focused on improving endothelial adhesion to biomaterial surfaces.

An approach to obtain EC-specific adhesion requires covalently bonding synthetic peptides to the graft material. These peptides are based on the receptor-binding domains of cell adhesion proteins. Integrins, synthesized and expressed on the surface of ECs, recognize the Arg-Gly-Asp (RGD) sequence.^{27–30} Holland et al³¹ showed increased in vitro EC adhesion, spreading, and growth for cells plated on RGD attached to a starch-coated polystyrene surface compared with a fibronectin-coated surface. Hubbell and colleagues^{32,33} also immobilized a synthetic peptide containing the sequence Arg-Glu-Asp-Val (REDV) on the otherwise nonadhesive glycophase glass and polyethylene terephthalate that had been surface-modified with polyethylene glycol. When the REDV sequence was immobilized on cell nonadhesive substrates, ECs attached and spread, but fibroblasts, vascular SMCs, and platelets did not. They also found that the endothelial monolayers on REDV-grafted substrates were nonthrombogenic. More recently, Tiwari et al³⁴ found that RGD and heparin that had covalently bonded onto MyoLink (Crecent Vascular Technologies, Ltd, Wrexham, Wales, UK) graft surfaces improved cell retention and provided an anti-thrombogenic surface for initial blood flow in vivo. The

approach to the selective adhesion of ECs coupled with nonadhesion of deleterious cell types may provide a solution to the failures of vascular grafts.

Although many studies in animals have indicated higher patency rates for EC-seeded grafts compared with unseeded grafts, early clinical trials of seeded grafts met with mixed results. This is most likely because direct comparison of these clinical trials is difficult because graft position, size, and endothelial seeding technique vary. Long-term clinical trials with EC-seeded vascular grafts have met with some success. One long-term trial has been reported serially in 1994,³⁵ 1997,³⁶ and 1999.³⁷ Over the entire 9-year follow-up period, autologous venous EC-seeded femoropopliteal bypass grafts, compared with unseeded grafts, demonstrated higher patency rates. The same group recently reported a primary patency of 83.7% after 4 years for 7-mm expanded polytetrafluoroethylene grafts lined with autologous ECs.³⁸ However, another trial reported conflicting results in patients undergoing distal femoropopliteal bypass with autologously endothelialized grafts.³⁹ In these patients, objective serum markers and platelet survival studies revealed incomplete endothelialization of the grafts, raising the question as to whether EC seeding would prove to be clinically successful. Another method of improving graft patency clinically has recently been reported by Karube et al.⁴⁰ They designed a tissue-engineered (TE) prosthesis made with autologous tissue embedded in the pores of the graft. Subcutaneous adipose tissue (a source of ECs) was obtained from patients and resuspended in saline solution. The Dacron graft was turned inside out, and the adipose tissue suspension was injected into the interstices of the graft. After 1, 2, 3, and 5 years, the primary patency rates were 85.3%, 83.3%, 73.8%, and 67.7%, respectively. Despite these results, it still remains to be seen whether EC seeding will prove clinically successful. A characteristic of vascular disease is endothelial dysfunction, and as long as autologous ECs are isolated from patients having varying degrees of vascular disease, these trials will most likely continue to produce conflicting results. However, further research into the EC seeding of vascular grafts remains a top priority in the treatment of advanced arterial disease.

TE Vascular Conduits

The development of a completely TE artery began with the coculture of ECs and SMCs in extracellular matrix components.^{41,42} For example, Weinberg and Bell⁴² developed a vascular conduit composed of SMCs cultured in a collagen tube. A layer of fibroblasts was then added around the outside of the tube, and ECs were seeded onto the luminal surface. A Dacron sleeve was added between the medial and adventitial layers to provide strength to withstand physiological pressures. Electron microscopy showed that the ECs lining the lumen and the SMCs in the wall were healthy, well differentiated, and biochemically active, producing von Willebrand factor and prostacyclin. Other natural materials have been considered for use as vascular constructs. Xenograft and allograft tissue (eg, decellularized porcine aortas) have been investigated for use as bioprosthetic vascular devices.^{43,44} Cryopreserved allografts have been used clinically as coro-

