Bioengineered Cardiac Grafts

A New Approach to Repair the Infarcted Myocardium?

Jonathan Leor, MD; Sharon Aboulafia-Etzion, MSc; Ayelet Dar, BSc; Lilia Shapiro, MSc; Israel M. Barbash, BMedSc; Alexander Battler, MD; Yosef Granot, PhD; Smadar Cohen, PhD

Background—The myocardium is unable to regenerate because cardiomyocytes cannot replicate after injury. The heart is therefore an attractive target for tissue engineering to replace infarcted myocardium and enhance cardiac function. We tested the feasibility of bioengineering cardiac tissue within novel 3-dimensional (3D) scaffolds.

Methods and Results—We isolated and grew fetal cardiac cells within 3D porous alginate scaffolds. The cell constructs were cultured for 4 days to evaluate viability and morphology before implantation. Light microscopy revealed that within 2 to 3 days in culture, the dissociated cardiac cells form distinctive, multicellular contracting aggregates within the scaffold pores. Seven days after myocardial infarction, rats were randomized to biograft implantation (n=6) or sham-operation (n=6) into the myocardial scar. Echocardiography study was performed before and 65±5 days after implantation to assess left ventricular (LV) remodeling and function. Hearts were harvested 9 weeks after implantation. Visual examination of the biograft revealed intensive neovascularization from the neighboring coronary network. Histological examination revealed the presence of myofibers embedded in collagen fibers and a large number of blood vessels. The specimens showed almost complete disappearance of the scaffold and good integration into the host. Although control animals developed significant LV dilatation accompanied by progressive deterioration in LV contractility, in the biograft-treated rats, attenuation of LV dilatation and no change in LV contractility were observed.

Conclusions—Alginate scaffolds provide a conducive environment to facilitate the 3D culturing of cardiac cells. After implantation into the infarcted myocardium, the biografts stimulated intense neovascularization and attenuated LV dilatation and failure in experimental rats compared with controls. This strategy can be used for regeneration and healing of the infarcted myocardium. **(*Circulation*. 2000;102[suppl III]III-56-III-61.)**

Key Words: cells ■ heart failure ■ myocardial infarction ■ myocytes ■ transplantation

Despite recent advances in the treatment of acute myocardial infarction (MI), the ability to repair extensive myocardial damage and to treat heart failure is limited.[1] The myocardium is unable to regenerate because cardiomyocytes cannot replicate after injury[2] and because there apparently are no muscle stem cells in the myocardium. The damage of MI is often progressive.[1] Patients who survive MI are prone to scar tissue formation and aneurysmal thinning of the damaged region. Even in the absence of cardiac aneurysm, the loss of viable myocardium can result in increased wall stress in the remaining myocardium, eventually triggering a sequence of molecular, cellular, and physiological responses that lead to left ventricular (LV) dilatation and heart failure.[1]

Cardiac transplantation is the only available treatment that significantly lengthens and improves quality of life. It is limited, however, due to a chronic shortage of donor hearts. A possible strategy to restore heart function after myocardial injury is to replace the damaged tissue with healthy tissue. Tissue engineering is a promising approach that makes possible the creation of new functional tissue to replace lost or failing tissue.[3] This new discipline combines isolated functioning cells and biodegradable 3-dimensional (3D) polymeric scaffolds. The scaffold temporarily provides the biomechanical support for the cells until they produce their own extracellular matrix. Because tissue-engineering constructs contain living cells, they may have the potential for growth and cellular self-repair and remodeling.[3] In the present study, we examined whether the tissue-engineering strategy within alginate scaffolds would result in the formation of alternative cardiac tissue that could replace the scar and improve cardiac function after extensive MI.

Methods

The study was performed in accordance with the guidelines of The Animal Care and Use Committee of Ben-Gurion University of the Negev, which conforms to the policies of the American Heart Association and the *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services, publication No. NIH 86-23).

From the Cardiac Research Center, Faculty of Health Sciences (J.L., S.A.-E., I.M.B., A.B.), Department of Biotechnology Engineering (A.D., L.S., S.C.), and Department of Life Sciences (Y.G.), Ben-Gurion University of the Negev, Beer-Sheva, Israel.

