Cell Transplantation, Vol. 16, pp. 927–934, 2007
Printed in the USA.

0963-6897/07 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM Clinical Trial): One Year Follow-Up

Juan C. Chachques,* Jorge C. Trainini,† Noemi Lago,† Osvaldo H. Masoli,† Jose L. Barisani,† Miguel Cortes-Morichetti,* Olivier Schussler,* and Alain Carpentier*

*Department of Cardiovascular Surgery, Pompidou Hospital, Paris, France
†Avellaneda Hospital, Buenos Aires, Argentina

Cell transplantation for the regeneration of ischemic myocardium is limited by poor graft viability and low cell retention. In ischemic cardiomyopathy the extracellular matrix is deeply altered; therefore, it could be important to associate a procedure aiming at regenerating myocardial cells and restoring the extracellular matrix function. We evaluated intrainfarct cell therapy associated with a cell-seeded collagen scaffold grafted onto infarcted ventricles. In 15 patients (aged 54.2 ± 3.8 years) presenting LV postischemic myocardial scars and with indication for a single OP-CABG, autologous mononuclear bone marrow cells (BMC) were implanted during surgery in the scar. A 3D collagen type I matrix seeded with the same number of BMC was added on top of the scarred area. There was no mortality and no related adverse events (follow-up 15 ± 4.2 months). NYHA FC improved from 2.3 ± 0.5 to 1.4 ± 0.3 ($p = 0.005$). LV end-diastolic volume evolved from 142 ± 24 to 117 ± 21 ml ($p = 0.03$), and LV filling deceleration time improved from 162 ± 7 to 196 ± 8 ms ($p = 0.01$). Scar area thickness progressed from 6 ± 1.4 to 9 ± 1.5 mm ($p = 0.005$). EF improved from 25 ± 7% to 33 ± 5% ($p = 0.04$). Simultaneous intramyocardial injection of mononuclear bone marrow cells and fixation of a BMC-seeded matrix onto the epicardium is feasible and safe. The cell-seeded collagen matrix seems to increase the thickness of the infarct scar with viable tissues and helps to normalize cardiac wall stress in injured regions, thus limiting ventricular remodeling and improving diastolic function. Patients' improvements cannot be conclusively related to the cells and matrix due to the association of CABG. Cardiac tissue engineering seems to extend the indications and benefits of stem cell therapy in cardiology, becoming a promising way for the creation of a "bioartificial myocardium." Efficacy and safety of this approach should be evaluated in a large randomized controlled trial.

Key words: Stem cell therapy; Myocardial regeneration; Tissue engineering; Heart failure; Ischemic heart disease; Bioartificial myocardium; Cellular cardiomyoplasty

INTRODUCTION

The objective of cellular cardiomyoplasty is to regenerate the myocardium by the implantation of living stem cells (2,23,25,27,33). Until now, in many clinical trials isolated cellular cardiomyoplasty failed to clearly demonstrate improvements of ventricular function (11,20,22, 28). Cardiac cell transplantation seems to be limited by poor graft viability and low cell retention (29). In addition, in ischemic disease both the contracting cells and the extracellular matrix are pathologically modified. Therefore, it could be important to associate a procedure aiming at regenerating myocardial cells and restoring the extracellular matrix function (1). The use of scaffolds may provide a means to reestablish a beneficial atmosphere for cell survival, multiplication, differentiation, and function. Preclinical investigations using tissue engineering technologies showed that this approach can contribute to improve the efficiency of cellular therapy for organ regeneration. These experimental studies demonstrated that collagen matrices enhance survival of transplanted cells and contribute to functional improvements and to the limitation of postischemic ventricular remodeling (5,17).

The goal of this clinical feasibility study was to evaluate the potential of a biodegradable three-dimensional collagen type I matrix seeded with bone marrow cells (BMC) and grafted onto the infarcted ventricle to support and regenerate postischemic lesions.

MATERIALS AND METHODS

Patients

In a phase I clinical trial, cellular cardiomyoplasty procedures associated with the implantation of a BMC-

Address correspondence to Juan C. Chachques, M.D., Ph.D., Department of Cardiovascular Surgery, Pompidou Hospital, 20 rue Leblanc, 75015 Paris, France. Tel: ++33613144398; Fax: ++33140728608; E-mail: j.chachques@brs.aphp.fr

seeded collagen matrix were performed in 15 consecutive patients (90% males), mean age 54.2 ± 3.8 years. Patients were in mean NHYA FC 2.3 ± 0.5, presenting with impaired LV function (mean radionuclide ejection fraction 25 ± 7%), LV wall postischemic scars (akinetic and metabolically nonviable), and surgical indication for CABG. The procedures were approved by the institutional review boards and informed written consent was obtained from each patient before the procedure.

Exclusion criteria included cardiogenic shock or congestive heart failure (NYHA FC IV), history of leukopenia or thrombocytopenia, evidence for malignant disease or terminal disease, patients under treatment with steroids, oncology drugs, or inmunological suppression, renal insufficiency (serum creatinine >2.5 mg/dl) or known hepatic insufficiency, and stroke or major surgery during the last month.