nary artery bypass conduits, but they have limited use because of their poor patency rates and problems with aneurysms.⁴⁵ The use of xenograft and allograft tissue has typically required chemical or physical pretreatment aimed at preserving the tissue by increasing its resistance to enzymatic or chemical degradation, reducing the immunogenicity of the material, and sterilizing the tissue.⁴⁵ Cross-linking techniques have been explored in an attempt to identify the ideal stabilization procedure. Decellularization approaches have also been used to reduce host immune responses. The most commonly used cross-linking reagent is glutaraldehyde. Cross-linking with glutaraldehyde can also sterilize and suppress immunologic recognition of the tissue.^{46–48} However, glutaraldehyde treatment does not completely eliminate the immune response to allografts and xenografts and has other undesirable properties, including cytotoxicity and calcification.⁴⁵ Therefore, there has been considerable effort in identifying alternative tissue treatments that preserve natural tissue but do not result in deleterious side effects. Several of these alternative approaches include the use of cyanamide,⁴⁹ adipyl dichloride,⁵⁰ hexamethyl diisocyanate,⁵¹ alginate azide,⁵² and dehydration approaches.^{49,53} Two of the most promising methods are chemical cross-linking with polyepoxy compounds and cross-linking catalyzed by dye-mediated photo-oxidation.⁴⁵

Investigators are increasingly using modified natural materials for implantation. For example, small intestinal submucosa has been used extensively as a conduit material.⁴⁵ Various studies in animals have shown its suitability in several applications, including vascular grafts.^{54–56} The primary advantage of small intestine submucosa is its ability to promote site-specific tissue remodeling and regeneration by the host.⁴⁵ High patency rates have been reported in the canine aorta, in carotid and femoral arteries, and in the superior vena cava.⁵⁷ However, in an experiment in which microvessel grafts were implanted in rats, none of the grafts remained patent beyond the first hour.⁵⁸ Huynh et al⁵⁹ demonstrated that a small-diameter (4-mm) graft constructed from a collagen biomaterial derived from small intestine submucosa and type I bovine collagen has the potential to develop into a functional blood vessel. In an animal model of rabbit arterial bypass, the collagen grafts displayed excellent hemostasis and patency 3 months after implantation. The grafts remodeled into cellularized vessels that responded physiologically to vasoactive agents. Further experiments aimed at elucidating the vascular response to small intestine submucosa should be performed before use of this conduit as a cardiovascular substitute can be embraced.

Although natural biomaterials by themselves have use as vascular replacements, they may also provide additional benefits as biomaterials for tissue engineering applications. In addition to inherent cell compatibility, natural materials possess the desired shape and the strength of the tissues from which they were derived.⁴⁵ Moreover, cell seeding of these materials would likely enhance their long-term function. Recent studies created living vascular tissue by seeding ECs and fibroblasts onto naturally derived biomaterials.^{60–64} Decellularized porcine aortas were seeded with ECs and myofibroblasts isolated from human saphenous vein and cultured

in a reactor under pulsatile flow.⁴³ The ECs proliferated to form a confluent monolayer on the lumen. Dixit et al⁶⁵ have performed studies to endothelialize photo-oxidized bovine pericardium and have found that both human and canine ECs attached and migrated well on this biomaterial. Natural biomaterials, when processed using techniques that do not induce cytotoxicity, may serve as an ideal material for tissue engineering applications in the vascular system.

As the field of tissue engineering has evolved over the last decade, many of the approaches have involved the use of synthetic polymeric materials as scaffolds to guide cell growth. Therefore, synthetic materials have been investigated for their role as arterial replacements. A pulmonary artery conduit was created by seeding tubular polyglactin/polyglycolic acid scaffolds with SMCs followed by ECs.⁶⁶ After an *in vitro* culture period, the conduits were implanted into the pulmonary arteries of lambs and evaluated between 11 and 24 weeks. In contrast to the acellular control, the TE scaffolds appeared histologically to be nearly identical to native arteries. Improvements in the mechanical properties of polyglycolic acid conduits have been achieved by culturing the cell-seeded scaffolds under pulsatile flow in a bioreactor.⁶⁷ SMCs were placed on the outside of the degradable polymers, and ECs were cultured on the inside of the polymers. The TE blood vessels were then grown in the presence of pulsatile flow, ie, similar to flow in native blood vessels. *In vivo* pig studies showed that these vessels remained patent for at least 1 month.

