Myocardial Tissue Engineering: Creating a Muscle Patch for a Wounded Heart

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ABSTRACT: Cardiac tissue engineering promises to revolutionize the treatment of patients with end-stage heart failure and provide new solutions to the serious problem of heart donor shortage. By its broad definition, tissue engineering involves the construction of tissue equivalents from donor cells seeded within three-dimensional polymeric scaffolds, then culturing and implanting of the cell-seeded scaffolds to induce and direct the growth of new, healthy tissue. Here, we present an up-to-date summary of research studies in cardiac tissue engineering, with an emphasis on the critical design principles.

KEYWORDS: biomaterial; cell; heart; three-dimensional scaffold

INTRODUCTION

Efforts to reduce the extent of myocardial damage from acute myocardial infarction (MI) have been successful in many ways, but the adverse complications of postinfarction scarring, expansion, dilatation, and consequent heart failure remain. The benefits of contemporary infarct-limiting strategies, such as early reperfusion and neurohormone inhibition, are all approaching their apparent limits. Consequently, researchers are intensively developing new strategies aimed at replacing infarcted myocardium with new tissue.

Tissue engineering is a rapidly emerging interdisciplinary field that applies the principles and knowledge of biology, medicine, material sciences, and engineering to the development of biocompatible substitutes for the restoration, maintenance, and improvement of human tissue functions.[1] Unlike blood or bone marrow tissues, which can be regenerated by intravenous injection of cells, regeneration of most anatomical tissues requires template scaffolding. The scaffold temporarily provides the biomechanical structural characteristics for the seeded cells, until they produce their own extracellular matrix, which provides the structural integrity and biomechanical profile for the replacement tissue. One of the most ambitious goals in the field of cardiovascular tissue engineering is the creation of an engineered heart muscle.[2,3]

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TISSUE ENGINEERING VERSUS CELL TRANSPLANTATION

In the last decade, several research groups have shown that direct injections of cell suspensions of fetal or neonatal cardiac myocytes into experimental myocardial infarcts improved remodeling and function of the heart.[4] Others replicated those encouraging findings by using skeletal myoblasts,[5] bone marrow–derived cells,[6] or embryonic stem cells.[7] Recent reports suggest that endogenous cardiac stem cells may be able to proliferate in the myocardium under certain circumstances and can migrate from bone marrow to the heart and possibly contribute to repair after cardiac disease.[8,9]

The concept of tissue engineering using three-dimensional scaffolds has certain advantages over the direct cell injection. (1) The three-dimensional scaffolds may replace the missing or damaged infrastructure (extracellular matrix) in the infarct area and provide a temporary support for self or implanted cells. (2) By tissue engineering, one can control the size, shape, strength, and composition of the graft *in vitro*. (3) Tissue engineering provides a solution to the problem of congenital or acquired heart defects and can be used to replace or reconstruct defective heart parts such as valves or vessels. Rather than competing with each other, these techniques can be complementary. Cellular therapy is applicable when the structure of the failing organ is relatively simple and small and when disease is localized rather than diffuse.

CRITICAL DESIGN ISSUES IN CARDIAC TISSUE ENGINEERING

Cells

The ideal candidate cell to create an engineered myocardial patch should be easy to harvest, proliferative, nonimmunogenic, and have the ability to differentiate into mature, functional cardiomyocytes. Donor cells are relatively easier to obtain but present immunologic problems. Autologous cells, on the other hand, are more difficult to obtain but have no immunologic barriers. Cell sources include differentiated and undifferentiated cells, progenitor or precursor cells, and embryonic stem cells. TABLE 1 describes potential cell sources. Each cell source has certain limitations. For example, mature cardiac myocytes have limited proliferative capacity. On the other hand, adult stem cells are rare and are technically difficult to isolate because of a lack of specific and accepted cell markers. Moreover, the process of differentiation of some cell types, such as human embryonic stem cells, is difficult to control and carry the risk of teratoma. In addition, the ethics of the use of human embryonic stem cells has been debated. One exciting concept of a potential endogenous cell source in the cardiovascular system is of particular interest: the potential for "self-repair" by induction of hyperplastic growth.[8,10] However, it is unclear yet whether this strategy can contribute to the development of effective functional myocardial grafts.