BMC Isolation and Preparation

Four hours prior to the cardiac surgical procedure and under local anesthesia, bone marrow (330 ± 28 ml) was aspirated from the ilium bone and processed to obtain mononuclear stem cells. The cell suspension was loaded on Ficoll-Paque density gradient (specific gravity 1.077, Amersham Biosciences, Arlington Heights, IL, USA), and centrifuged for 20 min at 2000 × *g*. BMC were isolated from the layer between the Ficoll-Paque reagent and blood plasma, and washed two times in phosphate-buffered saline (PBS; Sigma, St. Louis, MO, USA). An enriched suspension of BMC was obtained (CD34+ 8%, AC133+ 3%) and diluted in autologous patient serum. Two samples containing 250 ± 28 million cells each were prepared for intramyocardial injection and matrix seeding.

Collagen Matrix Preparation

Collagen matrix was prepared from a commercially available CE Mark collagen kit (Pangen 2, Urgo Laboratory, Chenove, France). This 3D biodegradable matrix (size: 5 × 7 × 0.6 cm) was manufactured using a lyophilized, nondenatured, native collagen (Fig. 1). The matrix pores measured 50–100 μm (Fig. 2). In the operating room matrices were placed in petri dishes. Afterwards 250 ± 28 million BMC suspended in medium were seeded onto each matrix. To promote a regular distribution of BMC into the matrix pores, petri dishes containing the collagen matrices were shaken continuously for 10 min at 120 rpm using an Orbital Shaker (Stuart Scientific, UK) (Fig. 3). This procedure can be also performed using centrifugation (10 min at 900 × *g*) of the matrix and cells.

Surgical Procedures

After sternotomy, a single OP-CABG was performed using LIMA. At the end of surgery, the intrainfarct implantation of the autologous BMC (250 ± 28 million cells) was performed into well-exposed LV ischemic areas, permitting 14 ± 3 injection points within and principally around the infarct, with a 25-gauge × 40-mm retrobulbar ophthalmic needle.

Afterwards a 3D collagen type I matrix (size 7 × 5 × 0.6 cm) seeded with the same number of BMC (250 ± 28 million cells) was added on top of the scarred area. It was fixed onto the epicardium by six single PDS sutures (6-0) and covered by a second noncellularized matrix (Fig. 4).

Postoperative Management and Clinical Follow-Up

All patients received oral amiodarone (200 mg/day) as antiarrhythmic drug, starting 1 week before surgery and up to the third postoperative month. Following surgery the patient's cardiac rhythm was monitored with continuous in-hospital telemetry. After discharge ECG-Holter monitoring was performed at 1, 2, and 3 months. Ventricular function and myocardial viability were evaluated by echocardiography, radionuclide ventriculography (MIBI Gated Spect), and MRI.

Statistics

Results were analyzed and reported as percentage or mean ± SD. Student's *t*-test was used to compare the groups. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

All patients had an uneventful recovery and were discharged from the ICU 3.6 ± 1.8 days following surgery. Patients were discharged from the hospital at 10 ± 3 postoperative days. At a mean follow-up of 15 ± 4.2 months without mortality and any related adverse events, no malignant cardiac arrhythmias were reported.

NYHA FC improved from 2.3 ± 0.5 to 1.4 ± 0.3 ($p = 0.005$). Blind radioisotopic/MRI analysis showed that 58 ± 9.3% of the cell-implanted segments improved their kinetics and viability (Fig. 5). LV EF improved from 25 ± 7% to 33 ± 5% (matrix, $p = 0.04$). Concerning postischemic remodelling and diastolic function, echocardiographic studies showed that LVEDVol evolved from 142 ± 24 to 117 ± 21 ml ($p = 0.03$) and LV filling deceleration time (DT) improved from 162 ± 7 to 196 ± 8 ms ($p = 0.01$). Scar area thickness progress from 6 ± 1.4 to 9 ± 1.5 mm ($p = 0.01$).

The association of CABGs with cellular cardiomyoplasty and matrix implantation was imposed by the in-

