

Muscle Cell Grafting for the Treatment and Prevention of Heart Failure

CHARLES E. MURRY,*† MARSHA L. WHITNEY,*† HANS REINECKE*

Seattle, Washington

ABSTRACT

Background: The review aims to highlight recent advances in cardiac and skeletal muscle cell grafting for myocardial infarct repair.

Results: Fetal and neonatal cardiomyocytes form new myocardium in normal or injured hearts, and this new myocardium differentiates toward an adult phenotype. Unfortunately, formation of new myocardium is limited by graft cell death, in large part because of ischemic injury. In contrast, skeletal myoblasts are ischemia-resistant and form larger grafts of mature skeletal muscle in the injured heart. Although contractile under field stimulation, skeletal muscle grafts do not express gap junction proteins and remain electrically insulated, suggesting they may not beat with host myocardium. When placed in coculture, however, cardiac and skeletal muscle form synchronously beating networks, where cardiomyocytes capture and pace skeletal muscle cells via intercalated disk-like structures containing gap junctions. This suggests that engineering skeletal muscle to express gap junction proteins in vivo may induce similar coupling with host myocardium. One major challenge to myocardial repair is getting sufficient graft cell mass without risking a tumor-like overgrowth. Recent experiments suggest it may be possible to control skeletal muscle graft size using a small, synthetic ligand, which activates the fibroblast growth factor signaling pathway only in genetically modified graft cells. Finally, a review of functional studies is presented that provides clear evidence that skeletal myoblast grafting is beneficial by limiting remodeling of the heart after infarction.

Conclusion: Given that clinical trials of skeletal myoblast grafting for myocardial repair are under way, it will be critically important to determine if these cells beat after grafting in the heart.

Key Words: Myocardial infarction, cell transplantation, skeletal myoblast, cell death, cell proliferation, gap junction

Myocardial infarction and subsequent heart failure can be viewed as a disease of cellular deficiency. It has long

been recognized that there is no functional regeneration of the myocardium after infarction.¹ Inability of the heart to regenerate stems from two factors: the apparent absence of a myogenic stem cell component in the infarct and the inability of surviving cardiomyocytes to reenter the cell cycle.² It has been suggested in studies that there may be a stem cell population that contributes new myocytes after infarction,³⁻⁶ and, similarly, that there can be limited DNA synthesis in surviving myocytes.⁷ If such responses truly exist, they clearly are physiologically insignificant in comparison to the mechanical requirements of the infarcted heart. For this reason, our group and others have explored cellular grafting as a strategy for repairing the infarcted heart.

*From the *Departments of Pathology and †Bioengineering, University of Washington School of Medicine, Seattle, Washington.*

These studies were supported, in part by NIH grants K08 HL03094, P01 HL03174, R01 HL61553, and R24 HL64387. Many of these studies were performed while Dr. Murry was the recipient of a Burroughs Wellcome Career Award in the Biomedical Sciences.

Reprint requests: Charles E. Murry, MD, PhD, Department of Pathology, Box 357470, Room D-514 HSB, University of Washington, Seattle, WA 98195.

Copyright 2002, Elsevier Science (USA). All rights reserved.

1071-9164/02/0806-0056\$35.00/0

doi:10.1054/jcaf.2002.129268

Several strategies for using cell grafts to repair the heart may be envisioned. The most intuitive is placing new muscle tissue into the infarct to restore systolic wall motion. This is clearly the most ambitious strategy and may take the longest time to achieve. A second strategy is the use of cellular grafts to induce angiogenesis. This strategy might be used after an acute infarction or to treat chronic ischemic heart disease. Finally, cellular grafting may be used to change the passive mechanical properties of the infarcted wall (e.g., wall thickness or compliance) to attenuate ventricular dilation and other adverse consequences of remodeling. This review will focus on the use of committed cardiac and skeletal muscle cells for myocardial repair.

Cardiac Myocyte Grafting

It seems logical that cardiac myocytes would be the best cell type to repair a myocardial infarct. Initial studies from Field's group⁸ generated significant excitement after they demonstrated that cardiomyocytes from fetal mice formed viable grafts after injection into normal myocardium of syngeneic hosts. Electron microscopic analysis showed formation of intercalated disks, complete with gap junctions, between graft and host cardiomyocytes. They subsequently showed successful engraftment using fetal canine cardiomyocytes into normal adult dog hearts,⁹ suggesting that this strategy might work for much larger hearts as well. Finally, they showed that mouse cardiomyocytes derived from embryonic stem cells formed stable grafts after injection into normal mouse hearts.¹⁰

Cardiomyocyte Grafts in Injured Hearts

Although the behavior of cardiomyocyte grafts in normal hearts appeared straightforward, their biology in the injured heart was less clear. Several groups reported that rat and fetal human cardiomyocytes could be grafted into infarcted or cryoinjured hearts,¹¹⁻¹⁴ whereas Watanabe and colleagues¹⁵ reported that fetal and neonatal pig cardiomyocytes died after implantation. Furthermore, even the studies reporting survival disagreed as to whether the graft cells differentiated normally. To sort out key variables, our group systematically surveyed how the developmental stage of implanted cardiomyocytes and the status of the recipient heart tissue (normal myocardium, acute necrosis, healing granulation tissue) influenced the success of grafting.¹⁶ We found that both fetal and neonatal rat cardiomyocytes formed viable grafts in normal myocardium, acutely necrotic myocardium, or 1-week-old granulation tissue. In contrast, adult