*J. Leor and S. Aboulafia-Etzion contributed equally to this study.

Correspondence to Jonathan Leor, MD, FACC, FESC, Neufeld Cardiac Research Institute, Sheba Medical Center, Tel-Hashomer 52621, Israel. E-mail jleor@bgumail.bgu.ac.il

***Circulation* is available at http://www.circulationaha.org**

Rat Model of MI

Our method for the induction of MI was previously described.[4] Female Sprague-Dawley rats were anesthetized with a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg). The chest was opened under sterile technique by left thoracotomy through the fourth intercostal space, the pericardium was removed, and the left main coronary artery was permanently occluded with an intramural stitch.

Cardiac Cell Isolation

Fetal cardiac cells were isolated, purified, and cultured as previously described for neonatal rat cells.[5] Briefly, female Sprague-Dawley rats that were carrying 16-day-old embryos were operated on while they were under deep anesthesia. The embryos were removed, and their hearts were placed in cold dissociation buffer. The ventricles were cut into 1- to 2-mm cubes and dissociated through alternating treatments at 24°C with[1] 0.1% trypsin and 0.002% DNase I in dissociation buffer for 5 minutes at 150 rpm and 2% calf serum in dissociation buffer for 5 minutes with gentle pipetting. The sequence was repeated until all tissue was dissociated. Freed cells were collected in cold M-199 culture medium that contained 0.5% calf serum and 0.002% DNase, centrifuged (0°C, 10 minutes at 2500 rpm), washed in culture medium, and preplated onto a 60-mm dish for 15 minutes at 37°C. An aliquot of the nonattached cells was counted and used for seeding within the alginate scaffold.

3D Culture Within Alginate Scaffolds

The isolated cardiac cells were seeded at a concentration of 3×10^5 cells per scaffold within cylindrical alginate scaffolds (6-mm diameter×1.0-mm height) placed in a 96-well plate. The scaffolds, with an average pore diameter of 100 μm, were prepared from sodium alginate (Protanal LF 120; Pronova Biopolymer) and calcium gluconate as the cross-linker according to a freeze-dry technique (Figure 1).[6] We seeded the cells into the dry alginate scaffolds by dropping the cell suspension on top of the scaffold. Due to their hydrophilic nature, the scaffolds were easily wetted by the culture medium, and cell seeding was efficient.[7] The cultures were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C, with daily medium changes.

The cell constructs were followed microscopically for 4 days before implantation. The viability and metabolic activities of the cardiac cells within the alginate scaffolds were assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures the ability of mitochondrial dehydrogenase enzymes to convert the soluble yellow MTT salt into insoluble purple formazan salt.[8] In addition, cells isolated from the scaffolds after dissolution of the matrix in PBS were recultured onto culture dishes. Two days later, the cultures were examined for contracting cells.

Biograft Implantation

Biograft transplantation was performed 7 days after MI. Rats were anesthetized, and under sterile technique, the chest was opened. The infarcted area was identified visually on the basis of surface scar and wall motion abnormality. Rats were randomized to implantation of biografts or sham-transplantation into the infarcted myocardium. Two scaffolds were attached, by 1 suture for each, to the scar. The control group consisted of rats subjected to sham-transplantation through the insertion of 1 suture into the scar. Air was expelled from the chest, and the surgical incision was sutured closed. In an initial series of pilot experiments, the technical aspects of the procedure were refined.

Nine weeks after transplantation, the rats were euthanized with phenobarbital overdose. The hearts were harvested, sectioned, and processed for histological and immunohistochemical examinations.

Histological and Immunohistochemical Examinations

Adjacent blocks of the harvested heart were embedded in paraffin, sectioned into 5-μm slices, and stained with hematoxylin-eosin.

