most probably need a graft different from one destined to replace failing myocardium in a 75-year-old patient.

5. With respect to the cells, Wagers and associates[5] have demonstrated that bone marrow stem cells have little developmental plasticity. Most of the tissue engineering approaches with bone marrow stem cells use whole bone marrow, rather than specific subpopulations of it, and they frequently lack identification of the inoculated cells because of missing labeling and reliable colocalization studies. What are we implanting? What happens to each particular cell population, and to what extent do these cells transdifferentiate into cardiomyocytes? Studies with myoblasts do no better. Reliability and interpretability of the results would be significantly enhanced if cell labeling and tracking methods would be used routinely. Some worth mentioning are the green fluorescent protein or carboxyfluorescein diacetate succinimidyl ester methods, the membrane fluorescent intercalated dye pkh26-gl method, and colocalization or confocal studies to identify cell identity, location, differentiation status and host immune response (Figure 1).

The commentary of this group will certainly initiate fruitful discussion on basic requirements for future scientific approaches to restoring injured myocardium.

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References

1. Osawa T, Mickle D, Weisel R, Koyama, N, Wong H, Ozawa S, Li RK. Histologic changes of nonbiodegradable and biodegradable biomaterials used to repair right ventricular heart defects in rats. *J Thorac Cardiovasc Surg*. 2002;1241157-63.
2. Yau TM, Tomita S, Weisel RD, Jia ZQ, Tumiati LC, Li RK. Beneficial effect of autologous cell transplantation on infarcted heart function: comparison between bone marrow stromal cells and heart cells. *Ann Thorac Surg*. 2003;75:169-77.
3. Eschenhagen T, Didie M, Heubach J, Ravens U, Zimmermann WH. Cardiac tissue engineering. *Transplant Immunol*. 2002;9:315-21.
4. Buckberg GD. Basic science review: the helix and the heart. *J Thorac Cardiovasc Surg*. 2002;124:863-83.
5. Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science*. 2002;27:2256-9.

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Reply to the Editor:

The letter of Kofidis and colleagues from Stanford University highlights several key issues that face regenerative medicine as it applies to the heart. Their insightful perspective will help spawn the critical discussion that is required to fulfill the promise of restoring cardiac structure and function after cardiac injury. Although we agree that engineered biomaterial cell-seeded patches and cellular transplantation are developing, less than a decade ago heart regeneration was considered science fiction. We hope that this discussion will generate more directed research in this emerging discipline.

With respect to cardiac geometry, living ventricular muscle constitutes a complex helical structure. It will be ideal to repair myocardial scar tissue or ventricular defects with asymmetric and anisotropic muscle tissue. However, scar tissue at site of reconstruction of ventricular aneurysm and synthetic material used for repair of congenital defects is not helical and lacks contractility and growth potential. An autologous cell-seeded conduit grows with the child[1] when used during correction of congenital defects and may reduce the need for reoperation. Similarly, in large ventricular aneurysms in which resection needs to be complemented with a patch, autologous cell-seeded biomaterials[2] may be preferable to the static materials currently used, such as pericardium, polytetrafluoroethylene, and Dacron polyester fabric.

Tissue engineering with cell-seeded biodegradable materials is a novel technology that allows the growth of muscle cells in three dimensions to regenerate injured muscle. The biodegradable material does not repair injured heart segments; rather, the biomaterial gradually degrades, and it is the cells in the biomaterial that secrete extracellular matrix proteins, stimulate angiogenesis, and form tissue resembling myocardial tissue.[3,4] The resulting tissue is fed by neovasculature and consists of muscle as well as a complex matrix that interdigitates with the host myocardium. Although the graft is initially isotropic, it mediates an increase in elasticity and strength of the scar tissue, prevents scar expansion, and halts ventricular dilation.[2] In addition, the engineered graft will remodel in response to ventricular pressure and stretch. Cellular hyperplasia and hypertrophy will enhance the strength of the graft as the biomaterial degrades. Importantly, the new muscle tissue has growth potential and would reduce the necessity for reoperation.[1,3,5]