In contrast to the aforementioned techniques of TE blood vessels, L'Heureux et al⁶⁸ reported a novel approach that was based exclusively on the use of cultured human cells without any synthetic or exogenous biomaterials. Human mesenchymal cells were cultured in the presence of vitamin C to create an extracellular matrix with characteristics similar to those observed *in vivo*. This cohesive cellular sheet was placed around a tubular support to produce the media of the vessel. A similar sheet of fibroblasts was wrapped around the media to produce the adventitia. The construct was placed in a bioreactor for a maturation period of ≈ 8 weeks, at which time the tubular support was removed, and the ECs were seeded in the lumen. This structure was implanted as a canine femoral arterial interposition graft and remained patent in 3 of 6 animals at 1 week. Campbell et al⁶⁹ also reported an innovative method of TE vascular conduits. Silastic tubing was inserted into the peritoneal cavity of rats or rabbits. After a 2-week period, the tubing had become covered by several layers of myofibroblasts, collagen matrix, and a single layer of mesothelium. The implants were harvested from the peritoneal cavity, the silastic tubing was removed, and the tube of living cells was everted to create a synthetic artery whose architecture now resembled that of a normal artery (Figure 1). The artificial artery was then grafted into the carotid artery or abdominal aorta of the same animal in which they were grown. The grafts remained patent for 4 months and developed structures resembling elastic lamellae.

Each of the studies discussed above has contributed significantly to the development of TE blood vessels that can serve as living grafts with growth and healing potential. However, before TE grafts can be embraced as a viable

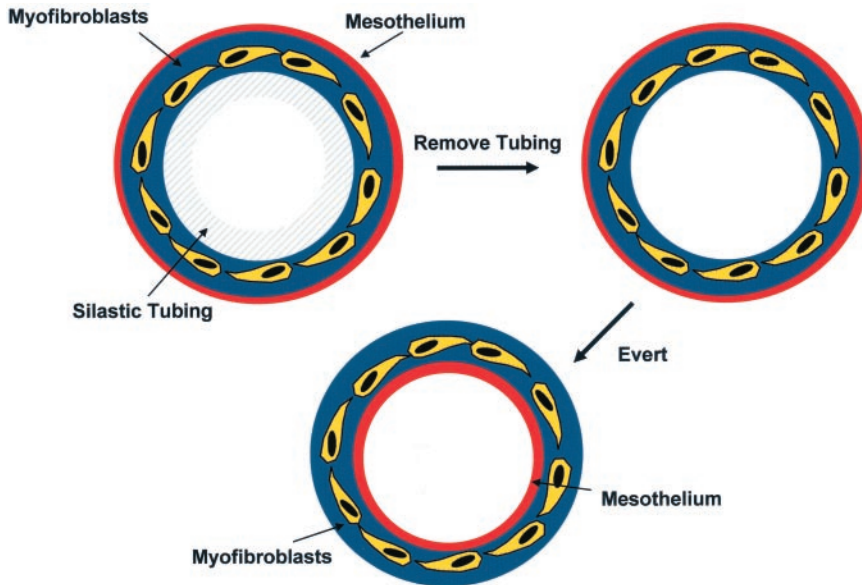


Figure 1. Creation of a TE vascular graft. The myofibroblast capsule, covered with a layer of mesothelium, is formed around silastic tubing in the peritoneal cavity. The silastic tubing is removed, and the capsule is everted such that the mesothelium lines the lumen. The tube, which resembled a blood vessel, was grafted into the severed carotid artery or abdominal aorta of the same animal in which they were grown. Diagram was modified from Campbell et al.⁶⁹

clinical option to replace native vessels, they will need to provide rates of thrombosis and tissue hyperplasia that are lower or comparable to those that are currently achieved with venous grafts.

TE Perivascular Implants

Tissue engineering enables the development of biological substitutes that restore, maintain, or improve tissue function. It also provides a tool with which to examine the structure-function relationship of specific tissues. Cells or tissue can be implanted at sites distant or in a different configuration from their original state, providing the opportunity to divorce the effects of cell secretory function on tissue biology from those imposed by maintaining tissue structure. It has recently been demonstrated that the biological effect of these cells on vascular repair is maintained when the cells are implanted at a site distant to their original location. ECs were cultured on 3D polymer matrices and implanted in the perivascular space of balloon-injured rat or pig carotid arteries.^{70–74} Perivascular ECs reduced intimal thickening in both rats and pigs. Compared with control porcine arteries, porcine arteries wrapped with ECs also had reduced occlusive thrombosis and higher patency rates at 1 and 3 months.^{71,73} These effects were not limited to mechanically injured arteries. Side-to-side arteriovenous fistulae were created with femoral arteries and veins in pigs.⁷⁴ Intimal thickening, observed within the venous segment at 1 and 2 months, was significantly reduced in the EC-treated animals compared with control animals.