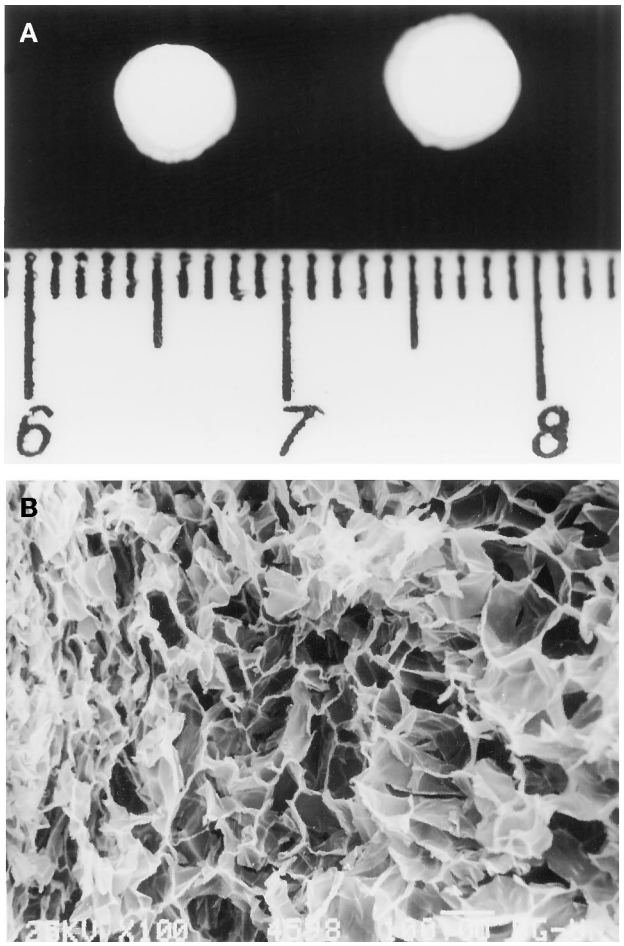

Figure 1. A, Photograph of alginate scaffold used in study. B, Scanning electron micrograph of a cross-section of alginate scaffold.

Serial sections were immunolabeled with antibodies against the gap junctional protein connexin 43 (Zymed).[9] By using an antibody against connexin 43, the presence of gap junctions in the biograft could be demonstrated and the estimated stage of development of the "artificial" tissue could be studied.

For further identification of the implanted cells, in a few experiments the cardiac cells were labeled with a fluorescent dye, dialkyl oxacarbocyanine (DiOC18; Molecular Probes) before seeding within the alginate scaffolds. This dye stains cell membranes green, without interference with cell viability or metabolic activity.[10]

Echocardiography to Evaluate Remodeling and Contractility

Transthoracic echocardiography was performed on all animals 5 to 7 days after MI, before implantation (baseline echocardiogram), and 65±5 days after implantation. Previous reports[11,12] have demonstrated the accuracy and reproducibility of transthoracic echocardiography in rats. Briefly, rats were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine. The chest was shaved, and the rats were placed supine. Echocardiograms were performed with a commercially available echocardiography system equipped with 7.5-MHz phased-array transducer (Hewlett-Packard). The transducer was positioned on the left anterior side of the chest after the precordium was shaved. The heart was first imaged in the 2-dimensional mode in the parasternal long- and short-axis views of the LV. By the use of these views, the M-mode cursor was positioned perpendicular to the ventricular septum and posterior wall; M-mode images were then obtained at the level below the tip of the mitral valve leaflets at the level of the papillary muscles. Care was taken to

avoid excessive pressure. Posterior wall thickness and LV internal dimensions were measured according to the leading edge method of the American Society of Echocardiography: maximal LV end-diastolic dimension (at the time of maximal cavity dimension), minimal LV end-systolic dimension (at the time of maximum anterior motion of the posterior wall), and fractional shortening as a measure of systolic function, which was calculated as FS (%)=[(LVIDd−LVIDs)/LVIDd]×100, where LVID is LV internal dimension, s is systole, and d is diastole. To further validate these measurements and to determine the accuracy and reproducibility of the technique, we carried out a reproducibility study in normal rats. All measurements were averaged for 3 consecutive cardiac cycles and were made by an experienced technician who was blinded to the treatment group.

Statistical Analysis

All values are shown as mean±SEM. Differences between measurements before and after implantation were compared by paired *t* test (InStat Version 3.01; GraphPad Software Inc). All tests were 2-tailed, and significance was accepted at $P<0.05$.