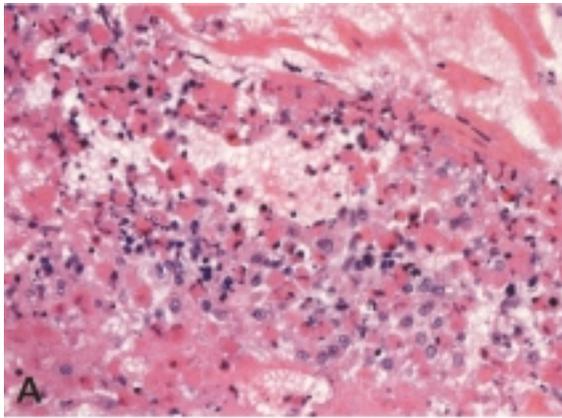
cardiomyocytes died irrespective of the type of tissue into which they were grafted.

A detailed time course analysis of neonatal cells implanted into acutely cryoinjured hearts revealed that the grafted cells underwent a normal differentiation program, including hypertrophy and formation of intercalated disks (Fig. 1, A-D). There was limited cardiomyocyte proliferation after grafting, with a peak PCNA (a cell cycle-associated antigen) index of 2%, 6 days after grafting. At early times after grafting (6 days) it was possible to demonstrate gap junctions between graft and host cardiomyocytes, suggesting electromechanical coupling. At later times, however, the grafted cardiomyocytes were more commonly separated from host myocardium by scar tissue.

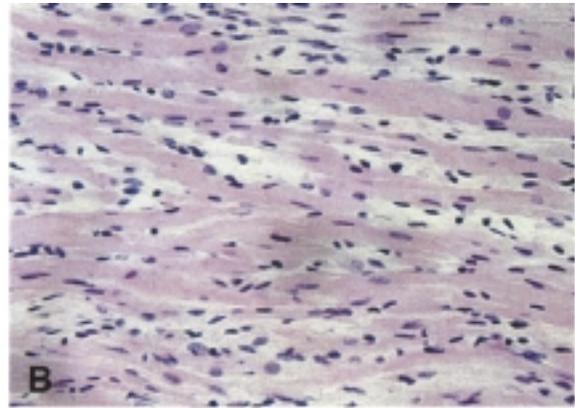
These studies showed that cardiomyocyte grafting could generate new, normal-appearing myocardium in injured hearts. This suggested that such an approach could improve myocardial function after infarction. To repair an infarct, however, grafts should replace a substantial fraction of the lost myocardium and ideally should be coupled to the host tissue for synchronous contraction. Li and colleagues¹¹ reported that cardiomyocyte grafting improved global ventricular function after cryoinjury in an isovolumic Langendorff preparation. Histology showed that the grafts were quite small compared to the lost myocardium and that the grafts were completely insulated from host myocardium by scar tissue. Thus their shift of the ventricular pressure-volume curve toward normal likely resulted from reduced ventricular dilation rather than restoration of systolic wall motion in the injured wall.

Cardiomyocyte Graft Cell Death

In an attempt to generate larger grafts in acutely cryoinjured rat hearts, we performed a dose-escalation study ranging from 3 to 25 million neonatal cardiomyocytes.¹⁷ (For reference, the normal adult rat heart contains ~20 million cardiomyocytes.¹⁸) Unfortunately, all grafts were small (<2.5% of left ventricular mass at 1 week), and there was no increase in graft size with increasing cell dose. This indicated that cell death was likely limiting the amount of new myocardium formed. Cell death was studied using terminal transferase UTP nick end-labeling (TUNEL) staining for DNA fragmentation and a constant dose of 5 million neonatal cardiomyocytes. This showed that, although cells were viable when injected, 32% were TUNEL-positive 1 day later (Fig. 1, E-G). TUNEL staining declined to 10% of graft cells at 4 days and was below 1% at 7 days. If one assumes that cells remain TUNEL-positive for 6 or 12 hours, this translates to 1 week survival of 1% or 10% of graft cells, respectively.