Three-Dimensional Polymeric Scaffolds

Three-dimensional polymeric scaffolds can function in several tissue engineering scenarios—for example, as a platform for the regeneration *in vivo* of remaining healthy tissues, and for guiding the formation of a tissue from dissociated implanted

TABLE 1. Proposed cell sources for myocardial tissue engineering

Cell source
Fetal cardiomyocytes
Stem cell–derived embryonic cardiomyocytes
Skeletal myoblasts
Bone marrow mononuclear cells
Bone marrow–derived hematopoietic stem cells
Mesenchymal stem cells
Smooth muscle cells
Fibroblasts
Genetically engineered fibroblasts
Umbilical cord blood–derived cells

cells, *ex vivo* and *in vivo.*[11] In the first application, the scaffold provides a desirable way to restore tissue structure and function by recruiting tissue ingrowth from the host surroundings. The scaffolds may contain molecular agents known to stimulate angiogenesis and regeneration or neutralize regeneration-inhibiting factors.[12] In the second application, the scaffold temporarily replaces the extracellular matrix for the seeded or implanted cells, until they produce their own matrix, which ultimately provides the structural integrity of the replacement "tissue." In both these functions, the scaffold serves only temporarily, and it dissolves with time—ideally, when the need for an artificial support diminishes.

The ideal scaffold for implantation must meet several stringent criteria. It must be biocompatible and non–foreign body reaction forming. It also should be resistant to stress and strain, be sterilizable, and match the biomechanical characteristics of the tissue it is replacing. Material degradation and resorption is another desirable property, and the degradation products must be nontoxic and readily evacuated from the body. From a macroscopic perspective, the scaffold should be porous, with interconnecting pore structure to enable the accommodation of a large number of cells and their organization into a functioning tissue. Pore size, of at least 50 μm, is needed to allow the vascularization of the scaffold after transplantation, to supply the seeded cells with nutrients, and to remove secretions. At the same time, the polymer scaffold should comprise good mechanical features to enable handling in culture and during transplantation. Finally, the scaffold should be able to release growth factors, gene signals, and other bioactive proteins, in a time-dependent fashion.

Biomaterials

In general, scaffold biomaterial for tissue engineering and regeneration can be divided into two categories: synthetic or biologically derived materials. Synthetic materials allow for precise control over properties such as molecular weight of the polymer, degradation time, mechanical properties, and hydrophobic/hydrophilic ratio. However, they may not interact favorably with cells as do biologically derived

materials. The most popular synthetic scaffold materials are the degradable polyesters composed of lactide (PLA) and glycolide (PLG) and their copolymers (PLGA). The degradation times of the polymers range from days to years, depending on the comonomer ratio and molecular weight. The polymers have a good safety profile, are approved by the United State Food and Drug Administration, and are easy to manufacture. However, these same polymers, when formulated into thick porous structures for cardiac and other tissue engineering applications, do not perform satisfactorily. With material degradation, the scaffolds tended to crumble and lose their mechanical strength rather rapidly.[13] Furthermore, because most of these polymers are hydrophobic in nature (especially those containing higher ratio of the comonomer LA), the cell seeding process onto the scaffold made of this polymer was not efficient, and most of the cells concentrated at the periphery of the scaffolds.[14] After scaffold implantation, tissue ingrowth and vascularization were limited, and most of the scaffold internal volume remained unfilled. Furthermore, degradation of polyester scaffolds is accompanied by accumulation of acidic degradation products that affects cell viability and causes adverse tissue reaction after implantation. Recently, Krupnick *et al.*[15] showed that the use of the PGA mesh within the heart results in an intense inflammatory response. Several of the drawbacks of the synthetic polyesters can be overcome by the synthesis of hydrogels, such as those based on polyethylene glycol. These hydrophilic materials mimic in many ways the native extracellular matrix, although they, like other synthetic polymers, do not possess the biological specifics of natural polymers such as collagen.

Natural polymers include both extracellular matrix (ECM) proteins and derivatives (e.g., collagen) and materials derived from plants and seaweed. Natural polymers derived from ECM, such as type I collagen and fibronectin, contain particular adhesive sequences, such as Arg-Gly-Asp (RGD) on their surfaces that can facilitate cell adhesion and maintain cell differentiation and are advantageous for tissue engineering applications. However, these materials do not possess sufficient mechanical strength, and unless they are chemically cross-linked they degrade rather rapidly in the body. In addition, batch-to-batch variations in material properties, as well as potential contamination when the materials are extracted from animal tissue, raise many concerns. Recombinant forms of human collagen and other materials are being produced, to avoid the use of animal products, by expressing them in cell lines including yeast.