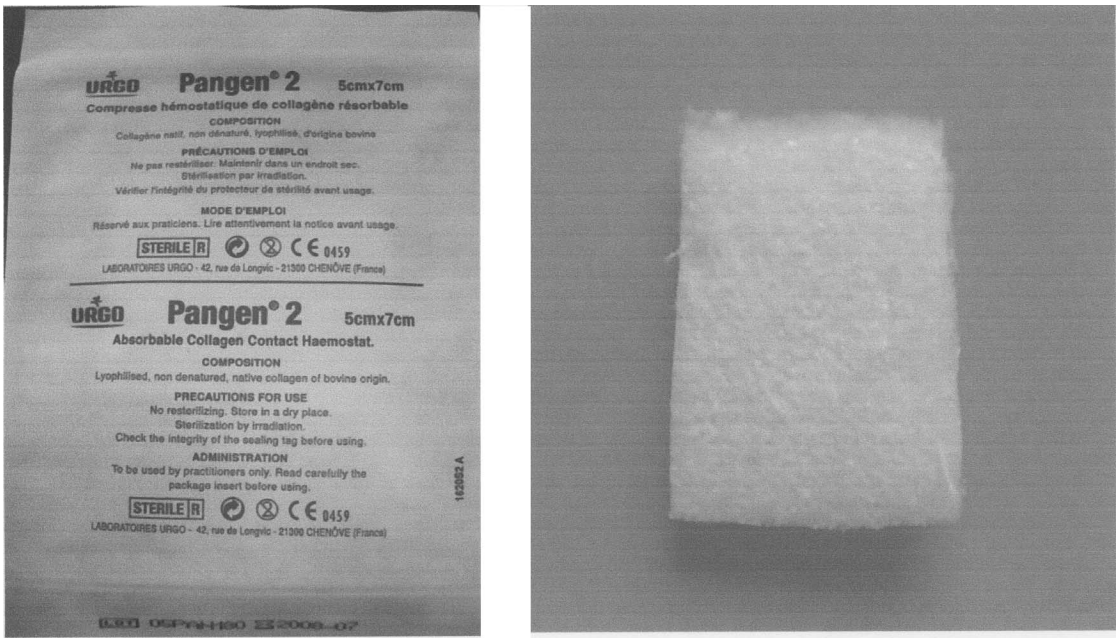

Figure 1. Macroscopic view of the collagen type I, 3D biodegradable matrix used for myocardial repair (size $7 \times 5 \times 0.6$ cm).

stitutional review board and thus the individual contribution of each procedure cannot be clearly elucidated at this stage of our clinical trial.

DISCUSSION

There are two types of collagen fibers in the normal adult heart, types I and III, produced by fibroblasts and myofibroblasts. The fiber type I represents 80% of collagen protein in the heart, and type III is near 10%. Collagen type I fibers mainly provide structural support and give the heart properties that include stiffness and resistance to deformation. Collagen type III fibers seem to play an important role as a link between contractile elements of adjacent myocytes, carrying some information useful for cell function. In the infarcted zone the extracellular myocardial matrix is modified; collagen type I can decreases from 80% to 40% and collagen type III may increase from 10% to 35%, creating a pathological fibrosis, resulting in ventricular remodeling, dilation, and diastolic/systolic dysfunctions (1,13,21).

The present clinical study performed in ischemic patients showed that bone marrow cell therapy associated with the surgical implantation onto the epicardium of a cell-seeded collagen type I matrix prevented myocardial wall thinning, limited postischemic remodeling, and improved diastolic function. The use of this biomaterial seems to create a microatmosphere where the exogenous and endogenous cells find the optimal microenvironment to repair with low scar production.

We hypothesize that the favorable effects of a cell-seeded matrix may be attributed to several mechanisms. First, the collagen matrix may support cell retention and cell survival, stimulating some paracrine effects. The BMC seeded in the collagen matrix is probably incorporated into the hosting myocardium through the epicardial channels created at the level of the needle injection sites, where the infarcts have been directly treated by cells. Secondly, the cell-seeded matrix helps prevents apoptosis in ischemic myocardium. This antiapoptotic effect can be mediated by improvement of myocardial perfusion by angiogenesis induced by endothelial cell progenitors. The antiapoptotic effect may be also mediated by a mechanical effect due to decrease LV parietal stress related to the reduction in LV dimensions (12).

Cell-based regenerative therapy is undergoing experimental and clinical trials in order to limit the consequences of decreased contractile function and compliance of damaged ventricles following myocardial infarction or in patients presenting nonischemic dilated cardiomyopathies. This biological approach is particularly attractive due to the potential for myocardial regeneration with a variety of myogenic and angiogenic cell types: skeletal myoblasts, bone marrow-derived mesenchymal stem cells, circulating blood-derived progenitor cells, endothelial and mesothelial cells, adipose tissue stem cells, and potentially embryonic cells. Over 800 patients have been treated worldwide with cell-based procedures for myocardial regeneration (3).