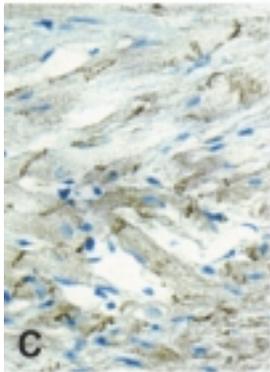


H&E, Day 1

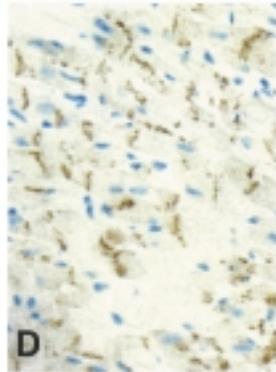


H&E, 2 Months

Cell Junctions, 2 Months

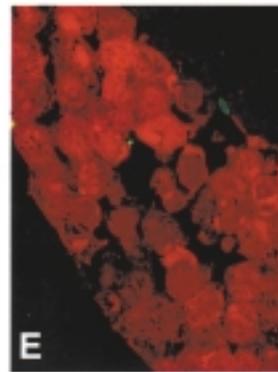


N-Cadherin

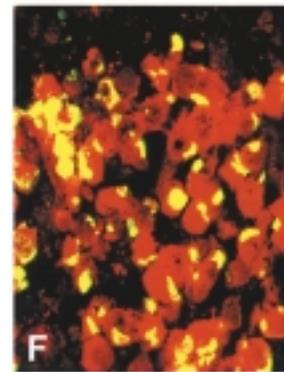


Connexin43

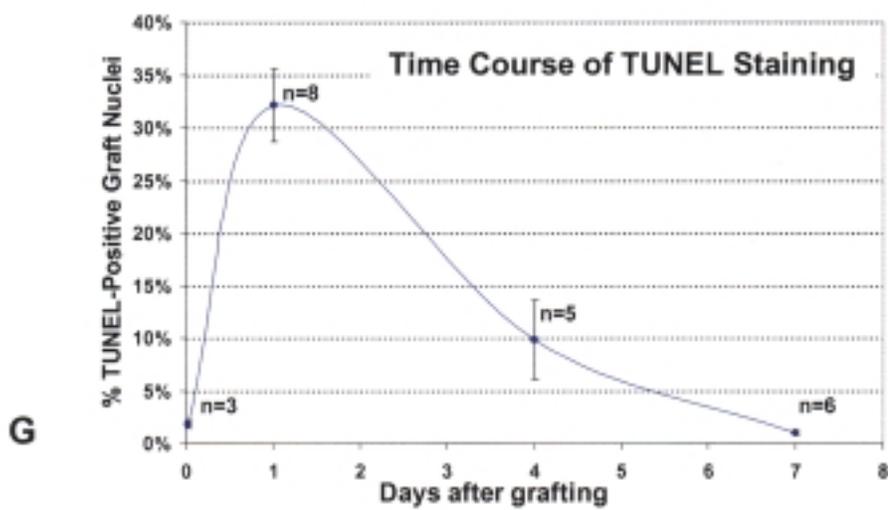
TUNEL/Orange Cell Tracker



30 min



18 Hours



To test whether ischemia contributed to poor cell survival, grafts were placed into highly vascularized 2-week-old cardiac granulation tissue or into normal myocardium. TUNEL staining 1 day after grafting was reduced by 53% in granulation tissue and by 86% in normal myocardium. This supports the hypothesis that ischemia plays a major role in causing cell death, although it does not rule out a contribution of acute inflammation. Modest benefits in survival were noted when the cytoprotective kinase, Akt, was overexpressed in the graft cells using adenoviral transduction. In contrast, heat shocking cardiomyocytes 1 day before grafting reduced TUNEL staining by 54%. Heat shock clearly induces multiple protective pathways (e.g., chaperones, antioxidants) and is therefore an imprecise intervention in mechanistic terms. On the other hand, it does provide a simple means of increasing graft cardiomyocyte survival.

In summary, these experiments show that it is possible to generate new myocardium by grafting cardiomyocytes into the injured heart. The amount of new myocardium that can be formed by this approach, however, is limited by cell death. That the fraction of dying cells increases with increasing cell number suggests that the cells are competing for limited resources (e.g., oxygen, survival factors). Current evidence points to ischemic injury as the principal culprit in causing cell death. Other paths to death, including inflammation, loss of matrix attachments (anoikis), or other apoptotic stimuli are also possible contributors to the poor survival of grafted cardiomyocytes.

Skeletal Muscle Grafting

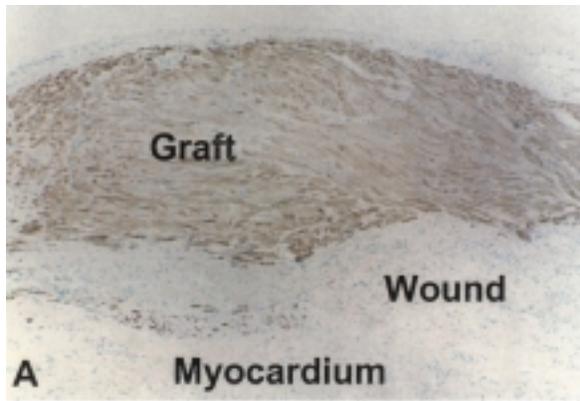
Because of some of the limitations of cardiomyocytes, our group and others have studied skeletal muscle as a repair cell for the infarcted heart. Before describing these studies, it is worthwhile to review a few points of basic skeletal muscle biology.¹⁹ Mature skeletal muscle fibers originate from undifferentiated, mononucleated progeni-

tor cells, which are termed myoblasts. Myoblasts proliferate in response to local mitogens, such as fibroblast growth factor (FGF) family members. When local growth factors are depleted, myoblasts withdraw irreversibly from the cell cycle, activate expression of muscle-specific genes (e.g., actins, myosins, creatine kinase) and fuse to form multinucleated cells called myotubes. Myotubes undergo progressive maturation and hypertrophy to form differentiated myofibers characteristic of adult skeletal muscle. Not all myoblasts fuse into myotubes, however. Rather, some become quiescent stem cells, or satellite cells, residing in close apposition to the muscle fiber. Satellite cells can reenter the cell cycle in response to muscle injury and are responsible for the ability of skeletal muscle to regenerate. No comparable cell population has been found in the heart. Several clinical trials are under way to determine whether autologous satellite cell/myoblast grafts in the heart are an effective strategy for myocardial infarct repair.²⁰