In addition to protein-based materials, there is significant activity in the area of natural polysaccharides. Alginate, a negatively charged polysaccharide from seaweed that forms hydrogels in the presence of calcium ions, is being developed for tissue engineering in native and modified forms.[16,17] In its hydrogel form, the alginate bears resemblance to glycocomponents of the extracellular matrix. We developed three-dimensional porous scaffolds from alginate, using a simple, all-aqueous process based on a freeze-dry technique.[17] The scaffolds were characterized by 90% porosity and pore sizes of 50–150 μm, depending on the freezing regimen.[18] The scaffolds supported the prolonged culture of various primary mammalian cells and facilitated the performance of rat hepatocytes in culture.[17] In a more recent study, we demonstrated the feasibility of bioengineering a cardiac tissue within alginate scaffolds.[19,20] After implantation onto rat infarcted myocardium, the cardiac biografts stimulated intense neovascularization from the neighboring coronaries and attenuated left ventricular dilatation and failure in an experimental rat model.[19]

GROWING A PATCH OF HEART MUSCLE

Several groups have reported encouraging results with various techniques for constructing beating cardiac patches for transplantation.[14,20–28] However, assembling vascularized three-dimensional myocardial tissue remains an enormous challenge. Shimizu *et al*.[23] grew rat cardiomyocytes on polymer surfaces that promoted the detachment of the thin cell layers when the temperature was reduced. The researchers put four of these sheets on top of each other until they fused and then implanted them under the skin of rats. Six months later, the researchers found that the engineered cardiac patch was beating and had been infiltrated by the host blood vessels. Eschenhagen and his colleagues created rings of engineered cardiac muscle using cardiomyocytes from neonatal rats.[2,3,21,22] As an alternative of seeding the cells on a scaffold, they mixed them into a collagen gel and cast them in a ring template. After a few days, they put the tissue-engineered patch on a stretching device that simulated the heart's contractions. When Zimmermann *et al*. implanted the engineered rings into rat heart, the stretched patches contracted more vigorously than unstretched patches.[2] Another technique that may accelerate and optimize tissue and organ assembly, including beating heart muscle, is "organ printing" technology.[29] A cell printer to print gels, single cells, and cell aggregates has been developed. Layer-by-layer sequentially placed and solidified thin layers of a thermoreversible gel served as "printing paper." This computer-aided, jet-based three-dimensional tissue engineering of living human organs suggests a new strategy for growing a patch of cardiac muscle.[29]

Bioreactors

The three-dimensional cell constructs that are developed *ex vivo* usually lack the vascular network that exists in normal vascularized tissues. Thus, the gas and nutrient supply to the scaffold-seeded cells depends merely on mass diffusion. In static cultivation, with no fluid mixing, large diffusional gradients are formed between the cell constructs and their surroundings so that the cells in the center of the construct do not get sufficient nutrients; the waste removal from the center is poor; and thus the cells eventually die. Oxygen transport is typically considered as the main limiting factor for nutrient exchange.[30]

To improve mass transport, researchers have designed several bioreactors, which exemplify different patterns of fluid dynamics and vessel geometry. A basic fluid-dynamic cultivation vessel is the spinner flask, which is a flask usually agitated at 50 rpm.[14,25] In these vessels, the cell constructs are subjected to turbulently mixed fluid that provides a well-mixed environment around the cell constructs and minimizes the stagnant layer at their surface. It has been shown that cultivation of cardiac cell constructs in spinner flasks produces engineered tissues that are superior, in almost every aspect (e.g., aerobic cell metabolism, DNA content, metabolic activity, and morphological appearance) to tissues cultivated under static conditions.[14,25,26] The spinner flask may not, however, be the optimal cultivation vessel for cardiac cells. The turbulent fluid flow at the surface of the constructs is usually characterized by eddies that destroy the seeded cells.

Bioreactors combined with mechanical signal stimuli improved the proliferation and distribution of the seeded human heart cells throughout the scaffold volume and

further stimulated the formation and organization of extracellular matrix, which contributed to the improvement in the mechanical strength of the cardiac graft.[21,22,31] Future bioreactors for cardiac tissue engineering should combine both perfusion and mechanical stimuli—for example, by allowing for adjustable pulsatile flow and varying levels of pressure. Such bioreactors are currently under development for engineering heart valves *ex vivo*.[32,33]

One of the major difficulties in cardiac tissue engineering is to grow three-dimensional structures that contain more than a few layers of muscle cells. Most bioreactors simply cannot supply enough nutrients and oxygen to the growing tissue. Whereas human heart muscle is approximately 1 cm thick, growth in a bioreactor typically stops once the tissue is approximately 100 micrometers, or less than 10 cell layers, thick. Beyond this thickness, the innermost cells are too far from the supply of fresh growth medium to thrive. After transplantation, rapid vascularization, adequate perfusion, survival, integration, and function of the engineered cardiac patch remain critical steps in the translation of *in vitro* achievements into an effective therapeutic tool.