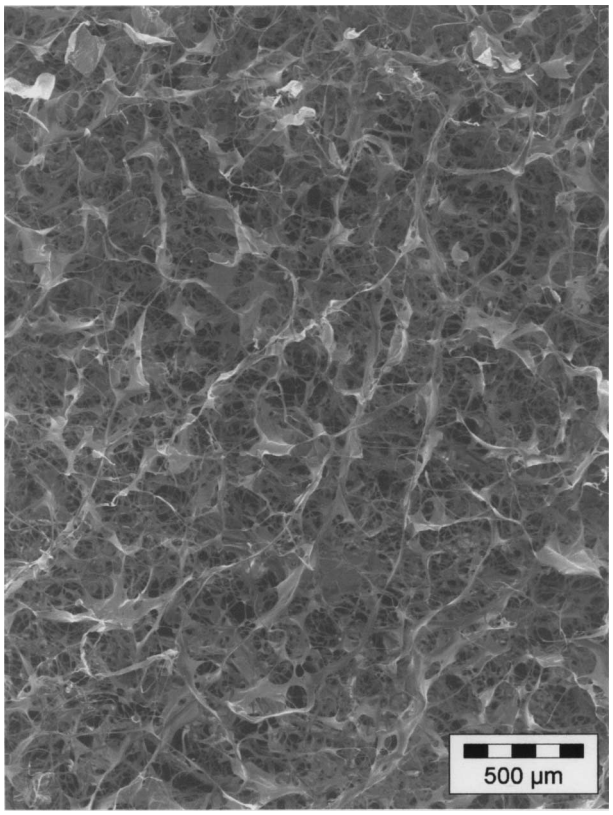

Figure 2. This image (scanning electron microscopy) shows the 3D arrangement of collagen fibers of the matrix used to be seeded with mononuclear bone marrow cells and grafted onto the LV infarcted wall.

The exact evolution of implanted cells into the host pathological myocardium has not been completely elucidated. Potentially this process may include transdifferentiation of transplanted stem cells (cellular plasticity), fusion of implanted cells with resident cells (chimerization), reactive angiogenesis or paracrine secretion of angiogenic growth factors, and modulation of inflammation at the level of the extracellular matrix (9,13,16,21).

Some published studies concerning cell-based regenerative therapy do not rule out objective effects of cell transplantation in patients with ischemic heart disease (11,20,22,28). Experiments to establish the existence of a pluripotent stem cell in adults are crucial. Currently, there is no clinical evidence of such a cell in adults. Moreover, large-scale studies are warranted to examine the potential effects of progenitor cell administration on morbidity and mortality. Further research is needed before this treatment can be recommended for patients with acute or chronic myocardial diseases (26).

Tissue engineering is an emerging field (starting in the 1970s) and is based on the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions. Probably the first definition of tissue engineering was by Langer and Vacanti (18), who stated it to be "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ."

Engineering materials can be implanted or "seeded" into a natural or synthetic structure capable of supporting three-dimensional tissue formation. These scaffolds are often critical, both ex vivo as well as in vivo, to recapitulating the in vivo milieu and allowing cells to influence their own microenvironments. Such scaffolds serve at least one of the following purposes: allow cell attachment and migration; deliver and retain cells and biochemical factors; enable diffusion of vital cell nutrients and expressed products; and exert certain mechanical and biological influences to modify the behavior of the cell phase. Specially designed bioreactor systems are used to manufacture tissue in vitro that will replace diseased tissue in vivo (7,8,14,34).

To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is essential because scaffolds need to be absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation; this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down, leaving the neotissue, newly formed tissue that will take over the mechanical load. Current clinical applications of tissue engineering include specialities such us neurosurgery, orthopedics, urology, dermatology, plastic surgery, dentistry, tracheal replacement, vascular surgery, and cardiac valvular surgery (19,31).

Our current study suggests that accelerated collagen accumulation caused by the epicardial placement of a cell-seeded type I collagen patch prevents myocardial wall thinning and limits postischemic remodeling. Collagen is one of the new biomimetic scaffold families that has now been commercialized and is impacting clinical tissue engineering. The functional integration of the collagen fibers provided by the patch into the infarcted area added at the physiological reparative fibrosis process may reinforce the infarcted LV wall in order to yield stress tolerance and limit ventricular expansion. The incorporation of new collagen fibers may improve LV failing heart matrix homeostasis by restoring deficient