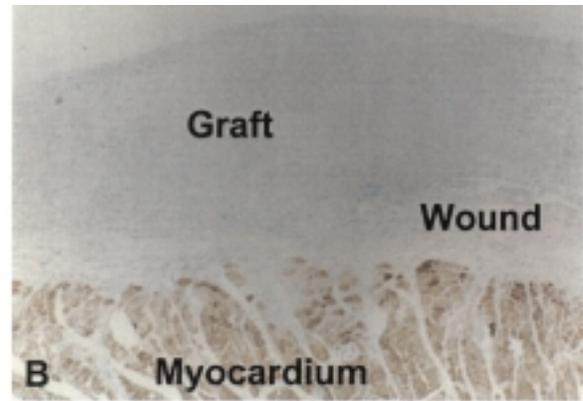
Differentiation, “Transdifferentiation,” and Electromechanical Coupling

In contrast to cardiac myocytes, skeletal muscle cells are among the most ischemia-tolerant in the body and, consequently, are capable of forming large grafts in the injured heart (Fig. 2A).²¹ When injected into acutely cryoinjured myocardium, skeletal myoblasts proliferate for up to 3 days and then differentiate to form multinucleated myotubes. Despite their ectopic location, the skeletal muscle cells undergo a normal maturation process, eventually forming hypertrophic cells with peripherally located nuclei. Several investigators have proposed that skeletal muscle cells will transdifferentiate into cardiomyocytes after cardiac engraftment.²²⁻²⁵ Our group has looked carefully at this question in several studies, most recently using BrdU prelabeling of the

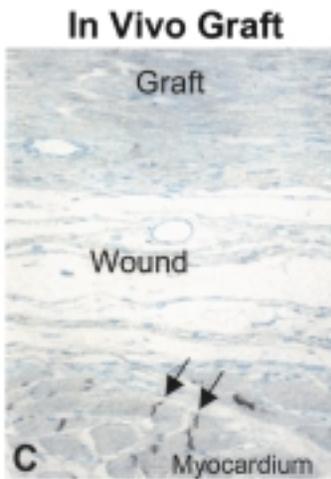
Fig. 1. Cardiomyocyte grafting for myocardial repair. Adult rat hearts were injured by a cryoprobe and 5 million syngeneic neonatal cardiomyocytes injected into the lesion immediately thereafter. (A) At 1 day after grafting the graft cardiomyocytes were round, undifferentiated-looking cells contained within the necrotic host myocardium. Note that some of the cells have plump nuclei while others have condensed, shrunken nuclei. (B) At 2 months the graft cardiomyocytes had rod-shaped morphology and formed interconnecting fibers, similar to normal host myocardium. (C, D) At 2 months the graft cardiomyocytes were coupled with one another by intercalated disks, containing N-cadherin and connexin43. (E, F) To study graft cell death, cells were pre-labeled with Orange Cell Tracker (Molecular Probes) and a fluorescent green TUNEL reaction performed to quantify DNA fragmentation. (The TUNEL reaction appears yellow in the merged, confocal image.) At 30 minutes after grafting, the cells had no DNA fragmentation, whereas by 18 hours there was extensive DNA fragmentation. (G) Time course of TUNEL staining. Cell counts were performed to quantify the amount of cell death after grafting. At 1 day ~32% of graft cells were TUNEL-positive. The incidence of cell death declined to 10% at day 4 and to 1% at day 7. Data are means \pm SEM. (A)-(D) reproduced from reference number 16; (E)-(G) reproduced from reference number 17.



A Emb. Skeletal Myosin, 7W



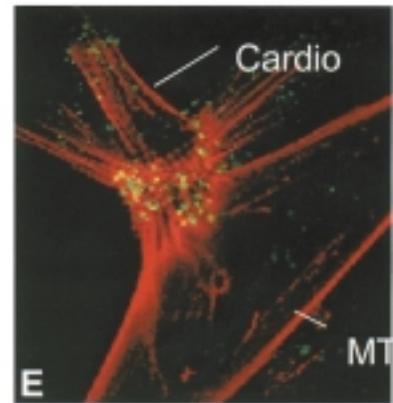
B α -Cardiac Myosin, 7W



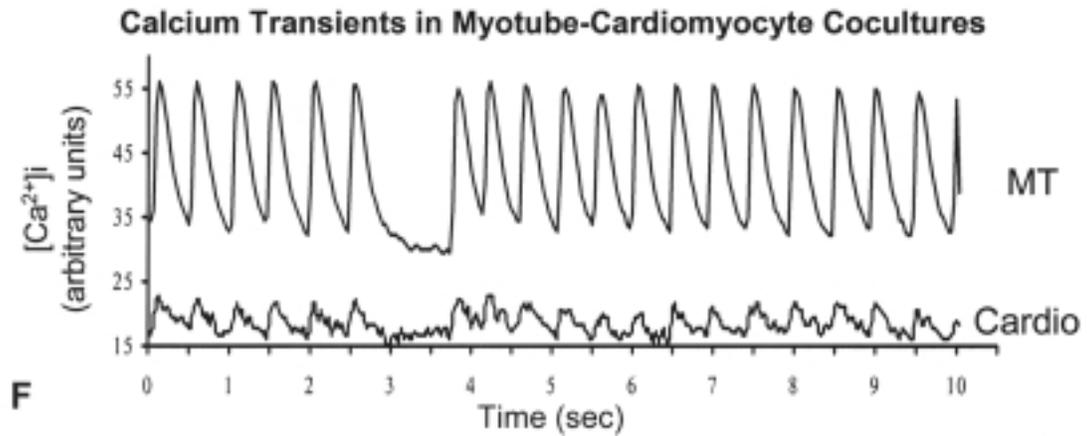
C N-cadherin



D N-cadherin



E Connexin43



skeletal muscle cells to follow their lineage, coupled with highly specific cardiac- or skeletal muscle-specific antibodies.²⁶ These studies have shown unambiguously that these grafts express skeletal muscle myosin heavy chains and fail to express cardiac markers such as α -myosin heavy chain (Fig. 2B), cardiac troponin-I and atrial natriuretic factor. Thus the skeletal myoblasts appear firmly committed to their fate and form only skeletal muscle in the heart.