Results

Overall, 16 infarcted rats were included in the present study. There were no operative deaths. Four rats were part of a pilot study to establish the feasibility and the technical aspects of the procedure. We implanted 1 scaffold without cells onto the infarcted myocardium of each rat. After 2 months, the histological examination of the engrafted scaffolds revealed that they were merged with the adjacent infracted tissue and were partially eroded. The echocardiographic study was performed on 12 rats. The experimental group (n=6) was treated with biograft implantation, and the control group (n=6) was treated with sham-transplantation. The survival rate after 65±5 days was 100% in the biograft-treated group and 83% (5 of 6) in the sham group.

Characteristics of Cell Constructs

The alginate scaffolds in the present study displayed a highly porous structure with interconnected pores and pore sizes that ranged between 100 and 150 μm in diameter as determined with scanning electron microscopy.[6] The scaffolds were easily wetted with the aqueous medium, and the hydrated matrix appeared to be transparent and displayed a soft consistency. Under in vitro cell culture conditions, the scaffolds were sufficiently stable, were easily handled, and did not contract as do some collagen-based materials.

Seeding of the cardiac cells within the alginate scaffolds was feasible due to the hydrophilicity of the alginate material, which allowed rapid wetting of the matrix. Because of the large pore diameter, the physical obstruction of liquid flow by the torturous pore pathway was minimal, allowing the transport of cells into the scaffold pores. The cells were uniformly distributed throughout the scaffold volume. MTT assay performed on the cell constructs on different days after seeding revealed that the cells were viable. In addition, cardiac cells isolated from the alginate scaffolds at 4 days after seeding were contracting after reculture onto tissue culture dishes.

The cardiac cells were located mainly within the scaffold pores. Phase contrast microscopy throughout the culture revealed that within 24 hours after seeding, most of the seeded cells within the alginate scaffolds were arranged in small viable multicellular 3D aggregates. Some of the aggregates within the scaffold were contracting spontaneously and rhythmically.

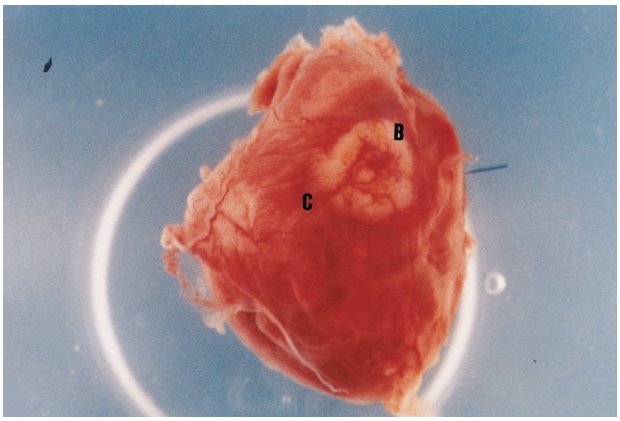

Figure 2. Photograph of heart at week 9 after implantation of cellular construct. Visual examination revealed intense neovascularization growth into implanted biograft (B). Note a coronary branch (C) that supplies biograft and covers it with extensive network of vessels (original magnification ×5).

Macroscopic and Histological Analyses

Visual inspection of the implanted biograft at 65±5 days after implantation revealed that the scaffold was covered by a thin lucent connective tissue that was enriched with blood vessels (Figure 2). The extensive neovascularization into the biograft emerged from the neighboring coronary network (Figure 2).

Histological examination of thin sections of the biograft identified differentiated forms of myocardial tissue (Figure 3). Well-formed myofibers with typical striation were found to grow in between collagen bundles. Cellular gap junctions, consistent with mechanical and electrical connections among cardiomyocytes, were also prevalent in the preparations (Figure 4). Some myofibers displayed the normal parallel arrangements of cardiomyocytes, whereas others were randomly oriented with abnormal intercellular connection, as determined with connexin 43 staining (Figure 4).