THERAPEUTIC POTENTIAL OF ENGINEERED CARDIAC GRAFT

Preformed three-dimensional cardiac grafts may allow support of the injured left ventricular free wall and may stimulate neovascularization, extracellular matrix formation, and possibly repair of congenital malformations and scar tissue. We have reported that the strategy of tissue-engineered myocardial patch transplantation can improve mycardial dysfunction in rats after MI.[19] Fetal cardiomyocytes were seeded into scaffolds composed of alginate sponges, and the cell constructs were cultivated for four days to evaluate their viability and function before implantation. After implantation of the cardiac cellular constructs into seven-day infarcted myocardium of rat, some of the cells appeared to differentiate into mature myocardial fibers. The implanted grafts were supplied by intensive neovascularization. The biografts prevented left ventricle dilatation and deterioration of heart function after infarction. The underlying beneficial effect of this process is unclear. A direct contribution of the biograft to contractility is unlikely because only a relatively small fraction of the biograft was composed of myocardial tissue. Attenuation of infarct expansion was attributed to the elastic properties of bioartificial grafts. It is possible that angiogenesis, induced by growth factors secreted from the embryonic cells, resulted in biological scaffolding and attenuation of infarct expansion.

Zimmermann *et al*.[21] have demonstrated that engineered heart tissue survives and beats for at least 28 days after transplantation into rat heart. The grafts were intensively vascularized and acquired a highly differentiated cardiac phenotype when transplanted in the heart of syngenic rats. Other investigators provided proof of concept that tissue engineered patch could be used to repair congenital heart defects.[15,34]

CHALLENGES AND FUTURE DIRECTIONS

The encouraging preliminary results of cardiac tissue engineering experiments in small animal models helped to develop new concepts and theories of myocardial tis-

sue repair. However, for tissue engineering technology to be effective in human patients, it is critical that we create an at least 1-cm-thick and strong muscular patch. Furthermore, after implantation, the engineered heart muscle should survive the ischemic period until new vessels invade the graft to maintain viability and function. This may take a few days and could have devastating results. Cardiomyocytes are very sensitive to prolonged ischemia and may respond in necrosis and apoptosis of the engineered myocardial graft. Stimulated by these challenges, our group is now testing *in situ* tissue engineering strategies. By implanting unseeded scaffolds on the damaged myocardium, we created a friendly environment and space to attract the implanted cells. After the implanted scaffold has been impregnated with new vessels and extracellular matrix, we injected cardiomyocytes into the preimplanted scaffolds. We are attempting, based on achievements in the field of cell transplantation, to create a scaffold-based cardiac patch *in vivo*. The implanted scaffold could be impregnated with growth and survival factors that improve viability and survival and may enhance stem cell homing and self-repair. An important advantage of this concept of *in situ* tissue engineering is the feasibility of using it with a catheter-based approach to avoid the need for surgical thoracotomy. The rapid innovations in tissue engineering research and stem cell biology will accelerate and optimize engineered tissue assembly; they may bring us to the point of being able to create an alternative tissue to repair or replace damaged heart muscle.

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REFERENCES

1. VACANTI, J.P. & R. LANGER. 1999. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet **354** (Suppl. 1): SI32–S134.
2. ZANDONELLA, C. 2003. Tissue engineering: the beat goes on. Nature **421:** 884–886.
3. ZIMMERMANN, W.H. & T. ESCHENHAGEN. 2003. Cardiac tissue engineering for replacement therapy. Heart Fail. Rev. **8:** 259–269.
4. REFFELMANN, T., J. LEOR, J. MULLER-EHMSEN, *et al*. 2003. Cardiomyocyte transplantation into the failing heart—new therapeutic approach for heart failure? Heart Fail. Rev. **8:** 201–211.
5. MENASCHE, P., A.A. HAGEGE, J.T. VILQUIN, *et al*. 2003. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J. Am. Coll. Cardiol. **41:** 1078–1083.
6. ORLIC, D., J.M. HILL & A.E. ARAI. 2002. Stem cells for myocardial regeneration. Circ. Res. **91:** 1092–1102.
7. GEPSTEIN, L. 2002. Derivation and potential applications of human embryonic stem cells. Circ. Res. **91:** 866–876.
8. ANVERSA, P. & B. NADAL-GINARD. 2002. Myocyte renewal and ventricular remodelling. Nature **415:** 240–243.
9. JACKSON, K.A., S.M. MAJKA, H. WANG, *et al*. 2001. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J. Clin. Invest. **107:** 1395–1402.
10. WILLIAMS, R.S. 1998. Cell cycle control in the terminally differentiated myocyte. A platform for myocardial repair? Cardiol. Clin. **16:** 739–754.