Studies with myocardial wound strips showed that the skeletal muscle grafts would contract when exogenously stimulated.²¹ The grafts showed the ability to undergo tetanic contraction under high-frequency stimulation, a property not shared by myocardium because of its refractory period after depolarization. As the electrical field stimulation was increased, the skeletal muscle grafts showed increasing twitch tension, indicating recruitment of additional fibers. Fiber recruitment implied that the skeletal muscle grafts were electrically insulated from one another, unlike cardiomyocytes, which are electrically coupled by gap junctions. These observations led us to explore expression of the intercalated disk proteins N-cadherin (mechanical junctions) and connexin43 (gap junctions) in skeletal muscle.²⁷ Cell culture experiments showed that proliferating skeletal myoblasts expressed abundant amounts of N-cadherin and connexin43. When cells differentiated into myotubes, however, both proteins were markedly downregulated. Immunostaining revealed that skeletal muscle grafts in the heart had undetectable levels of N-cadherin and connexin43, indicating that the grafts were not electromechanically coupled with one another or with host myocardium (Fig. 2C). These findings make it unlikely that skeletal muscle grafts in the heart are beating synchronously with the host myocardium.

Much to our surprise, however, when skeletal and cardiac muscle cells were placed in coculture, the cells formed a synchronously beating network.²⁷ The β -adrenergic agonist isoproterenol increased synchronous beating rates, suggesting cardiomyocytes were the

pacemakers. Conversely, the gap junction blocker, heptanal, stopped skeletal muscle contractions and restricted individual cardiomyocytes to their intrinsic pacemaker frequency, suggesting the two cell types were coupled with gap junctions. Fluorescent calcium imaging studies showed that cardiomyocytes and skeletal muscle cells had synchronous calcium transients, indicating tight coupling between the cell types (Fig. 2F). Microinjection studies showed that the gap junction permeant dye, Lucifer yellow, could pass from skeletal muscle cells to cardiomyocytes. Finally, confocal microscopy revealed the presence of N-cadherin-mediated adherens junctions and connexin43-mediated gap junctions between skeletal muscle cells and cardiomyocytes (Fig. 2D, E).

Taken together, these experiments indicate that cardiomyocytes have the capacity to form electromechanical junctions with skeletal muscle cells and to use these junctions to induce synchronous beating in the skeletal muscle. Why does this coupling not occur *in vivo* after grafting? Skeletal muscle cells in culture are less differentiated than *in vivo* graft cells, and in culture the cells still have low levels of N-cadherin and connexin43. It appears that this low-level expression is sufficient to permit physiologic coupling. As the graft cells mature *in vivo*, however, N-cadherin and connexin43 appear to be downregulated to undetectable levels, thereby precluding coupling. Studies are currently directed at inducing expression of these two genes in grafted skeletal muscle cells to determine if this permits coupling between skeletal and cardiac muscle *in vivo*.

Control of Graft Cell Proliferation

For a graft to restore contractile function, it must replace a substantial fraction of the myocardium lost to infarction. A simple approach to increasing graft size would be to add more cells. As mentioned previously, this strategy will not work for cardiomyocytes because

Fig. 2. Skeletal muscle grafting and coculture. (A) Seven-week-old syngeneic skeletal muscle graft in a cryoinjured rat heart, immunostained for embryonic skeletal myosin heavy chain. Skeletal muscle cells formed a large mass of muscle in the injured heart. Grafts were typically separated from underlying myocardium by scar (wound) tissue. Note that the underlying myocardium does not express embryonic skeletal myosin. (B) A serial section immunostained for the cardiac marker α -myosin heavy chain. Note that the graft does not stain, indicating no transdifferentiation to cardiac muscle has occurred. (C) Skeletal muscle graft stained for the intercalated disk adherens junction protein, N-cadherin. Although the intercalated disks of the host myocardium stain vigorously for N-cadherin (arrows), the skeletal muscle graft does not express this protein. Similar results were obtained after immunostaining for the gap junction protein, connexin43 (not shown). (D, E) Skeletal myotubes (MT) and cardiomyocytes in coculture, stained for F-actin (red) and N-cadherin or connexin43 (green). In contrast to the grafting results, in coculture the two cell types formed intercalated disk-like junctions containing both N-cadherin and connexin43. (F) Calcium imaging in cocultures using Oregon Green. Synchronous calcium transients were observed in the cardiomyocyte and the coupled skeletal MT. Note that the pause at 3 seconds and the synchronous resumption of calcium fluxes. (A) and (B) reproduced from reference number 21; (C)-(F) reproduced from reference number 27.

adding more cells simply increases the fraction that dies. We performed a simple dose-escalation study with primary cultures of skeletal myoblasts grafted into normal and cryoinjured rat hearts.²⁸ Unlike cardiomyocytes, we found that graft size generally increased with increasing graft cell number. Unfortunately, this relationship was very noisy, with a wide variation in graft size at any given number of cells. Most of the imprecision appears to be due to variation in initial seeding efficiency. Müller-Ehmsen and colleagues showed that injecting cells in tissue culture medium resulted in highly variable retention, ranging from 15% to 80%.²⁹ In our studies we observed that with a constant dose of cells, some grafts were quite small, some nicely replaced the zone of injury, and some caused massive distention of the ventricular wall, bulging both the epicardial and endocardial contours.²⁸