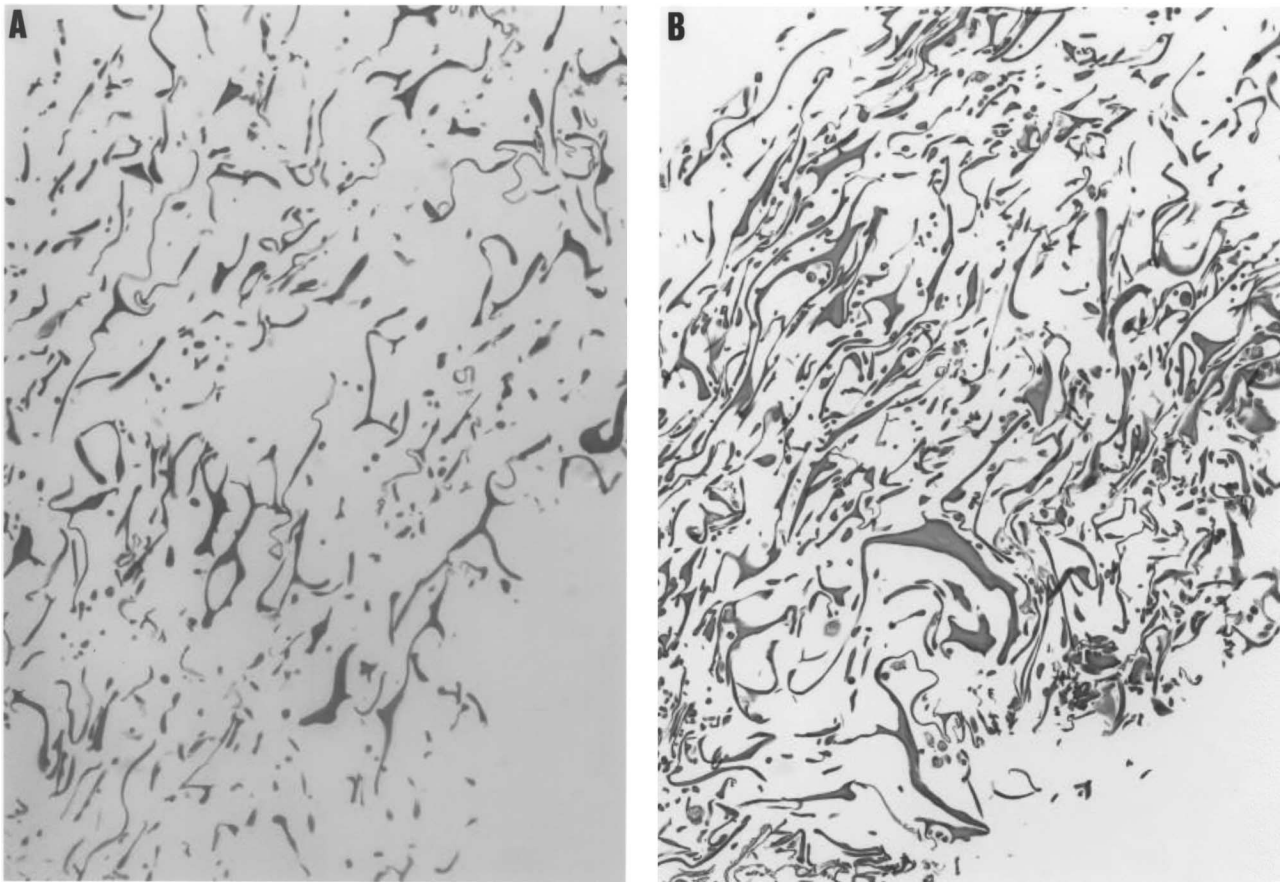

Figure 3. (A) Histological study of the original collagen type I matrix. Hematoxylin-eosin staining (original magnification 100×). (B) Histological study of the collagen matrix after bone marrow cells seeding. Hematoxylin-eosin staining (original magnification 100×).

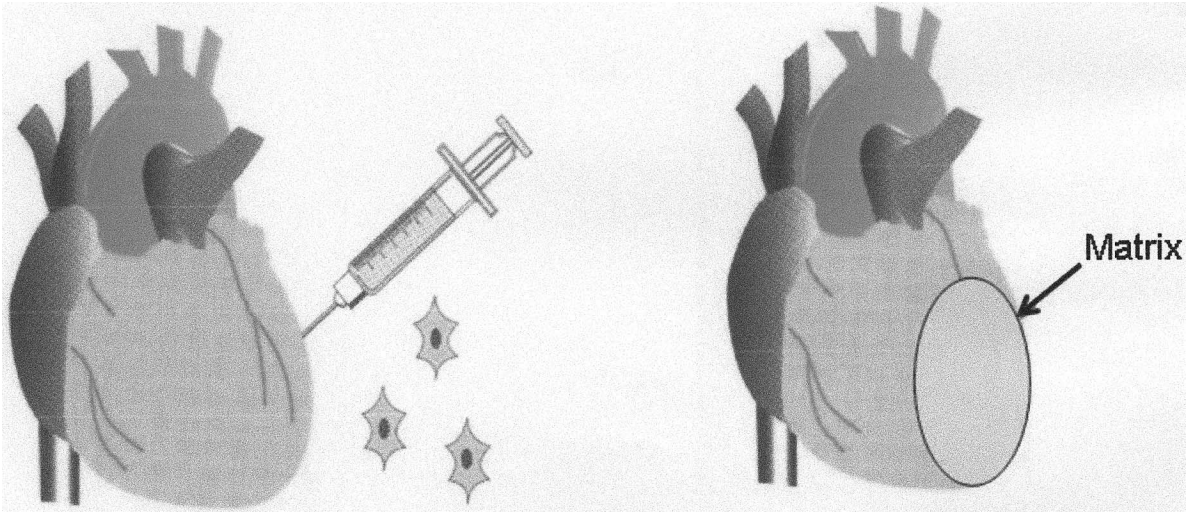

Figure 4. Surgical procedure associating the intrainfarct implantation of stem cells followed by the fixation of a cell-seeded matrix onto the epicardial surface.