With respect to cardiac hemodynamics, early biomaterials were unable to withstand wall stress of the left ventricle. However, modification of the biomaterials has increased their strength. We have evaluated[2,4] a composite biomaterial consisting of a spongy central core made of 50% ϵ-caprolactone and 50% L-lactic acid that encourages tissue ingrowth. The outer layers are reinforced with knitted poly-L-lactide fabric, which provides sufficient strength to prevent disruption when placed in the left ventricle. The exciting feature of this new biomaterial is that the spongy portion, which supports the initial cell inoculum, is absorbed within 2 months and the fibrous portion, which provides the strength, persists for 1 to 2 years. We have shown that smooth muscle cells seed and survive within this material in vitro and form muscle tissue in vivo.[3] The modified biomaterial continued to withstand right ventricular pressures at 6 months after implantation.[5] We also used the cell-engineered graft (poly-L-lactide fabric with smooth muscle cells) to repair transmural defects of left ventricular free wall in adult rat hearts after myocardial infarction. The graft withstood left ventricular pressures and prevented ventricular dilation in this model.[2] A similar material was used as an autologous cell-seeded patch in the repair of a congenital defect.[1]

These promising results will need to be evaluated in larger animal models that require thicker and more extensive grafts. The thicker grafts may be limited by the extent of tissue ingrowth in vitro and of neovascularization in vivo. The use of decellularized tissue provides the promise of a preformed vascular supply. However, the existing structure of the material will be quickly dissolved by the remodeling pro-

cess, and cell survival will depend on the graft's ability to attract neovascularization. Whereas the field of therapeutic angiogenesis remains nascent, both mechanical support and vascular supply to the engineered graft could be enhanced by surgical strategies such as the placement of a pedicled muscular graft around the biomaterial.

With respect to microscopic structure, the microscopic appearance before implementation is rapidly altered by the remodeling process after engraftment. Preformed channels for nerves, arterioles, and lymphatics are rapidly replaced by host and transplanted cells, remodeling the region in response to chemical, electrical, physical, and hemodynamic stimuli.

With respect to electrical properties, those of the newly formed muscle tissue require further characterization. Skeletal myoblast implantation resulted in an increased risk of ventricular arrythmia after bypass grafting and autologous cell transplantation in patients with ischemic cardiomyopathy.[6] Although this presents a hurdle to be overcome, it also provides the potential to convert electrically inert scar to tissue that is capable of passively conducting action potentials. Indeed, preliminary experiments in our laboratory have demonstrated that a QRS complex can be detected in a cell-transplanted scar where it had been electrically silent before implantation (unpublished observations). The electrical resynchronization may improve systolic performance, as reported from many centers, in the absence of myogenesis. Similar to the process by which multisite pacing has been shown to improve systolic function,[7] cell transplantation may improve hemodynamics by allowing the entire myocardium to beat in unison again.

With respect to issues of storage, conservation, and scale, we agree that a child with a congenital defect will need a different graft than a 75-year-old patient with a ventricular aneurysm. There is no question that a variety of grafts need to be developed to tailor the surgical strategy to the patient. The autologous cell-seeded tissue engineered patch uses the component cells for repair. Aside from the choice of biomaterial, therefore, the tissue-engineered graft can be seeded with various combinations of cell types.

With respect to the cells, issues vary by cell type. Because congenital cardiac defects can be diagnosed in the fetal stage, umbilical cord blood cells could be used to engineer tissue.[8,9] Experience with this cell type, however, is limited.

Bone marrow contains stem cells that can differentiate into myogenic cells with proper induction.[10,11] In fact, bone marrow mesenchymal stem cells have extensive plasticity and have the potential to recreate all of the cell types in the heart. Because the tissue is easily obtained, these cells are excellent candidates for autologous tissue engineering.

Skeletal myoblasts have been used for cell transplantation to repair damaged hearts.[6] The animal data and clinical trials have been relatively encouraging. The implanted cells formed muscle tissue in the myocardial scar tissue and improved heart function. These cells can certainly be used for autologous tissue engineering.

Smooth muscle cells represent another candidate cell type for tissue engineering. The smooth muscle cells can be harvested from a number of sources in the patient to construct an autologous cell patch. The cells are easily cultured, should induce angiogenesis in the graft, and can respond to in vivo mechanical stretch by hyperplasia and hypertrophy to prevent patch dilatation and thinning.[2,5]

With respect to cell fate, the fate of the transplanted cells and the mechanism by which they enhance myocardial function remain largely unknown. We agree with Kofidis and colleagues that appropriate tracking methods, such as those presented in their letter, will aid in answering these questions.