An alternative strategy to getting the right graft size would be to introduce a smaller number of cells initially and then control their proliferation to a desired level. As mentioned previously, skeletal myoblasts proliferate well in response to basic FGF (bFGF) treatment. FGF also induces proliferation of multiple other cell types (e.g., fibroblasts), which might cause fibrosis rather than graft proliferation. We therefore sought to genetically modify skeletal muscle cells so they would activate the FGF signaling pathway in response to a synthetic ligand.³⁰ A chimeric receptor was created, consisting of the cytoplasmic domain of FGF receptor-1, fused to a drug binding domain. We used a modified FK506 binding protein, F36V, which bound to a bifunctional synthetic ligand (AP20187, ARIAD Pharmaceuticals, Cambridge, Massachusetts). The chimeric receptor was transfected in a stable manner into an FGF-dependent mouse myoblast line, MM14, and cells were tested for response to the dimerizer. When exposed to the dimerizer *in vitro*, transfected myoblasts activated the mitogen-activated protein kinase pathway and proliferated to levels comparable to treatment with bFGF (Fig. 3A). Untransfected myoblasts showed no response to dimerizer. The dimerizer also blocked differentiation into myotubes and expression of myosin heavy chain in a manner indistinguishable from bFGF treatment (Fig. 3B). Furthermore, cells continuously stimulated with dimerizer for 30 days differentiated normally upon dimerizer withdrawal, demonstrating reversibility of the effect. These experiments show a promising strategy for control of skeletal muscle graft size *in vivo*.

Effects on Myocardial Function

Several groups have explored the functional consequences of transplanting skeletal myoblasts into the infarcted heart. Taylor and colleagues²² implanted autologous myoblasts into adult rabbit hearts 1 week after

cryoinjury. Using sonomicrometry crystals implanted at the border of the injured and noninjured region, they reported improvements in segmental stroke work in 5 of 12 myoblast-engrafted hearts, with an additional 2 showing borderline improvement. Individual data were not provided for the 5 sham-injected hearts, although group data showed a significantly lower stroke work than the myoblast-engrafted animals. Jain and colleagues³¹ implanted 1 million neonatal skeletal myoblasts into the hearts of syngeneic adult rats injured 1 week previously by a 60-minute coronary occlusion followed by reperfusion. Although infarct size in both groups averaged 30% of the left ventricle, the myoblast-engrafted animals had greater maximal exercise capacity, presumably resulting from enhanced *in vivo* cardiac performance. *Ex vivo* analysis of cardiac function in a Langendorff apparatus showed that the myoblast-engrafted hearts shifted their developed pressure vs. end-diastolic volume relation toward normal. Interestingly, the improved function in myoblast-grafted hearts was associated with a significant reduction in ventricular dilation.

Most recently, Pouzet and colleagues³² reported on the effects of engrafting autologous skeletal myoblasts into rat hearts 1 week after permanent coronary occlusion. Although baseline echocardiographic function was not different between groups, the myoblast-engrafted group showed a marked improvement in ejection fraction at 1 and 2 months after engraftment, whereas vehicle-injected hearts showed a progressive decline in ejection fraction. Importantly, these investigators performed a dose-response study and showed that there was a linear relation between improvement in ejection fraction and myoblast dose. Although doses of 1 million myoblasts were insufficient to improve ejection fraction 2 months later, when 6 million myoblasts were implanted, the ejection fraction at 2 months averaged twice the baseline.

Finally, Menasche and colleagues have reported the initial results from one patient in which 800 million autologous skeletal myoblasts were grafted into the site of an old myocardial infarct.²⁰ Five months later, the engrafted site showed glucose uptake by positron emission tomography imaging, and global function of the heart was improved. Although this is encouraging, it should be stressed that this patient also received coronary artery bypass grafting, which obscures the interpretation of the functional data.

Taken together, these studies provide good evidence that skeletal myoblast grafting results in improved global contractile function. It is not clear, however, how this benefit is achieved. In all cases where histology was provided, the skeletal muscle graft was surrounded by scar tissue that was well removed from host myocardium. Furthermore, as reported previously, the absence of junctional proteins in skeletal muscle grafts make them unlikely to couple electrically with host cardiomyo-