When cell-based therapies such as these are coupled with molecular modification technology, they offer new tools to dissect the complex biology of vascular repair. Stably transfected clones of bovine ECs were generated to express high levels of an antisense vector targeting domain III of perlecan, an important secreted EC-derived regulator of vascular homeostasis.⁷² When matrices containing the transfected ECs were implanted around balloon-injured porcine carotid arteries, an interesting divergence of biological effects was observed. Whereas the parent cells prevented occlusive throm-

bosis, the perlecan-deficient cells were completely ineffective and had patency rates similar to those of the control group. However, the ability of the transfected cells to inhibit intimal hyperplasia was abrogated only in part by perlecan suppression. These results suggest that although perlecan is necessary to inhibit thrombosis after vascular injury, it provides only a portion of the regulatory control over intimal thickening. These data may begin to explain why promising preclinical findings do not always translate into clinical benefit. The human lesion involves a complex combination of cellular events. Modification of one molecular pathway may have a profound effect in an animal model in which only that pathway is specifically and intentionally altered. When multiple pathways are introduced, as in human disease, they are not affected identically, and escape from control inevitably ensues. These observations support the development of cell-based therapies. As it becomes increasingly difficult to isolate every biological pathway or cell-secreted product involved in vascular repair, only intact cells may be able to restore vascular physiology.

Heart Valves

Heart valve replacement by mechanical or biological valve prostheses remains the most common treatment for advanced valvular heart disease.⁷⁵ The major limitations to mechanical valves include the need for lifelong anticoagulation that is associated with the possibility of hemorrhage, the risk of thromboembolic events, and predisposition to lifelong risks of infection.^{76,77} Prosthetic valves are prone to disintegration and failure and are unable to repair themselves, remodel, or grow, which are vital characteristics for pediatric populations.⁷⁸ Biological valves have superior hemodynamics, are relatively resistant to infection, and do not require anticoagulation. However, biological valves have limited durability, which is most likely due to their immunogenic potential or to detergent fixation, which may lead to an inability to be repopulated by autologous cells.^{79,80} The creation of a TE living heart valve may provide a solution to these problems.



Figure 2. TE heart valve after 14 days of in vitro conditioning in a bioreactor. All leaflets were intact, mobile, and pliable, and valve constructs were competent during valve closure. Control valves, grown in static culture, were fragile and began to lose structural integrity after 14 days in culture. Reprinted from Hoerstrup SP, Sodian R, Daebritz S, Wang J, Bacha EA, Martin DP, Moran AM, Guleserian KJ, Sperling JS, Kaushal S, Vacanti JP, Schoen FJ, Mayer JE. Functional living trileaflet heart valves grown in vitro. *Circulation*. 2000;102(suppl III):III-44–III-49, by permission of the American Heart Association ©2000.

Although TE valve replacements have problems of their own (eg, the valves must perform in a dynamic mechanical environment and endure considerable bending and shear stresses),⁸¹ significant progress has been made in their design. Steinhoff et al⁸⁰ recently reported a study in which allogeneic acellularized heart valves were seeded with autologous myofibroblasts, followed by ECs. The in vivo function was evaluated in a sheep model of orthotopic pulmonary valve conduit transplantation. Unseeded control valves showed partial degeneration and no interstitial valve reconstitution. In contrast, TE valves showed complete histological reconstitution of valve tissue and confluent endothelial coverage in all cases.

Another approach to creating a TE heart valve was developed by Hoerstrup et al.⁸² They used an in vitro pulse duplicator system and a novel rapidly bioabsorbable composite scaffold material. Nonwoven polyglycolic acid mesh was coated with a thin layer of poly-4-hydroxybutyrate (P4HB). P4HB is a biologically derived rapidly absorbable biopolymer that is strong, pliable, and thermoplastic so that it can be molded into almost any shape. They fabricated trileaflet scaffolds out of the polyglycolic acid/P4HB composite material by using a heat-application welding technique. Myofibroblasts were seeded onto the scaffolds, and after a 4-day culture, the scaffolds were seeded with ECs and transferred to the pulse duplicator system (Figure 2). The valve constructs were implanted into lambs, and the valves were explanted at 1 day and at 4, 6, 8, 16, and 20 weeks. Echocardiography demonstrated mobile functioning leaflets without stenosis,

TABLE 2. Cell Types Investigated for Cardiomyoplasty Applications

Cell Type	References
Fetal cardiomyocytes	91
Autologous skeletal myoblasts	88–90
Smooth muscle cells	94
Genetically labeled myoblasts	95
Syngeneic skeletal myoblasts	96
Fibroblasts	97
Adult tumor-derived cardiac cells	87
Embryonic stem cells	92
Bone marrow-derived stromal cells	98
Bone marrow-derived stem cells	93

thrombus, or aneurysm up to 20 weeks. The polymer materials were completely degraded by 8 weeks, and mechanical properties were comparable to those of native tissue at 20 weeks. As long as the risks of heart valve replacement surgery persist, efforts to produce a completely biological “living” functional heart valve will continue to be a main concern among cardiac surgeons.

