THE ANATOMICAL RECORD 263:367–371 (2001)

Tissue Engineering in the Cardiovascular System: Progress Toward a Tissue Engineered Heart

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ABSTRACT

Achieving the lofty goal of developing a tissue engineered heart will likely rely on progress in engineering the various components: blood vessels, heart valves, and cardiac muscle. Advances in tissue engineered vascular grafts have shown the most progress to date. Research in tissue-engineered vascular grafts has focused on improving scaffold design, including mechanical properties and bioactivity; genetically engineering cells to improve graft performance; and optimizing tissue formation through in vitro mechanical conditioning. Some of these same approaches have been used in developing tissue engineering heart valves and cardiac muscle as well. Continued advances in scaffold technology and a greater understanding of vascular cell biology along with collaboration among engineers, scientists, and physicians will lead to further progress in the field of cardiovascular tissue engineering and ultimately the development of a tissue-engineered heart. Anat Rec 263:367–371, 2001.

Key words: tissue engineering; cardiac muscle; heart

Cardiovascular disease remains a significant cause of morbidity and mortality in the United States. Successful treatment has been limited in many situations by the poor performance of synthetic materials used for tissue replacement. Recently, the Life Initiative, an international consortium of tissue engineers headed by Dr. Michael Sefton, has proposed the goal of developing a completely tissue-engineered heart. Clearly, realization of this ambitious goal will require substantial improvements in cellular and scaffold technologies. Furthermore, progress toward this goal will be made through the parallel development of effective tissue engineered components of the cardiovascular system that will later be synthesized into a larger organ structure, namely a heart. Achievements made during the development of these individual tissue components will provide many therapeutic advances and enhance our understanding of fundamental issues involved in cardiovascular tissue engineering. This article presents the current progress toward development of some of the different tissue components, namely arterial replacements, heart valves, and cardiac muscle. Tissue engineering techniques may also be used within the cardiovascular system to improve the function of the native tissue, for instance for treatment of restenosis or congestive heart failure.

ENGINEERING OF TISSUE-BASED ARTERIAL REPLACEMENTS

Approximately 500,000 coronary artery bypass surgeries are performed each year in the United States. Natural tissue, primarily saphenous vein or internal mammary artery, is generally used for coronary artery replacement. The results have been quite favorable for these procedures, with patency rates generally ranging from 50 to 70%. Failures may be caused by intimal thickening, due in large part to adaptation of the vessel in response to increased pressure and wall shear stress, compression, inadequate graft diameter, and disjunction at the anastomoses.

The development of plastics and other polymers led to the first synthetic materials used as blood vessel replacements in the 1950s. These synthetic materials included polyesters, polyethylene terephthalate (PET, Dacron), and expanded polytetrafluoroethylene (ePTFE, Gore-Tex). However, patency rates of synthetic grafts are signifi-

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Received 10 November 2000; Accepted 31 January 2001

cantly lower than those observed with natural vessels, particularly in small-diameter applications. Some of the problems plaguing synthetic vascular grafts include platelet adhesion and activation and a decreased compliance compared with the adjacent arterial tissue. These problems have led to investigations of tissue engineered alternatives.

Before considering design of tissue engineered arterial replacements, it is important to understand aspects of the anatomy and physiology of the normal arterial wall. The arterial wall is composed of three distinct tissue regions: the intima, with a nonthrombogenic endothelial cell lining; the media, with smooth muscle cells and elastin fibers aligned circumferentially for optimal mechanical properties; and the adventitia, composed primarily of fibroblasts and connective tissue. An ideal replacement would mimic the properties of each of the tissue layers. In addition, the artery must withstand dynamic forces, including the transmural pressure acting normal to the vessel wall and the shear stress acting tangentially to the wall. An arterial replacement should have mechanical properties closely matching those of the normal vessel to withstand the pressures associated with blood flow and to avoid potential compliance mismatch at the anastomoses. Furthermore, it is important to remember that the normal endothelial cell lining is antithrombogenic, actively preventing platelet adhesion and thrombus formation. The lining of any arterial replacement should be similarly antithrombogenic.