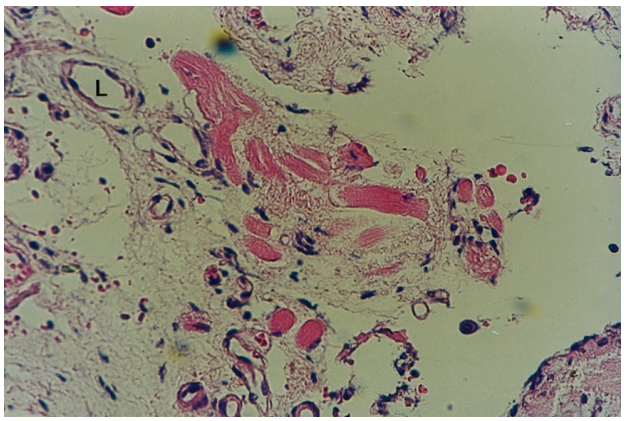

Figure 3. Hematoxylin-eosin–stained, high-power micrograph of biograft 9 weeks after implantation. Well-formed myofibers were found to grow in between collagen bundles. Some myofibers displayed normal parallel arrangements of cardiomyocytes, whereas others were randomly oriented. Significantly, biograft was populated with newly formed vessels (L), embedded within matrix of collagen bundles (original magnification ×200).

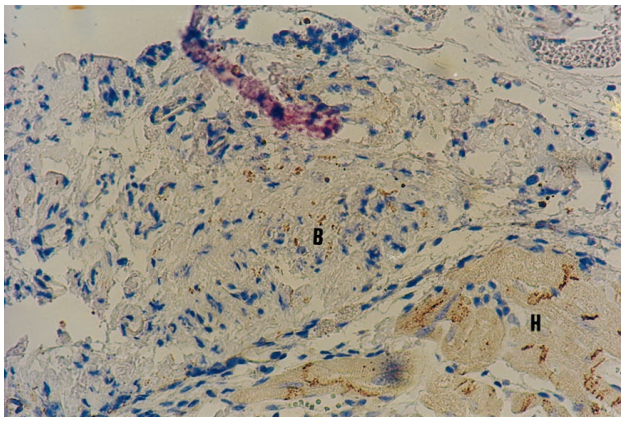

Figure 4. Microscopic image of biograft labeled for connexin 43. Connexin 43 was localized in normal parallel arrangements in host myocardium (H) and randomly oriented in biograft (B) (original magnification ×200).

Significantly, the biografts were populated with newly formed capillaries and arterioles (Figure 3) embedded within the collagen bundle matrix. Typical fibroblasts, macrophages, and lymphocytes were present in the grafts (Figure 5). At many anchorage sites, the bioartificial graft showed integration with the scar tissue, and the specimens showed almost complete disappearance of the scaffold. In experiments in which the implanted cells were prelabeled with the fluorescent dye, examination under a fluorescent microscope confirmed that the biografts contained many of the seeded cells.