Cell Transplantation to Repair Injured Myocardium

Despite recent advances in the treatment of acute myocardial infarction, the ability to repair extensive myocardial damage is limited. The adult heart is incapable of effective cardiomyocyte regeneration after injury or infarction.⁸³ Cardiomyocytes do not regenerate after birth, and the adult heart lacks a reserve of precursor or stem cells. Loss of cardiomyocytes leads to regional contractile dysfunction, with the injured myocardium becoming a noncontracting fibrous scar that alters the workload of the surrounding tissue.⁸³ If the injured area is large, the remaining myocardium will ultimately deteriorate, leading to congestive heart failure. Current treatments for acute myocardial infarction and subsequent heart failure include mechanical support using left ventricular assist devices and, ultimately, cardiac transplantation.^{84,85} These treatments have limitations such as rejection, infection, and organ donor shortages.⁸⁶ Cell transplantation has been proposed as a new approach and holds enormous potential to repair or regenerate the injured myocardium. Cellular cardiomyoplasty (cell transplantation for cardiac repair)⁸³ has been attempted with many types of cells (Table 2), including fetal cardiomyocytes, autologous skeletal myoblasts, adult cardiac-derived cells, embryonic stem cells, and bone marrow stem cells.^{87–98} Although several of these cell types have shown promise in animal studies, skeletal myoblasts have been the first to be evaluated in a clinical setting.⁸³

Damaged skeletal muscle is capable of regeneration after injury because of undifferentiated myoblasts (eg, satellite cells) present in the tissue.⁸¹ Autologous myoblasts obtained from skeletal muscle, implanted into damaged canine myocardium, formed new tissue that resembled cardiac muscle at the site of injury.⁹⁹ Improved myocardial function has also been reported in many other animal studies.^{88,96,100,101} However, a recent study performed by Reinecke et al¹⁰² has raised

doubt that adult skeletal muscle stem cells undergo transdifferentiation into cardiomyocytes after grafting into the heart. Rat skeletal muscle stem cells were grafted into normal syngeneic rat hearts. At 4 and 12 weeks, the graft cells formed multinucleated cross-striated myofibers that expressed fast skeletal myosin heavy chain, indicating a skeletal muscle phenotype. Clinical trials based on the use of skeletal myoblasts for cardiomyoplasty have been initiated in Europe and the United States. Preliminary observations showed an improvement in ejection fraction and a new-onset metabolic viability within the infarcted area.¹⁰³ Although the feasibility of myoblast transplantation and safety appear satisfactory, there is an increased risk of arrhythmias, which raises serious concerns and needs to be further investigated.¹⁰⁴ Although most of the animal and preliminary clinical studies on cardiomyoplasty using skeletal myoblasts appear positive, the long-term results of such a therapy remain unclear. If the transplantation of skeletal myoblasts is to be a solution to myocardial repair, the cells must transdifferentiate into cardiomyocytes and be able to survive for many years in the heart. The infarcted area and the surrounding region do not provide an optimal environment for cell survival. Cardiomyocytes might ultimately be the best cell type for myocardial repair. However, given the inability of adult cardiomyocytes to replicate either *in vitro* or *in vivo*, it does not seem likely that they will be readily available as a cell source. Transplanted fetal cardiomyocytes can limit scar expansion and prevent postinfarction heart failure. Soonpaa et al¹⁰⁵ were the first to report that fetal cardiomyocytes survive after injection into the heart. Fetal cardiomyocytes, isolated from transgenic mice, formed stable intracardiac grafts in syngeneic hosts up to 2 months after implantation, with no evidence of cardiac arrhythmia or chronic immune rejection. Recently, Sakakibara et al¹⁰⁶ described a study in which fetal rat cardiomyocytes were transplanted into rats with myocardial infarction. They also demonstrated that prevascularizing the ischemic region before cell transplantation with fibroblast growth factor-2–incorporated microspheres enhanced the benefits of cardiomyocyte transplantation. However, the use of fetal cardiomyocytes is unfeasible because human fetal tissue cannot be obtained in sufficient quantities.¹⁰⁷ Progress in stem cell biology may provide a source of cardiomyocytes in the future. Recent reports have demonstrated the existence of pluripotent stem cells in adult tissue.^{108,109} Tomita et al¹¹⁰ transplanted bone marrow stromal cells, which had been induced to differentiate to a myogenic phenotype with 5-azacytidine, into the myocardial infarct region in pigs. After 4 weeks, the transplanted cells formed islands of cardiac-like tissue, induced angiogenesis, prevented thinning and dilation of the infarct region, and improved regional and global contractile function.