The first attempts toward developing tissue engineered vascular grafts involved seeding cells, particularly endothelial cells, onto surfaces of synthetic grafts to provide a more natural and antithrombogenic blood-contacting surface. This approach has been limited by poor retention of endothelial cells on biomaterial surfaces upon exposure to blood flow. Thus, significant efforts have been made to improve the adhesion strength of endothelial cells on biomaterials. Electrostatic cell seeding methods have been shown to significantly improve retention of endothelial cells on ePTFE after exposure to flow for 2 hr (Bowlin et al., 1998). Alterations of the graft surface have also been investigated for improved cell retention. Modifying the surface, either covalently or by means of adsorption, with cell adhesion ligands has shown promise. For example, Schneider et al. coated ePTFE grafts with fibronectin or fibronectin and mixed components of extracellular matrix (ECM) before endothelial cell seeding (Schneider et al., 1997). The grafts were then subjected to shear stress for a period of 1 hr. Grafts coated with the ECM mixture retained significantly more cells after the exposure to shear stress. In addition, in grafts implanted in vivo, the ECM coating was found to promote endothelial cell coverage. This may be due to the adhesive proteins or growth factors in the ECM mixture. Substances used for graft coating should ideally have a high specificity for endothelial cells to avoid problems with increased platelet adhesion or neointimal hyperplasia. The peptide REDV, derived from fibronectin, has been shown to be selective for endothelial cell adhesion (Hubbell et al., 1991). An alternative approach is to seed the graft surface with a cell type that adheres well to biomaterials, such as a smooth muscle cell or a fibroblast, but that has been genetically engineered to have antithrombogenic properties. Transfection of smooth muscle cells with genes for nitric oxide synthase and GTP cyclohydrolase has been shown to result in nitric oxide production rates similar to endothelial cells, resulting in drastic reductions in platelet adhesion (Scott-Burden and Frazier, 1995). Another method for developing biohybrid grafts has been to incorporate materials into synthetic grafts to promote tissue ingrowth. For example, synthetic grafts have been impregnated with basic fibroblast growth factor; this type of treatment has been shown to significantly increase the amount of endothelialization occurring in situ (Greisler et al., 1992; Doi et al., 1996).

Efforts to develop tissue-based replacements for arteries began in the 1970s with the coculture of endothelial cells and smooth muscle cells in extracellular matrix components. Weinberg and Bell (1985, 1986) developed a construct consisting of smooth muscle cells grown in collagen (the medial layer), shaped into a tube, and contracted for a week with a layer of fibroblasts in collagen gel around the outside (the adventitial layer) and endothelial cells seeded onto the luminal surface (the intimal layer). These engineered tissues were not strong enough to withstand physiological pressures, so a Dacron sleeve was added between the medial and adventitial layers. Smooth muscle cells in collagen gels have been shown to orient circumferentially, if allowed to contract around a mandrel (Ziegler et al., 1995a,b). Ishibashi and Matsuda (1994) also used knitted Dacron for support of their construct, with an intimal layer of endothelial cells, a medial layer of smooth muscle cells, and an adventitial layer of fibroblasts in a mixed gel of collagen I and dermatan sulfate. After 12 weeks of implantation in dogs, the collagen fibers in the intimal layer were longitudinally oriented, whereas those in deeper layers were circumferentially oriented. Elastin deposition was also observed. Researchers have also looked at other approaches using natural materials. For example, small-intestine submucosa has been used as a vascular graft in dogs (Badylak et al., 1989; Lantz et al., 1990; Sandusky et al., 1992) and has been combined with type I bovine collagen to create a scaffold material (Huynh et al., 1999). Both have been shown to remodel in vivo to histologically resemble native vessel. Another approach has used seeded xenografts (Teebken et al., 2000). Decellularized porcine aortas were seeded with endothelial cells and myofibroblasts isolated from human saphenous vein and cultured in a reactor under pulsatile flow where the endothelial cells grew to form a monolayer on the lumen. The addition of a releasable growth factor from a collagen graft has also been investigated as a way to improve growth of endothelial cells seeded on the graft (Wising et al., 2000). Basic fibroblast growth factor was bound to heparin that was immobilized on a cross-linked collagen graft and found to increase proliferation of endothelial cells.

Synthetic bioresorbable materials are also being investigated for use in tissue engineering of arterial replacements. Polyglycolic acid scaffolds have been used to form arterial replacements, but these were found to be prone to aneurysmal dilation and rupture (Galetti et al., 1988). Polydioxanone scaffolds have also been used (Greisler et al., 1987). These were shown to have a lower incidence of aneurysm, presumably due to the slower degradation rate of the scaffold material. A scaffold made from a copolymer of polyglycolic acid and polyhydroxyalkanoate has been investigated recently as an arterial replacement (Shum-Tim et al., 1999). Scaffolds seeded with autologous cells and grafted into the abdominal aorta remained patent and had no aneurysms out to 5 months. At that point, biolog-

ical characteristics, such as collagen and DNA content and the presence of elastic fibers, approached those of native vessel. Improvements in mechanical properties when using polyglycolic acid scaffolds have been made by culturing the scaffolds with smooth muscle cells under pulsatile flow in a bioreactor (Niklason et al., 1999), although further in vivo analysis is needed.