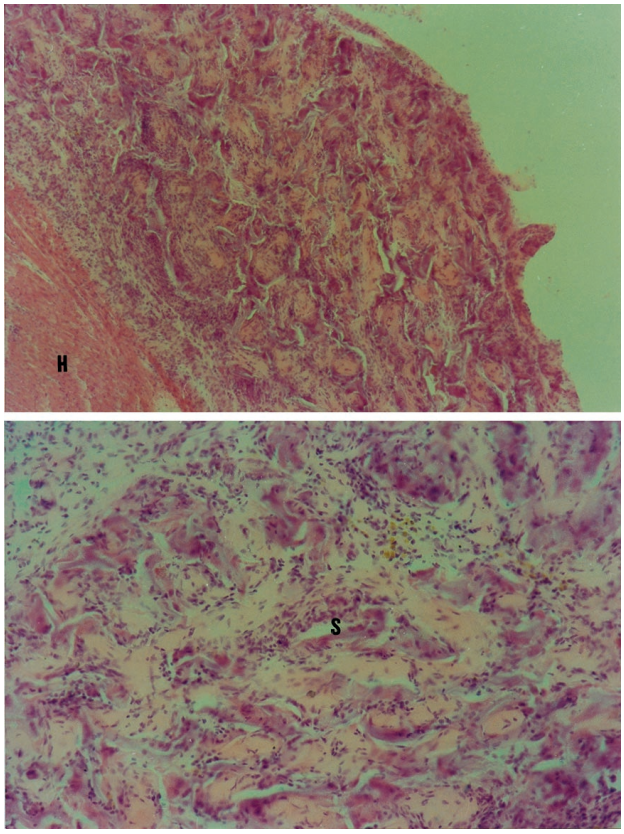

Figure 5. Photomicrograph of biograft at 9 weeks after implantation. A, Hematoxylin-eosin staining demonstrates inflammatory infiltrates and fibrous strands of collagen. At many anchorage sites, bioartificial graft showed integration with host myocardium (H) and specimens showed almost complete disappearance of scaffold (original magnification ×100). B, High-power photomicrograph of biograft. Typical fibroblasts, macrophages, and lymphocytes were present in biograft. Remainder of scaffold is identified (S) (original magnification ×400).

Results of Echocardiography Study

	Before Transplantation	After Transplantation	*P*
Sham group			
LV internal diameter, cm			
End diastole	0.64±0.03	0.84±0.05	0.03
End systole	0.33±0.02	0.55±0.06	0.02
Fractional shortening, %	47±2	33±4	0.005
LV wall thickness, cm			
Anterior	0.10±0.004	0.09±0.01	0.34
Posterior	0.12±0.006	0.13±0.002	0.07
Biograft-treated group			
LV internal diameter, cm			
End diastole	0.64±0.04	0.69±0.02	0.32
End systole	0.32±0.04	0.37±0.04	0.52
Fractional shortening, %	53±4	47±5	0.52
LV wall thickness, cm			
Anterior	0.09±0.004	0.10±0.007	0.21
Posterior	0.11±0.006	0.12±0.007	0.44

Echocardiographic Functional Study

To evaluate the influence of the biografts on LV remodeling and function, a series of echocardiography studies were conducted. After permanent left coronary artery occlusion, the sham group developed a typical course of LV remodeling and heart failure that complicated anterior MI.[2] After 3 months, LVIDd and LVIDs increased progressively, by 31% and 65%, respectively (Table, sham group). Progressive LV dilatation was also accompanied by significant deterioration in LV performance, as shown by the deterioration of FS (from 47±2% at baseline to 33±4%; $P=0.005$) at the end of the present study.

Conversely, in the biograft-treated rats, we observed attenuation of all LV remodeling indices (Table, biograft-treated group). During the follow-up period, there was no significant change in LVIDs and LVIDs (0.64±0.04 versus 0.69±0.02 cm, $P=0.31$; 0.32±0.04 versus 0.37±0.04 cm, $P=0.52$, respectively). The beneficial effect of the biografts on LV remodeling was translated into prevention of LV function deterioration as reflected by preservation of FS after implantation (53±4% versus 47±5%, $P=0.52$).

Discussion

In the present report, we tested the feasibility of bioengineering cardiac tissue within novel 3D porous alginate scaffolds. We found that the seeded fetal cardiac cells retained viability within the scaffolds and that they formed multicellular beating aggregates within 24 hours. After implantation of the cellular constructs into the infarcted myocardium, some of the cells appeared to differentiate into mature myocardial fibers.

The implanted grafts were supplied by intensive neovascularization, which evidently contributed to the prolonged survival of the cells in the grafts. The biografts attenuated LV dilatation and heart function deterioration.

Recently, Li et al,[13] Sakai et al,[14] Scorsin et al,[12] Taylor et al,[15] and our group[16] reported that the transplantation of isolated fetal cardiomyocytes or skeletal myoblasts can enhance cardiac function after myocardial injury. The potential added advantage of the tissue-engineering approach is better control of the tissue formation process, of the shape and size of the graft, and of the ability to determine the consistency of the graft (eg, number of cells, cell-to-cell ratio). Optimization of the scaffold and techniques for cell manipulation in culture will further encourage the cells to express their inherent biological potential to form differentiated tissue.

In the present study, we specifically chose to seed partially differentiated fetal cardiomyocytes that have been used successfully by us[16] and others[12–15] in isolated cell transplantation experiments. Our study showed that fetal cardiac cells are able to differentiate within the alginate scaffold, in situ, into mature cardiomyocytes and to organize into bundles of myofibers. A large portion of the biograft was occupied by collagen bundles with scattered fibroblasts. It appears that the extracellular matrix produced by the biograft replaces the temporary support provided by the alginate scaffold. This may eventually contribute to the complete integration of the engineered biograft to the host.