TE Myocardial Constructs

In addition to the transplantation of isolated cells, there has also been substantial work on therapies to transplant functional TE heart grafts. Zimmermann et al¹¹¹ have developed 3D engineered heart tissue from rat neonatal cardiomyocytes in collagen matrices. The cardiac cells in the engineered heart tissue displayed morphological features resembling adult

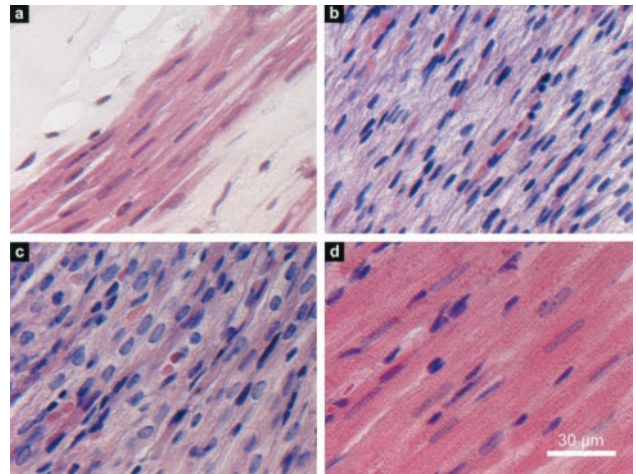


Figure 3. Photomicrographs of hematoxylin/eosin–stained paraffin sections of engineered heart tissue (a) and native myocardium from newborn rats (b), 6-day-old rats (c), and adult rats (d). Although the cardiac cells in the engineered heart tissue were obtained from neonatal rats, the morphology of the cardiac bundles in the engineered heart tissue (a) more closely resembles the morphology of the adult heart (d). Reprinted from Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res.* 2002;90:223–230, by permission of the American Heart Association ©2002.

differentiated tissue (Figure 3). Li et al⁹¹ have shown that TE cardiac graft transplantation using a biodegradable gelatin mesh replaces both myocardial scar and right ventricular outflow track defects. Alginate has also been used as a scaffold to facilitate the 3D culturing of cardiac cells. Leor et al¹¹² implanted rat fetal cardiac cells, grown within porous alginate scaffolds, into rat infarcted myocardium. The grafts stimulated intense neovascularization and attenuated left ventricular dilation. The authors also reported an almost complete disappearance of the scaffold and good integration of the grafted cells into the host. More recently, Shimizu and colleagues^{113,114} reported a cell manipulation technique to construct 3D cardiac tissue grafts using temperature responsive cell culture surfaces. These surfaces were grafted with the temperature-responsive polymer poly(*N*-isopropylacrylamide) (PIPAAm), producing slightly hydrophobic and cell-adhesive surfaces under culture conditions at 37°C, which change reversibly to hydrophilic and non–cell-adhesive surfaces at <32°C. Neonatal rat cardiomyocyte sheets detached from PIPAAm-grafted surfaces were overlaid to construct cardiac grafts. The sheets were then transplanted into the subcutaneous tissue of nude rats. Three weeks later, surface electrograms originating from transplanted grafts were detected, and spontaneous beating was macroscopically observed. Recent advances in methods of cardiomyocyte isolation and 3D culture show promise and may contribute to the creation of bioengineered tissue to be used for surgical repair of the injured myocardium.

Gene Transfer in Cardiovascular Tissue Engineering

Animal and preliminary clinical data support the continued development of cardiomyoplasty as an approach to repair an

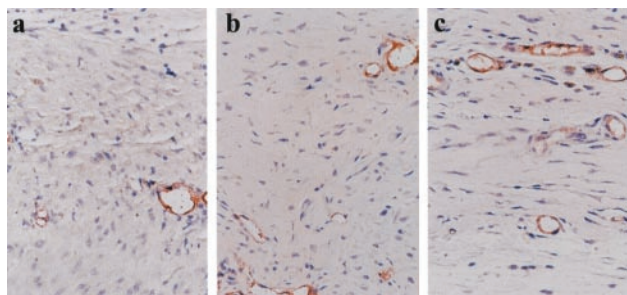


Figure 4. Vascular density in rat hearts transplanted with culture medium alone (a), untransfected heart cells (b), or VEGF-transfected heart cells (c). Sections were stained with antibodies against factor VIII. Vascular density was the greatest in hearts transplanted with transfected cells, intermediate in hearts transplanted with untransfected heart cells, and the lowest in control hearts. Reprinted from Yau TM, Fung K, Weisel RD, Fujii T, Mickle DA, Li R-K. Enhanced myocardial angiogenesis by gene transfer with transfected cells. *Circulation*. 2001;104(suppl 1):I-218–I-222, by permission of the American Heart Association ©2001.