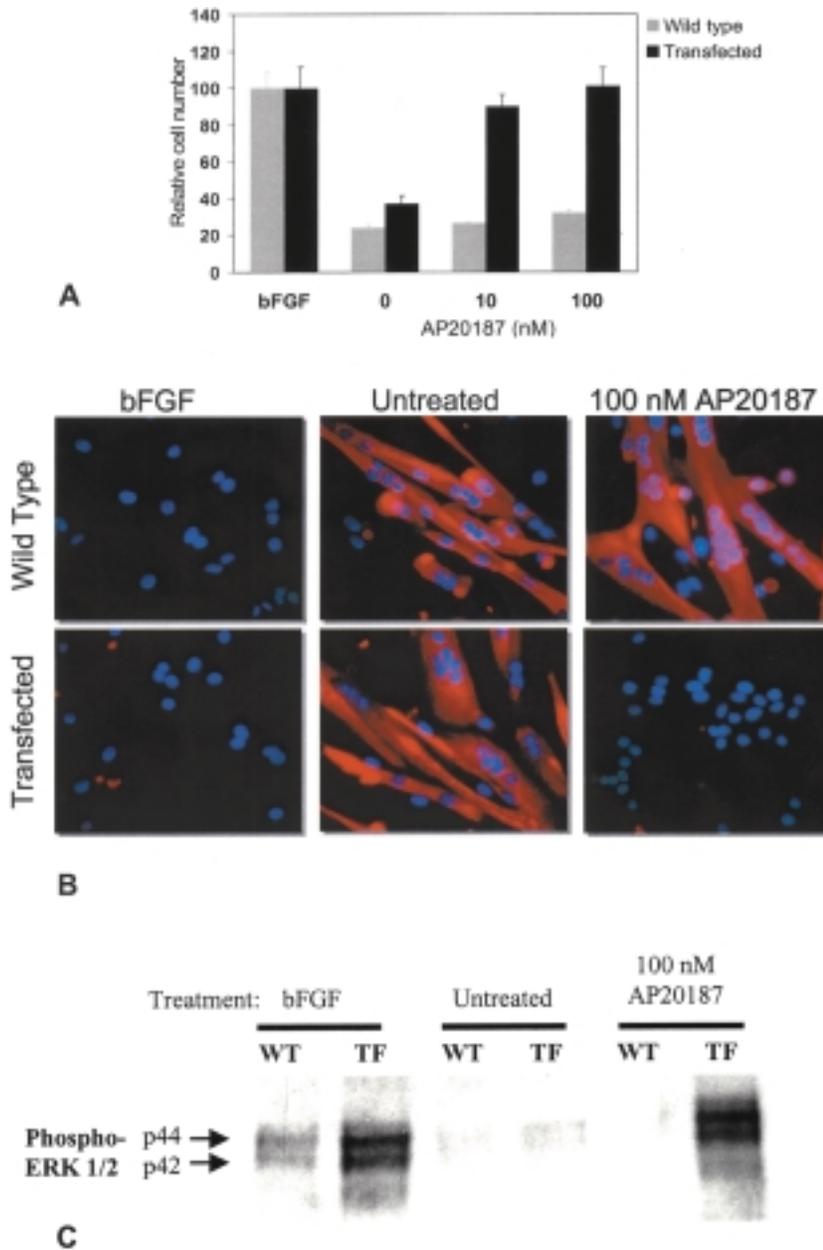


Fig. 3. Control of skeletal myoblast proliferation using a synthetic ligand. Mouse myoblasts (MM14) were stably transfected with a chimeric gene encoding the signaling domain of the FGF receptor-1, fused to a drug binding protein. The bifunctional drug AP20187 was used to activate receptor signaling in transfected cells. (A) Both wild-type and transfected myoblasts proliferated in response to bFGF. The transfected myoblasts proliferated to a similar extent after exposure to the dimerizer AP20187, whereas the drug had no effect on wild-type cells. (B) Sarcomeric myosin heavy chain staining (red). Treatment with bFGF blocked fusion into myotubes and the expression of myosin heavy chain. Both wild-type and transfected cells formed myosin-positive myotubes after bFGF withdrawal. When exposed to the dimerizer (AP20187), differentiation was blocked in transfected cells, similar to bFGF treatment, whereas wild-type cells differentiated normally. (C) Immunoblot for phospho-ERK 1/2. Both wild-type (WT) and transfected (TF) cells phosphorylated ERK after bFGF treatment and showed comparably low ERK phosphorylation after bFGF withdrawal. After treatment with AP20187, transfected cells phosphorylated ERK similar to bFGF treatment, whereas wild-type cells were unaffected. Reproduced from reference number 30.

cytes. The critical question for myoblast grafting is: Do these grafts beat? Although seemingly simple, this question has not been adequately answered. Taylor's sonomicrometry data reporting improved work provides the best data for regional function.²² Although consistent with beating graft cells, there are alternative explanations that must be considered. Despite having a transmural cryoinjury, all of the injured segments, both engrafted and sham, showed positive stroke. This indicates that the sonomicrometry crystals were not measuring function exclusively in the cryoinjured region (which would have zero or a negative stroke work). Thus it seems equally plausible that the enhanced work loops after myoblast

grafting could have resulted from global changes in function resulting from effects on myocardial remodeling. Given that three clinical trials for skeletal muscle grafting in the heart are currently under way, determining whether the graft cells beat will be a very important point for future studies.

In contrast to the controversy regarding restoration of systolic wall motion, there are very good data indicating that skeletal myoblast grafting significantly attenuates ventricular remodeling after an infarct. Jain and colleagues showed a leftward shift of the ventricular pressure-volume curve and directly demonstrated a reduced ventricular volume in the myoblast-engrafted

hearts.³¹ This study definitively shows that myoblast grafting is an effective strategy to target remodeling after infarction.

Summary and Future Directions

Cellular grafting has significant promise to enhance function of the heart after myocardial infarction. In the near term, the most promising target would appear to be using skeletal myoblasts to target postinfarction remodeling. An intermediate-term strategy may be to use cellular grafts to enhance angiogenesis in the ischemic region. Although not covered in this review, several studies suggest that either circulating endothelial progenitor cells³³ or bone marrow mononuclear cells³⁴ can enhance myocardial vascularization in models of chronic ischemia. The “holy grail” of regenerative therapies will remain restoration of systolic wall motion after infarction. This could be achieved either through the use of cardiomyocytes or skeletal muscle cells. For cardiomyocytes, the critical obstacles are defining a reliable cell source for human applications (likely derivation from stem cells) and either preventing cell death or enhancing cell proliferation after engraftment. For skeletal muscle, the critical obstacles are inducing electromechanical coupling of skeletal and cardiac muscle, and developing strategies to reliably control the size of grafts after they are in place. Although promising experimental leads are present, it will take a considerable amount of careful, basic research before the promise of cell therapy can be reduced to a clinical practice.