To avoid the risk of aneurysm, it will be important to have rapid and extensive tissue growth and extracellular matrix production before scaffold resorption. Incorporation of growth factors into scaffolds may also increase the rate of tissue formation. Transforming growth factor-beta tethered to a polyethylene glycol (PEG) scaffold has been shown to increase matrix production of smooth muscle cells (Mann et al., in press). Ideally, scaffold resorption should be linked to tissue formation, for instance through targeted proteolytic degradation of materials (West and Hubbell, 1999). In addition, it may be desirable to use thromboresistant scaffold materials in these applications. Bots et al. (1986) used a copolymer of polyethylene oxide (PEO) and polypropylene oxide (PPO) as a biodegradable vascular graft. PEO has been extensively evaluated for its thromboresistance (Lee et al., 1989; Hill-West et al., 1994). The PEO/PPO copolymer was shown to have high resistance to platelet adhesion and mechanical properties similar to a natural vessel. Photopolymerized PEG hydrogels with grafted cell adhesion peptides have also been used as scaffold materials (Mann et al., in press). Although the base material, PEG, is intrinsically resistant to cell adhesion, the scaffolds can support adhesion and growth of seeded cells through interactions between the adhesion peptides and receptors on the cell surface.

There has been report of successful development of an arterial substitute based on in vitro culture of vascular cells without the use of a scaffold (L'Heureux et al., 1998). A sheet of smooth muscle cells in their own extracellular matrix was wrapped around a tube, covered with a sheet of fibroblasts in their own extracellular matrix, and then the luminal surface was seeded with endothelial cells. This construct reportedly had a burst strength of over 2,000 mmHg. In addition, the smooth muscle cells re-expressed desmin, and the endothelial cells strongly inhibited platelet adhesion in vitro. However, when these grafts were implanted in dogs, they had a patency rate of approximately 50%. In addition, the grafts required 3 months for production.

Another interesting approach to engineering a blood vessel was investigated by Campbell et al. (1999). Silastic tubing was implanted in the peritoneal cavity of a rat or rabbit. After 2 weeks, the tube was covered with layers of myofibroblasts, collagen matrix, and a single layer of mesothelium. The Silastic tubing was then removed, and the tube of tissue was everted, with the tissue now resembling a blood vessel in structure: the layer of mesothelial cells became the intima; layers of myofibroblasts, collagen, and elastin became the media; and an outer collagenous layer became the adventitia. This tissue tube was then grafted into the carotid artery or abdominal aorta of the same animal where the tubes remained patent for at least 4 months.

TISSUE ENGINEERED HEART VALVES

Valve replacement with prosthetic devices or bioprosthetic valves (glutaraldehyde-fixed xenograft valves or cryopreserved homograft valves) is commonly used as treatment for end-stage valvular disease. In adults, these materials have been largely efficacious, although they do carry some risk of infection and thromboembolic complications. However, a significant portion of the affected patient population are children with congenital defects. Therefore, a severe limitation of all of the treatment modalities currently available are the inability to grow and remodel with the surrounding tissue. Theoretically, because a tissue engineered valve leaflet would be an autologous living structure, it should be able to sense and respond to the normal biological signals for growth and development.

Design of tissue engineered heart valves has been encumbered by the complex, dynamic mechanical environment in which the final tissue must perform. The valves must open and close at a frequency of approximately 1 Hz, resulting in considerable bending stresses, and be exposed to high shear stresses associated with blood flow. Nonetheless, significant progress has been made toward the development of tissue-engineered valve replacements, with approaches focusing primarily on seeding cells onto polyglycolic acid matrices or onto decellularized xenograft tissues.

Recently, a trileaflet valve conduit was made of a combination of polyglycolic acid and polyhydroxyalkanoate (Sodian et al., 1999), resulting in a more flexible scaffold than polyglycolic acid alone. Cells were seeded onto the scaffold and cultured under pulsatile flow in a bioreactor, where the cells proliferated, deposited matrix, and oriented themselves in the direction of flow. Subjecting valve constructs to hemodynamic stresses in a bioreactor might aid in new tissue formation. Hoerstrup et al. (1999) have shown that hydroxyproline content of valve constructs is increased when they are subjected to tension and, therefore, may lead to better mechanical stability of new tissue.