injured heart. However, there remain a few difficulties with this type of therapy. Irrespective of the issues surrounding the choice of an optimal cell source, the infarcted region remains a problematic environment for cell survival. Myocardium, in and around the infarct, is inadequately perfused and exposed to dysfunctional forces and altered biochemical environments.¹⁰⁷ Survival, attachment, and incorporation of transplanted cells in this area is often limited. To this end, gene transfer may play an expanding role in cardiovascular tissue engineering.

Recently, Miyagawa et al⁸⁶ reported a novel method of myocardial regeneration therapy for heart failure. They hypothesized that transfection of the gene for human hepatocyte growth factor, an angiogenic factor that also enhances cell-cell and cell-matrix interactions, combined with cardiomyoplasty would regenerate an impaired myocardium. They found improved cardiac performance in rats that received neonatal rat cardiomyocytes combined with HVJ-liposomes bearing the human hepatocyte growth factor gene. In a study by Yau et al,¹¹⁵ donor rat heart cells were transfected with plasmids encoding vascular endothelial growth factor (VEGF) and were transplanted into rats that had previously undergone left ventricular cryoinjury. After 5 weeks, they found that heart cells transfected with VEGF induced greater angiogenesis than did unmodified cells (Figure 4). Capillary density in the center of the scar and in the border zone around the scar and regional blood flow within the scar were all significantly higher in transfected rats compared with control rats. Although the authors found no difference in left ventricular function attributable to the transfected cells at 5 weeks, these data suggest an important role for gene therapy as an adjunct to cardiovascular tissue engineering.

The transplantation of endothelial progenitor cells (EPCs) represents a novel therapeutic approach to enhance neovascularization. EPCs are present in the systemic circulation. They have been harvested from the peripheral circulation, expanded *ex vivo*, and administered to animals with limb or myocardial ischemia.^{116,117} High rates of limb salvage and improvement in myocardial function have been observed in

these animal models. Kaushal et al¹¹⁸ have also used sheep EPCs to seed decellularized porcine iliac vessels. When EPC-seeded grafts were implanted as carotid interposition grafts in sheep, they remained patent for 130 days. Non-seeded grafts occluded within 15 days. When explanted, the EPC-seeded grafts exhibited contractile activity and NO-mediated vascular relaxation that was similar to that of native carotid arteries. Iwaguro et al¹¹⁹ recently investigated *ex vivo* phenotypic modulation as a method to enhance EPC function and to reduce the number of EPCs required for transplantation. EPCs transduced with adenovirus encoding VEGF were transplanted into athymic nude mice with hind limb ischemia. Neovascularization and blood flow were both improved in the treated animals. Limb necrosis and autoamputation were reduced by 63.7% in treated compared with control animals. Moreover, the number of EPCs required to observe these effects was 30 times less than that required in previous animal studies to improve ischemic limb salvage. A limitation of EPC transplantation is the volume of blood that would be required to produce an optimal number of EPCs for autologous transplantation. The use of VEGF-transduced EPCs may provide a solution to this problem. The combination of gene therapy with the techniques of cell transplantation represents a promising method for the treatment of myocardial or limb ischemia. However, further experiments will be required to determine the time course of gene transfer, limiting amounts of the gene and transfected cells as well as long-term effects.

Cell Source and In Vitro Conditions for Optimal TE Cardiovascular Constructs

An issue with all TE constructs designed as cardiovascular replacements is cell source. Human vascular ECs are important for the development of engineered vessels. Autologous cells are better tolerated than allogeneic or xenogeneic cells; however, the hallmark of vascular disease is endothelial dysfunction. Patients with obstructive vascular disease may have dysfunctional ECs, precluding the use of their own cells for tissue engineering applications. The use of autologous ECs could pose another set of problems. A vascular graft seeded with autologous cells would need to be created in time for surgery, and often, there is little time between the diagnosis of vascular disease and surgery. To provide a TE conduit lined with autologous ECs, there must be adequate time for cell harvest, isolation, identification, selective growth, cell seeding, incubation, characterization, and implantation. Potential sources of human ECs for these applications are stem cells. Levenberg et al¹²⁰ recently developed a method for the isolation of ECs derived from human embryonic stem cells. They described a series of differentiation steps of the stem cells into ECs that were then isolated by using fluorescently labeled platelet EC adhesion molecule-1 antibodies. Fluorescently labeled cells were isolated by using a flow cytometry cell sorter. In addition, they showed that when cultured on Matrigel, these cells formed tube-like structures, and that when they were transplanted into SCID mice, the cells appeared to form microvessels containing mouse blood cells. Fluorescence-activated cell sorting (FACS) has also been used to separate mixed cells obtained from a human aorta.¹²¹ Pure isolations of myofibro-

blasts and ECs were obtained by FACS and seeded sequentially onto polymer scaffolds. These results suggest that FACS may be a reliable and safe method to produce pure isolations of human cells for tissue engineering applications.