An equally important issue is the role that the host's immune response plays in the field of cell transplantation and biomaterial engineering. This has been an oft neglected issue. Recent evidence from our laboratory indicates that even transplantation of syngeneic cells grown in vitro for 2 weeks elicits a response that includes T- and B-cell infiltration (unpublished observations). The appropriate modulation of the immune response may improve the already promising results of cell transplantation.

In closing, we are grateful to Kofidis and colleagues for bringing these important issues to critical discussion. The area of regenerative medicine is promising and in our mind forms the basis for our future therapies. Although we have a long way to go, we must not forget that we have come an even longer way, in spite of the Achilles heels.

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References

1. Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med*. 2001;344:532-3.
2. Matsubayashi K, Fedak PW, Mickle DA, Weisel RD, Ozawa S, Li RK. Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. *Circulation.* 2003;108(10 Suppl 1):II219-25.
3. Ozawa T, Mickle DA, Weisel RD, Koyama N, Wong H, Ozawa S, et al. Histologic changes of nonbiodegradable and biodegradable biomaterials used to repair right ventricular heart defects in rats. *J Thorac Cardiovasc Surg*. 2002;124:1157-64.
4. Ozawa T, Mickle DA, Weisel RD, Koyama N, Ozawa S, Li RK. Optimal biomaterial for creation of autologous cardiac grafts. *Circulation*. 2002;106(12 Suppl 1):I176-82.
5. Ozawa T, Mickle DA, Weisel RD, Matsubayashi K, Fujii T, Fedak PW, Koyama N, et al. Tissue engineered grafts matured in the right ventricular outflow tract. *Cell Transplant.* In press.
6. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol*. 2003;41:1078-83.
7. Yu CM, Lin H, Fung WH, Zhang Q, Kong SL, Sanderson JE. Comparison of acute changes in left ventricular volume, systolic and diastolic functions, and intraventricular synchronicity after biventricular and right ventricular pacing for heart failure. *Am Heart J*. 2003;145:E18.
8. Hoerstrup SP, Kadner A, Breymann C, Maurus CF, Guenter CI, Sodian R, et al. Living, autologous pulmonary artery conduits tissue engineered from human umbilical cord cells. *Ann Thorac Surg*. 2002;74:46-52.
9. Kadner A, Hoerstrup SP, Tracy J, Breymann C, Maurus CF, Melnitchouk S, et al. Human umbilical cord cells: a new cell source for cardiovascular tissue engineering. *Ann Thorac Surg*. 2002;74:S1422-8.
10. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg*. 2002;73:1919-25.
11. Tomita S, Mickle DA, Weisel RD, Jia ZQ,

Tumiati LC, Allidina Y, et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. *J Thorac Cardiovasc Surg*. 2002;123:1132-40.

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Coagulation, fibrinolysis, and cell activation in patients and in shed mediastinal blood during coronary artery bypass grafting with a new heparin-coated surface

To the Editor:

Johnell and colleagues[1] recently reported the improved biocompatibility of a new heparin-coated surface. They also presented results after modifications of the anticoagulation protocol. They concluded that a low dose of systemic heparin might not be sufficient to maintain the antithrombotic activity and that a high dose resulted in increased blood activation.