Most recently, Li et al[17] reported that a bioengineered cardiac graft can be made of fetal cardiac cells and 3D gelatin mesh. The cells in the graft formed heart-like tissue and contracted spontaneously. Implantation of the biografts into the skeletal muscle was associated with new vessel formation. However, LV developed pressure was lower in hearts in which either a cell-seeded or -unseeded graft had been implanted compared with controls. Li et al[17] proposed that inappropriate sizing of the grafts interferes with the contractility of the viable myocardium.

The expansion of anterior MI produces progressive deterioration in LV function.[1] An encouraging finding in the present study was the association between biograft implantation and the improved remodeling indices and LV function. The mechanism behind this beneficial effect is still unclear but is in agreement with recent reports on the impact of fetal cardiomyocyte transplantation on improvement in cardiac function.[12–16] A direct contribution of the biograft to contractility is unlikely because only a relatively small portion of the biograft was composed of myocardial tissue and we did not observe full integration of these artificial fibers within the host myocardium. Attenuation of infarct expansion by virtue of the elastic properties of bioartificial grafts is possible. Most recently, Kelley et al[18] showed that restraint of the expansion of the LV with mesh placed over the infarcted myocardium preserves LV geometry and resting function in a sheep model of MI. Angiogenesis induced by growth factors secreted from the embryonic cells, resulting in improved collateral flow and augmentation of contractility, is also a possible mechanism. Finally, a nonspecific immune response against the implanted biograft followed by cytokine release could enhance scar formation and cardiac function.

We are aware of certain limitations of the present study. The rat MI model of coronary artery ligation can results in infarcts of different sizes. Because of this, we prefer to evaluate changes in remodeling and function through serial echo studies with each animal used as its own control. Based on the echo study at baseline (Table), the extent of myocardial damage was almost identical in the treated and control groups.

It is possible that measurements with additional reference points or with other methods, such as the Langendorf preparation, to evaluate remodeling and function might further strengthen our conclusions.

The presence of inflammatory infiltrates of macrophages and lymphocytes in the biograft may be attributed to the seeding of allogenic donor cells without immunosuppression and the use of calf serum when culturing the cardiac cells. Currently, we are using syngeneic inbred rats and serum-free culture medium to minimize immune response.

Another issue that warranted further investigation is the effect of the scaffold on LV remodeling and function. Our control group was allocated to sham-implantation. It is possible that unseeded grafts could elicit similar results as those obtained with seeded grafts. However, the implantation of an unseeded scaffold is associated with significantly less neovascularization compared with a seeded graft (J. Leor, unpublished data, 1999).

Finally, the finding that only a relatively small portion of the biograft consisted of myofibers may be related to the relatively small number (300 000) of seeded cells. To achieve therapy, total transplanted “myocardial” tissue mass is critically important. In future experiments, we plan to increase the number of seeded cardiomyocytes by at least 10-fold in each biograft.

Conclusions and Implications

Our results so far suggest that the strategy of tissue engineering could be used for regeneration and healing of the infarcted myocardium and attenuation of wall stress, infarct expansion, and LV dilatation. These beneficial effects could be translated into the prevention of heart failure progression.

Candidate cells for cardiac tissue engineering could be myoblasts derived from mesenchymal[19] or embryonic stem cells[20] or genetically modified fibroblasts.[21] The cells can be expanded, genetically modified, and tissue engineered into implantable biograft that secretes therapeutic protein.[22]

Recent advances in methods of cardiomyocyte isolation and 3D culture[6,7,23–25] show promise and will contribute to cardiac tissue engineering in vitro. The bioengineered tissue could be used for surgical repair of the infarcted myocardium or of congenital cardiac defects.[17] The ability to provide the means of replacing damaged myocardium will have a dramatic impact on the way in which cardiovascular medicine is practiced. It will become increasingly important as the population continues to age and the number of patients with heart failure increases.[1]

Acknowledgments

This work was supported in part by grant 95-00294/1 from the United States–Israel Binational Science Foundation (BSF), grant 3619 from the Ministry of Health Sciences, and grant 52/99-1 from

The Israel Science Foundation. We are grateful to Judy Knopf for editorial assistance and Maya Rovner for technical assistance in the echo studies.