Studies in cardiovascular tissue engineering have shown the importance of the in vitro culture conditions used to produce the constructs. Formulation methods generally involve 3 techniques: (1) delivering cells to the scaffold, (2) optimizing cell attachment, and (3) in vitro culture to initiate tissue formation. Cell seeding may be performed under static or dynamic conditions. Although static seeding methods have been successful when seeding polymer films and thin scaffolds, higher seeding efficiencies and more uniform cell distribution are observed for thicker scaffolds seeded under dynamic conditions. Precoating the scaffolds with attachment factors such as fibronectin, laminin, or collagen generally increases cell attachment and subsequent proliferation.¹²² In a recent study, a 20-fold increase in myoblast number was obtained on polyglycolic acid meshes coated with laminin compared with noncoated control meshes.¹²³

Recent publications have also highlighted the beneficial effects of shear stress for the optimization of cell attachment and mechanical properties in creating both heart valves and arterial replacements.^{124,125} Improvements in the mechanical properties of TE blood vessels were achieved by generating the vessels with applied shear stress. The vessels had a higher myosin heavy chain and collagen content as well as higher burst strength than vessels generated under static conditions.⁶⁷ Although it has been shown that TE conduits generated in the presence of flow outperform those generated in a nonflow environment, less is known about the optimal conditions of the applied shear stress.^{126,127} It has been hypothesized that fetal-like conditions may be the optimal conditions for new cardiovascular tissue formation in bioreactors. Stock and Vacanti¹²⁸ recently provided a review of cardiovascular physiology during fetal development and suggested parameters that may have potential in the design of bioreactors for cardiovascular tissue engineering. Data obtained from fetal lambs and humans have indicated that cardiovascular development does not require large quantities of shear stress. In fact, it has been shown in vitro that high shear stress inhibits proliferation of ECs and SMCs and increases cell-polymer detachment.^{129,130} However, static conditions are not beneficial for tissue maturation. Therefore, careful consideration needs to be given to the design of bioreactors used for generating cardiovascular tissue.

Microfabrication technology provides additional avenues for improving the strength and viability of TE cardiovascular constructs. Microelectromechanical systems (MEMS) fabrication technology allows for the creation of complex structure for cell seeding, eg. polymer wafers containing branched, 2D, vascular-like spaces.¹³¹ The MEMS process entails the use of a micro-etched wafer as a master mold for pattern transfer to a biocompatible polymer material. Such patterns can be designed to mimic the fluid dynamic properties of specific vascular structures. Recent studies have demonstrated the feasibility of creating ordered branched arrays of channels lined with living ECs.^{131–133} Anderson et al¹³⁴ and Chiu et al¹³⁵ have described a procedure for making topolog-

ically complex 3D microfluidic channel systems in poly(dimethylsiloxane). Specific vascular systems might then be created to support large, viable, morphologically complex tissue constructs both in vitro and in vivo. It may soon be possible to fabricate 3D polymer scaffolds that degrade at controlled rates to form new tissues with intact vascular systems devoid of foreign materials.

Conclusion

The development of TE conduits for use in the cardiovascular system has, to date, been quite promising, especially in the area of EC-seeded vascular grafts. A wide range of techniques now exists for the formulation of arterial and heart valve replacements as well as cellular cardiomyoplasty for the injured myocardium. The transition from experimental models to routine clinical use of these technologies may be enhanced by further elucidation of the biological mechanisms that determine growth and response after acute or prolonged vascular injury. Future advances will likely be made in polymer scaffold design, in optimal culture conditions, and in obtaining a cell source specific for the cardiovascular application. Further experiments are needed to determine the optimal conditions for new cardiovascular tissue formation in vitro. Cell source is an ongoing problem, and stem cell technologies coupled with gene therapy may provide a solution. The innovative application of tissue engineering as cardiovascular therapy will necessitate successful collaboration among the fields of polymer chemistry, engineering, cell biology, vascular biology, cardiovascular surgery, and clinical cardiology.

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