11. LANGER, R. & J.P. VACANTI. 1993. Tissue engineering. Science **260:** 920–926.
12. PERETS, A., Y. BARUCH, F. WEISBUCH, *et al*. 2003. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J. Biomed. Mater. Res. **65A:** 489–497.
13. KIM, B.S. & D.J. MOONEY. 1998. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol. **16:** 224–230.
14. CARRIER, R.L., M. PAPADAKI, M. RUPNICK, *et al*. 1999. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol. Bioeng. **64:** 580–589.
15. KRUPNICK, A.S., D. KREISEL, F.H. ENGELS, *et al*. 2002. A novel small animal model of left ventricular tissue engineering. J. Heart Lung Transplant. **21:** 233–243.
16. ROWLEY, J.A., G. MADLAMBAYAN & D.J. MOONEY. 1999. Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials **20:** 45–53.
17. SHAPIRO, L. & S. COHEN. 1997. Novel alginate sponges for cell culture and transplantation. Biomaterials **18:** 583–590.
18. ZMORA, S., R. GLICKLIS & S. COHEN. 2002. Tailoring the pore architecture in 3-D alginate scaffolds by controlling the freezing regime during fabrication. Biomaterials **23:** 4087–4094.
19. LEOR, J., S. ABOULAFIA-ETZION, A. DAR, *et al*. 2000. A new approach to repair the infarcted myocardium? Circulation **102:** III56–III61.
20. DAR, A., M. SHACHAR, J. LEOR & S. COHEN. 2000. Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. Biotechnol. Bioeng. **80:** 305–312.
21. ZIMMERMANN, W.H., M. DIDIE & G.H. WASMEIER, *et al*. 2002. Cardiac grafting of engineered heart tissue in syngenic rats. Circulation **106:** I151–I157.
22. ZIMMERMANN, W.H., K. SCHNEIDERBANGER, P. SCHUBERT, *et al*. 2002. Tissue engineering of a differentiated cardiac muscle construct. Circ. Res. **90:** 223–230.
23. SHIMIZU, T., M. YAMATO, Y. ISOI, *et al*. 2002. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ. Res. **90:** e40.
24. SHIMIZU, T., M. YAMATO, T. AKUTSU, et *al*. 2002. Electrically communicating three-dimensional cardiac tissue mimic fabricated by layered cultured cardiomyocyte sheets. J. Biomed. Mater. Res. **60:** 110–117.
25. CARRIER, R.L., M. RUPNICK, R. LANGER, *et al*. 2002. Perfusion improves tissue architecture of engineered cardiac muscle. Tissue Eng. **8:** 175–188.
26. PAPADAKI, M., N. BURSAC, R. LANGER, *et al*. 2001. Tissue engineering of functional cardiac muscle: molecular, structural, and electrophysiological studies. Am. J. Physiol. Heart Circ. Physiol. **280:** H168–H178.
27. BURSAC, N., M. PAPADAKI, R.J. COHEN, *et al*. 1999. Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies. Am. J. Physiol. **277:** H433–H444.
28. AKINS, R.E., R.A. BOYCE, M.L. MADONNA, *et al*. 1999. Cardiac organogenesis in vitro: reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells. Tissue Eng. **5:** 103–118.
29. MIRONOV, V., T. BOLAND, T. TRUSK, *et al*. 2003. Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol. **21:** 157–161.
30. COLTON, C.K. 1995. Implantable biohybrid artificial organs. Cell Transplant. **4:** 415–436.
31. AKHYARI, P., P.W. FEDAK, R.D. WEISEL, *et al*. 2002. Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. Circulation **106:** I137–I142.
32. SODIAN, R., J.S. SPERLING, D.P. MARTIN, *et al*. 2000. Fabrication of a trileaflet heart valve scaffold from a polyhydroxyalkanoate biopolyester for use in tissue engineering. Tissue Eng. **6:** 183–188.
33. DOHMEN, P.M., S. OZAKI, E. VERBEKEN, *et al*. 2002. Tissue engineering of an autoxenograft pulmonary heart valve. Asian Cardiovasc. Thorac. Ann. **10:** 25–30.
34. SAKAI, T., R.K. LI, R.D. WEISEL, *et al*. 2001. The fate of a tissue-engineered cardiac graft in the right ventricular outflow tract of the rat. J. Thorac. Cardiovasc. Surg. **121:** 932–942.