Results of implantation of a tissue engineered valve leaflet in a lamb model have been reported (Shinoka et al., 1995, 1996; Shinoka and Mayer, 1997). In these studies, ovine endothelial cells and myofibroblasts, the cell types normally found in the valve structure, were seeded onto highly porous polyglycolic acid materials under static conditions. The surface of these constructs consisted of an endothelial cell monolayer to ensure blood compatibility. After in vitro culture, the tissue engineered leaflets were implanted in the right posterior pulmonic valve in lambs. Results showed no valvular stenosis and only slight regurgitation with autologously derived valve constructs. Furthermore, these tissues continued to grow or remodel after implantation, as evidenced by changes in the extracellular matrix composition and mechanical properties. Implantation of an unseeded polymer scaffold did not result in de novo tissue formation.

Seeded xenografts are also being investigated as potential valve replacement materials (Alexander et al., 1995; Naughton et al., 1995). Human fibroblasts were seeded onto decellularized porcine valves under physiologic flow conditions. The valve was then cultured in a pulsatile bioreactor to simulate the variable pressure changes across the valve observed physiologically. Dynamic conditioning seems to stimulate extracellular matrix protein synthesis and may be important for developing optimal mechanical properties in the engineered tissue. Although no in vivo data have been reported to date, cell viability was retained in the construct for 4 weeks.

CARDIAC MUSCLE

Tissue-engineered cardiac muscle replacement will certainly be central to the development of a functional heart, but will also have potential use in treatment of congestive heart failure and myocardial infarction. Cardiac muscle becomes terminally differentiated shortly after birth, causing these cells to lose their ability to divide. As a result, cardiac muscle will not regenerate after injury, such as that caused by myocardial infarction. In addition, all muscle cells lose their ability to contract effectively when cultured in vitro. These issues will significantly hamper the efforts to develop tissue-engineered cardiac muscle. Alternative methods to ex vivo growth of harvested cells from the appropriate tissue on a scaffold will have to be examined. Advances in stem cell technology and genetic engineering are likely to provide suitable replacement cells.

Several cell types have been investigated for cardiac muscle replacement. Damaged skeletal muscle is capable of regeneration after injury due to the presence of undifferentiated myoblasts, termed satellite cells, within the tissue. Autologous satellite cells, obtained from skeletal muscle and cultured in vitro, were implanted into damaged myocardium in a canine model (Chiu et al., 1995). These cells survived and formed new tissue that resembled cardiac muscle at the site of injury. Similar studies have been performed by using embryonic cardiac myocytes (Soonpaa et al., 1994), myoblast cell lines (Koh et al., 1993), and cardiomyocytes, smooth muscle cells, and fibroblasts seeded onto scaffolds (Li et al., 1999, 2000; Carrier et al., 1999). Carrier et al. (1999) have also begun to look at the influence of seeding conditions and different bioreactors when culturing seeded scaffolds. Well-mixed conditions for seeding and a high initial seeding density aided in maintaining structural integrity of the construct, whereas seeding under laminar flow conditions rather than turbulent flow resulted in higher seeding efficiencies and less cell damage. Additionally, culturing the constructs under laminar flow conditions led to maintenance of metabolic parameters, higher construct cellularity, more aerobic metabolism, and a more elongated cell shape.

An entirely different approach has been the use of skeletal muscle as a replacement. Ventricles have been formed from the latissimus dorsi muscle of dogs, and it was possible to harvest sufficient work from these structures to fully replace ventricular function (Pochettino et al., 1990). Appropriate innervation of a cardiac muscle replacement tissue is another issue that will have to be addressed.

CONCLUSIONS

The progress toward development of individual cardiac components to date has been quite encouraging, particularly in the development of tissue-engineered vascular grafts, although significant advances must be made before these will achieve routine clinical use. Many of the advances that will be required will be in scaffold technology: improved biocompatibility and mechanical properties, better control over scaffold ultrastructures, development of novel bioactive biomaterials that control cell adhesion and behavior, and materials that can sense and respond to their biological environment. Other advances will be in the milieu of vascular cell biology. Better understanding of the interactions between cells and their extracellular matrix as well as mechanisms of vascular wound healing will greatly facilitate tissue engineering efforts. Cells derived from cardiovascular tissues clearly respond to dynamic mechanical conditioning; the mechanisms of these responses need to be elucidated. Stem cell technologies and gene therapy will also likely contribute significantly to the field of cardiovascular tissue engineering. Interdisciplinary cooperation between engineers, scientists, and physicians will allow innovations and discoveries to be rapidly applied to development of clinically useful, tissue-engineered, cardiovascular implants and ultimately a tissue-engineered heart.

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