References

1. Mann DL. Mechanisms and models in heart failure. *Circulation*. 1999; 100:999–1008.
2. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res*. 1998;83:15–26.
3. Vacanti JP, Langer R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet*. 1999;354(suppl 1):SI32–SI34.
4. Leor J, Patterson M, Quinones MJ, et al. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat: a potential method for repair of infarcted myocardium. *Circulation*. 1996;94(suppl II):II-332–II-336.
5. Aharonovitz O, Aboulafia-Etzion S, Leor J, et al. Stimulation of 42/44 kDa mitogen activated protein kinases by arginine vasopressin in rat cardiomyocytes. *Biochim Biophys Acta*. 1998;1401:105–111.
6. Shapiro L, Cohen S. Novel alginate sponges for cell culture and transplantation. *Biomaterials*. 1997;18:583–590 .
7. Glicklis R, Shapiro L, Agbaria R, et al. Hepatocyte behavior within three-dimensional porous alginate scaffolds. *Biotechnol Bioeng*. 2000;67:344–353.
8. Ciapetti G, Cenni E, Pratelli L, et al. In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials*. 1993;14:359–364.
9. Kanter HL, Laing JG, Beau SL, et al. Distinct patterns of connexin expression in canine Purkinje fibers and ventricular muscle. *Circ Res*. 1993;72:1124–1131.
10. Kroesen B-J, Mesander G, ter Haar JG, et al. Direct visualisation and quantification of cellular toxicity using two color fluorescence. *J Immunol Methods*. 1992;156:47–54.
11. Litwin SE, Katz SE, Morgan JP, et al. Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat. *Circulation*. 1994;9:345–354.
12. Scorsin M, Hagege AA, Marotte F, et al. Does transplantation of cardiomyocytes improve function of infarcted myocardium? *Circulation*. 1997; 96(suppl II):II-188–II-193.
13. Li RK, Jia ZQ, Weisel RD, et al. Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg*. 1996;62:654–661.
14. Sakai T, Li RK, Weisel RD, et al. Fetal cell transplantation: a comparison of three cell types. *J Thorac Cardiovasc Surg*. 1999;118:715–725.
15. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med*. 1998;4:929–933.
16. Leor J, Aboulafia-Etzion S, Barbash IM, et al. Influence of fetal cardiomyocyte transplantation on the progression of heart failure. *J Am Coll Cardiol*. 1999;33(suppl):215A. Abstract.
17. Li RK, Jia ZQ, Weisel RD, et al. Survival and function of bioengineered cardiac grafts. *Circulation*. 1999;100(suppl II):II-63–II-69.
18. Kelley ST, Malekan R, Gorman JH, et al. Restraining infarct expansion preserves left ventricular geometry and function after acute anteroapical infarction. *Circulation*. 1999;99:135–142.
19. Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation*. 1999; 100(suppl II):II-247–II-256.
20. Klung MG, Soonpaa MH, Koh GY, et al. Genetically selected cardiomyocytes from differentiating embryonic stem cell form stable intracardiac grafts. *J Clin Invest*. 1996;98:216–224.
21. Leor J, Prentice H, Sartorelli V, et al. Gene transfer and cell transplant: an experimental approach to repair a “broken heart.” *Cardiovasc Res*. 1997; 35:431–441.
22. Bonadio J, Smiley E, Path P, et al. Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. *Nat Med*. 1999;5:753–759.
23. Bursac N, Papadaki M, Cohen RJ, et al. Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies. *Am J Physiol*. 277:H433–H444.
24. Carrier RL, Papadaki M, Rupnick M, et al. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol Bioeng*. 1999;64:580–599.
25. Akins RE, Boyce RA, Madonna ML, et al. Cardiac organogenesis in vitro: reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells. *Tissue Eng*. 1999;5:103–118.