The design of the study merits discussion; otherwise, the conclusion might be misunderstood. In particular, the reduction of anticoagulation, even combined with a thromboresistant extracorporeal circuit, might appear detrimental for patients undergoing cardiopulmonary bypass (CPB). The basis for a safe low anticoagulation protocol in combination with heparin-coated CPB circuits was described by Aldea and associates[2] in 1998. It includes many parameters derived from the scientific literature from the 1990s, such as controlled suction with cell-saving devices allowing washing before retransfusion, limitation of air-blood contact with closed circuits, specifically adapted anticoagulation through precise heparin and protamine titration, low prime volume facilitated by retrograde autologous prime, and normothermia. This tailored approach of CPB, adopted by our own center, has been found clinically beneficial[3] and is therefore justified for routine surgical practice. One of the most important parameters omitted by the authors in the management of a low heparinized CPB is the retransfusion of highly activated blood into the circulation because cardiotomy suction has been used. The contact of blood with air or with the surgical field through the tissue pathway is followed by activation of inflammation and hemostasis disturbances, despite the use of a significant amount of fluid heparin, which appears to be an unperfected anticoagulant for CPB although it is universally used. When a cell-saving device is used instead of cardiotomy suction, all the different markers of blood activation are excluded and not retransfused into the circulation. With this approach, it has been proved that circulating F_{1+2} levels do not correlate with activated clotting time (the lowest activated clotting time does not imply the highest F_{1+2}) and that a low anticoagulation protocol is safe for the patient.[2] Therefore some of the results presented by Johnell and colleagues[1] need to be taken with caution.

On the other hand, the study of Johnell and colleagues[1] provides important data about the detrimental effects of heparin, which could justify a low anticoagulation protocol. Evidence of proinflammatory and procoagulant effects of high-dose heparin was found, as in previous reports. The reduction of heparin dose during CPB with heparin-coated circuits reduces leukocyte adhesion on artificial surfaces[4] and better preserves antithrombin III levels.[5] In addition, low anticoagulation requires a low dose of protamine for titration and reduces the amount of heparin-protamine complexes known to activate the classic pathway of complement cascade.

The biocompatibility of new equipment must be assessed, particularly with respect to thromboresistance. Johnell and colleagues[1] successfully demonstrated the reduction of contact activation. However, it is fundamental that the experimental design respect some principles that have been previously elucidated when they are expected to be of major importance in the outcome. This is particularly true if CPB is managed with low anticoagulation.

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References

1. Johnell M, Elgue G, Larsson R, Larsson A, Thelin S, Siegbahn A. Coagulation, fibrinolysis, and cell activation in patients and shed mediastinal blood during coronary artery bypass grafting with a new heparin-coated surface. *J Thorac Cardiovasc Surg*. 2002;124:321-32.
2. Aldea GS, O'Gara P, Shapira OM, Treanor P, Osman A, Patalis E, et al. Effect of anticoagulation protocol on outcome in patients undergoing CABG with heparin-bonded cardiopulmonary bypass circuits. *Ann Thorac Surg*. 1998;65:425-33.
3. Baufreton C, de Brux JL, Binuani P, Corbeau JJ, Subayi JB, Daniel JC, et al. A combined approach for improving cardiopulmonary bypass in coronary artery surgery: a pilot study. *Perfusion*. 2002;17:407-13.
4. Nakajima T, Kawazoe K, Ishibashi K, Kubota Y, Sasaki T, Izumoto H, et al. Reduction of heparin dose is not beneficial to platelet function. *Ann Thorac Surg*. 2000;70:186-90.
5. Ranucci M, Cazzaniga A, Soro G, Isgro G, Frigiola A, Menicanti L. The antithrombin III–saving effect of reduced systemic heparinization and heparin-coated circuits. *J Cardiothorac Vasc Anesth*. 2002;16:316-20.

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Myogenesis after myocardial stem cell transplantation

To the Editor:

I read the article of Chedrawy and colleagues,[1] "Incorporation and Integration of Implanted Myogenic and Stem Cells Into Native Myocardial Fibers: Anatomic Basis for Functional Improvements," in the Journal with great interest. Chedrawy and colleagues[1] described regenerated isogenic myofebrile grafts injected into nonischemic normal myocardium. By 4 to 6 weeks, fully differentiated myocytes could be seen to interconnect among native cardiomyocytes. The authors attributed this desirable incorporation to cell-cell electromechanical junction. However, in a clinical setting with scar tissue and akinetic ventricular wall, the microenvironment is not conducive to electromechanical activity to induce a desirable environment to trigger phenotypic changes for transplanted cells. Is it possible that cytokines, such as transforming growth factor β or insulin growth factor, generated because of the presence of macrophage and monocytes, play a role in the genesis of proliferation and transformation of new myofibrils?

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Reference

1. Chedrawy EG, Wang JS, Nguyen DM, Shum-Tim D, Chiu RC. Incorporation and integration of implanted myogenic and stem