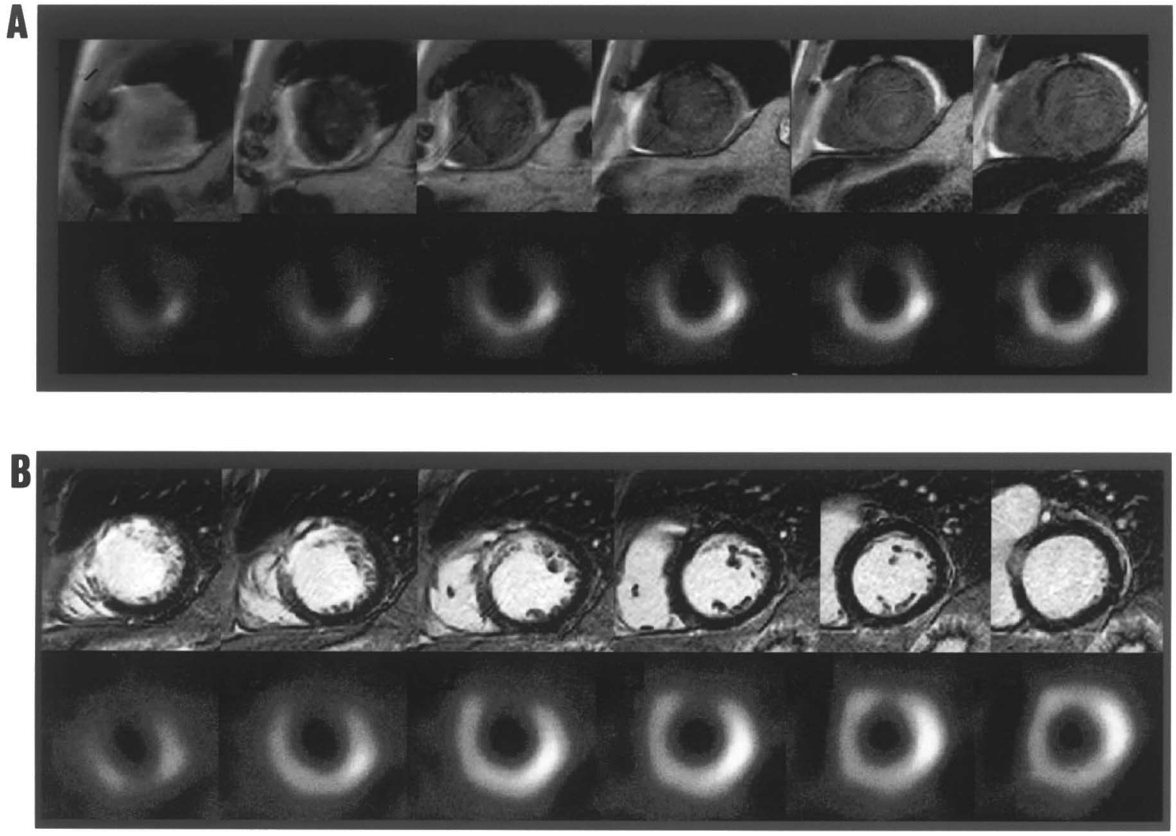

Figure 5. (A) Cellular cardiomyoplasty associated with the implantation of a cell-seeded matrix. MRI-SPECT studies at baseline showing a LV myocardial infarct extension of 64% (11/17 segments). (B) MRI-SPECT at 1 year follow-up, showing the reduction of the pathological area, now the myocardial infarct extension is of 41% (7/17 segments).

interstitial matrix components, and also by improving spatial organization of extracellular matrix elements and by limiting matrix degradation (5,17).

Another option in cardiac tissue engineering is to inject a laminin-collagen type IV gel (e.g., matrigel) to stiffen the infarct (15). However, currently available collagen gels are contraindicated for clinical use due to origin (EHS mouse sarcoma) and the toxicity of the solution components. An additional risk for this approach is the creation of a dissected area within the myocardial tissue, followed by a dead space after adsorption of the material. This situation could be compared with the noncompacted myocardial pathology (32).

The use of efficient scaffolds allows reestablishing a beneficial atmosphere for cell survival, multiplication, differentiation, and function. Interesting stem cells are in evaluation for myocardial support and regeneration (10,30). Cardiac tissue engineering (e.g., using collagen matrix seeded with stem cells) emerges as a new therapeutic tool and extends even more the amazing possibilities of cell therapies in cardiology, becoming a promising way for the creation of a "bioartificial myocardium."

PERSPECTIVES

Future matrix improvements would include the cross-linking of antiapoptotic and angiogenic factors (4); the cell microenvironment could be custom designed in order to obtain the releasing of determined factors or control cellular adhesion or mechanical function. Other matrix improvements include the decrease of inflammatory response and prolongation of the absorption delay by additional chemical and/or physical cross-linking treatments. Electrostimulation and shear stress could be incorporated to precondition matrices. Novel technologies, such as nanofabrication, molecular bioimaging, and genetic reprogramming, or cell fusion techniques will contribute to the development of this field (6,24).

ACKNOWLEDGMENTS: The authors would like to acknowledge J. Genovese, M.D., A. Patel, M.D., C. Spadaccio, M.D., and E. Chachques, B.S., from the McGowan Institute for Re-

generative Medicine (Pittsburg, PA, USA) and Marc Marie, M.D., from Urgo Laboratory (Chenove, France) for the histological studies of matrices and for the illustrations in Figures 1, 2, and 3.

REFERENCES

1. Brown, L. Cardiac extracellular matrix: A dynamic entity. Am. J. Physiol. Heart Circ. Physiol. 289:H973–974; 2005.
2. Chachques, J. C.; Acar, C.; Herreros, J.; Trainini, J. C.; Prosper, F.; D'Attellis, N.; Fabiani, J. N.; Carpentier, A. Cellular cardiomyoplasty: Clinical application. Ann. Thorac. Surg. 77:1121–1130; 2004.
3. Chachques, J. C.; Salanson-Lajos, C.; Lajos, P.; Shafy, A.; Alshamry, A.; Carpentier, A. Cellular cardiomyoplasty for myocardial regeneration. Asian Cardiovasc. Thorac. Ann. 13:287–296; 2005.
4. Chachques, J. C.; Duarte, F.; Cattadori, B.; Shafy, A.; Lila, N.; Chatellier, G.; Fabiani, J. N.; Carpentier, A. Angiogenic growth factors and/or cellular therapy for myocardial regeneration: A comparative study. J. Thorac. Cardiovasc. Surg. 128:245–253; 2004.
5. Cortes-Morichetti, M.; Frati, G.; Schussler, O.; Van Huyen, J. P.; Lauret, E.; Genovese, J. A.; Carpentier, A. F.; Chachques, J. C. Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration. Tissue Eng. 13:2681–2687; 2007.
6. Davis, M. E.; Motion, J. P.; Narmoneva, D. A.; Takahashi, T.; Hakuno, D.; Kamm, R. D.; Zhang, S.; Lee, R. T. Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. Circulation 111:442–450; 2005.
7. Eschenhagen, T.; Zimmermann, W. H. Engineering myocardial tissue. Circ. Res. 97:1220–1231; 2005.
8. Eschenhagen, T.; Zimmermann, W. H.; Kleber, A. G. Electrical coupling of cardiac myocyte cell sheets to the heart. Circ. Res. 98:573–575; 2006.
9. Fedak, P. W.; Szmitko, P. E.; Weisel, R. D.; Altamentova, S. M.; Nili, N.; Ohno, N.; Verma, S.; Fazel, S.; Strauss, B. H.; Li, R. K. Cell transplantation preserves matrix homeostasis: A novel paracrine mechanism. J. Thorac. Cardiovasc. Surg. 130:1430–1439; 2005.
10. Henning, R. J.; Burgos, J. D.; Ondrovic, L.; Sanberg, P.; Balis, J.; Morgan, M. B. Human umbilical cord blood progenitor cells are attracted to infarcted myocardium and significantly reduce myocardial infarction size. Cell Transplant. 15:647–658; 2006.
11. Janssens, S.; Dubois, C.; Bogaert, J.; Theunissen, K.; Deroose, C.; Desmet, W.; Kalantzi, M.; Herbots, L.; Sinnaeve, P.; Dens, J.; Maertens, J.; Rademakers, F.; Dymarkowski, S.; Gheysens, O.; Van Cleemput, J.; Bormans, G.; Nuyts, J.; Belmans, A.; Mortelmans, L.; Boogaerts, M.; Van de Werf, F. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet 367:113–121; 2006.
12. Jessup, M.; Brozena, S. Heart failure. N. Engl. J. Med. 348:2007–2018; 2003.
13. Jugdutt, B. I.; Joljart, M. J.; Khan, M. I. Rate of collagen deposition during healing and ventricular remodelling after myocardial infarction in rat and dog models. Circulation 94:94–101; 1996.
14. Kofidis, T.; de Bruin, J. L.; Hoyt, G.; Ho, Y.; Tanaka, M.; Yamane, T.; Lebl, D. R.; Swijnenburg, R. J.; Chang, C. P.; Quertermous, T.; Robbins, R. C. Myocardial restoration with embryonic stem cell bioartificial tissue transplantation. J. Heart Lung Transplant. 24:737–744; 2005.
15. Kofidis, T.; Lebl, D. R.; Martinez, E. C.; Hoyt, G.; Tanaka, M.; Robbins, R. C. Injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart following myocardial injury. Circulation 112(9 Suppl.):I173–177; 2005.
16. Kresh, J. Y. Cell replacement therapy: The functional importance of myocardial architecture and intercellular gap-junction distribution. J. Thorac. Cardiovasc. Surg. 131: 1310–1313; 2006.
17. Kutschka, I.; Chen, I. Y.; Kofidis, T.; Arai, T.; von Degenfeld, G.; Sheikh, A. Y.; Hendry, S. L.; Pearl, J.; Hoyt, G.; Sista, R.; Yang, P. C.; Blau, H. M.; Gambhir, S. S.; Robbins, R. C. Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. Circulation 114(1 Suppl.):I167–173; 2006.
18. Langer, R.; Vacanti, J. P. Tissue engineering. Science 260:920–926; 1993.
19. Leor, J.; Landa, N.; Cohen, S. Renovation of the injured heart with myocardial tissue engineering. Expert Rev. Cardiovasc. Ther. 4:239–252; 2006.
20. Lunde, K.; Solheim, S.; Aakhus, S.; Arnesen, H.; Abdelnoor, M.; Egeland, T.; Endresen, K.; Ilebekk, A.; Mangschau, A.; Fjeld, J. G.; Smith, H. J.; Taraldsrud, E.; Grøgaard, H. K.; Bjørnerheim, R.; Brekke, M.; Müller, C.; Hopp, E.; Ragnarsson, A.; Brinchmann, J. E.; Forfang, K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N. Engl. J. Med. 355: 1199–1209; 2006.
21. Lunkenheimer, P. P.; Redmann, K.; Westermann, P.; Rothaus, K.; Cryer, C. W.; Niederer, P.; Anderson, R. H. The myocardium and its fibrous matrix working in concert as a spatially netted mesh: A critical review of the purported tertiary structure of the ventricular mass. Eur. J. Cardiothorac. Surg. 29(Suppl. 1):S41–49; 2006.
22. Meyer, G. P.; Wollert, K. C.; Lotz, J.; Steffens, J.; Lippolt, P.; Fichtner, S.; Hecker, H.; Schaefer, A.; Arseniev, L.; Hertenstein, B.; Ganser, A.; Drexler, H. Intracoronary bone marrow cell transfer after myocardial infarction: Eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. Circulation 113: 1287–1294; 2006.
23. Patel, A. N.; Geffner, L.; Vina, R. F.; Saslavsky, J.; Urschel, Jr., H. C.; Kormos, R.; Benetti, F. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: A prospective randomized study. J. Thorac. Cardiovasc. Surg. 130:1631–1638; 2005.
24. Pedrotty, D. M.; Koh, J.; Davis, B. H.; Taylor, D. A.; Wolf, P.; Niklason, L. E. Engineering skeletal myoblasts: Roles of three-dimensional culture and electrical stimulation. Am. J. Physiol. Heart Circ. Physiol. 288:H1620–1626; 2005.
25. Rajnoch, C.; Chachques, J. C.; Berrebi, A.; Bruneval, P.; Benoit, M. O.; Carpentier, A. Cellular therapy reverses myocardial dysfunction. J. Thorac. Cardiovasc. Surg. 121: 871–878; 2001.
26. Schwartz, R. S. The politics and promise of stem-cell research. N. Engl. J. Med. 355:1189–1191; 2006.
27. Sherman, W.; Martens, T. P.; Viles-Gonzalez, J. F.; Simi-

niak, T. Catheter-based delivery of cells to the heart. Nat. Clin. Pract. Cardiovasc. Med. 3(Suppl. 1):S57–64; 2006.
28. Stamm, C.; Kleine, H. D.; Choi, Y. H.; Dunkelmann, S.; Lauffs, J. A.; Lorenzen, B.; David, A.; Liebold, A.; Nienaber, C.; Zurakowski, D.; Freund, M.; Steinhoff, G. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: Safety and efficacy studies. J. Thorac. Cardiovasc. Surg. 133:717–725; 2007.
29. Teng, C. J.; Luo, J.; Chiu, R. C.; Shum-Tim, D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: Implications for cellular cardiomyoplasty. J. Thorac. Cardiovasc. Surg. 132:628–632; 2006.
30. Tran, N.; Li, Y.; Maskali, F.; Antunes, L.; Maureira, P.; Laurens, M. H.; Marie, P. Y.; Karcher, G.; Groubatch, F.; Stoltz, J. F.; Villemot, J. P. Short-term heart retention and distribution of intramyocardial delivered mesenchymal cells within necrotic or intact myocardium. Cell Transplant. 15:351–358; 2006.
31. Vacanti, C. A. History of tissue engineering and a glimpse into its future. Tissue Eng. 12:1137–1142; 2006.
32. Weiford, B. C.; Subbarao, V. D.; Mulhern, K. M. Noncompaction of the ventricular myocardium. Circulation 109:2965–2971; 2004.
33. Wollert, K. C.; Drexler, H. Clinical applications of stem cells for the heart. Circ Res. 96:151–163; 2005.
34. Zimmermann, W. H.; Melnychenko, I.; Wasmeier, G.; Didié, M.; Naito, H.; Nixdorff, U.; Hess, A.; Budinsky, L.; Brune, K.; Michaelis, B.; Dhein, S.; Schwoerer, A.; Ehmke, H.; Eschenhagen, T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat. Med. 12:452–458; 2006.