Acknowledgments

The authors are grateful to Veronica Poppa for many of the immunohistochemical studies presented in this manuscript.

References

- Mallory GK, White PD, Salcedo-Salgar J: The speed of healing of myocardial infarction: a study of the pathologic anatomy in 72 cases. *Am Heart J* 1939;18:647–671
- Soonpaa MH, Field LJ: Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res* 1998;83:15–26
- Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA: Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;107:1395–1402
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;410:701–705
- Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 2001;98:10344–10349
- Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P: Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci* 2001;938:221–229
- Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P: Evidence that human cardiac myocytes divide after myocardial infarction. *New Engl J Med* 2001;344:1750–1757
- Soonpaa MH, Koh GY, Klug MG, Field LJ: Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science* 1994;264:98–101
- Koh GY, Soonpaa MH, Klug MG, Pride HP, Cooper BJ, Zipes DP, Field LJ: Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs. *J Clin Invest* 1995;96:2034–2042
- Klug MG, Soonpaa MH, Koh GY, Field LJ: Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 1996;98:216–224
- Li RK, Jia ZQ, Weisel RD, Mickle DA, Zhang J, Mohabeer MK, Rao V, Ivanov J: Cardiomyocyte transplantation improves heart function. *Ann Thor Surg* 1996;62:654–660
- Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA: Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 1996;94:II332–II336
- Connold AL, Frischknecht R, Dimitrakos M, Vrbová G: The survival of embryonic cardiomyocytes transplanted into damaged host rat myocardium. *J Muscle Res Cell Motil* 1997;18:63–70
- Scorsin M, Marotte F, Sabri A, Le Dref O, Demirag M, Samuel JL, Rappaport L, Menasché P: Can grafted cardiomyocytes colonize peri-infarct myocardial areas? *Circulation* 1996;94:II337–II340
- Watanabe E, Smith DM Jr, Delcarpio JB, Sun J, Smart FW, Van MC Jr, Claycomb WC: Cardiomyocyte transplantation in a porcine myocardial infarction model. *Cell Transplantation* 1998;7:239–246
- Reinecke H, Zhang M, Bartosek T, Murry CE: Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation* 1999;100:193–202
- Zhang M, Methot D, Poppa V, Fujio Y, Walsh K, Murry CE: Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol* 2001;33:907–921
- Olivetti G, Capasso JM, Sonnenblick EH, Anversa P: Side-to-side slippage of myocytes participates in ven-

- tricular wall remodeling acutely after myocardial infarction in rats. *Circ Res* 1990;67:23–34
19. Hauschka SD: The embryonic origin of muscle. In Engel AG, Franzini-Armstrong C, eds. *Myology*. McGraw-Hill, New York, 1994, pp 3–73
 20. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP: Myoblast transplantation for heart failure. *Lancet* 2001; 357:279–280
 21. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD: Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest* 1996;98:2512–2523
 22. Taylor DA, Atkins BZ, Hungspreugs P, Jones TR, Reedy MC, Hutcheson KA, Glower DD, Kraus WE: Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med* 1998;4:929–933
 23. Kao RL, Chin TK, Ganote CE, Hossler FE, Li C, Browder W: Satellite cell transplantation to repair injured myocardium. *Card Vasc Regen* 2000;1:31–42
 24. Chiu RC, Zibaitis A, Kao RL: Cellular cardiomyoplasty: myocardial regeneration with satellite cell implantation. *Ann Thorac Surg* 1995;60:12–18
 25. Atkins BZ, Lewis CW, Kraus WE, Hutcheson KA, Glower DD, Taylor DA: Intracardiac transplantation of skeletal myoblasts yields two populations of striated cells in situ. *Ann Thorac Surg* 1999;67:124–129
 26. Reinecke H, Poppa V, Murry CE: Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 2002;34:241–249
 27. Reinecke H, MacDonald GH, Hauschka SD, Murry CE: Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. *J Cell Biol* 2000; 149:731–740
 28. Reinecke H, Murry CE: Transmural replacement of myocardium after skeletal myoblast grafting into the heart. Too much of a good thing? *Cardiovasc Pathol* 2000;9: 337–344
 29. Muller-Ehmsen J, Whittaker P, Kloner RA, Dow J, Sakoda T, Long TI, Laird PW, Kedes L: Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol* 2002;34:107–116
 30. Whitney ML, Otto KG, Blau CA, Reinecke H, Murry CE: Control of myoblast proliferation with a synthetic ligand. *J Biol Chem* 2001;276:41191–41196
 31. Jain M, DerSimonian H, Brenner DA, Ngoy S, Teller P, Edge AS, Zawadzka A, Wetzel K, Sawyer DB, Colucci WS, Apstein CS, Liao R: Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. *Circulation* 2001; 103:1920–1927
 32. Pouzet B, Vilquin JT, Hagege AA, Scorsin M, Messa E, Fiszman M, Schwartz K, Menasche P: Factors affecting functional outcome after autologous skeletal myoblast transplantation. *Ann Thorac Surg* 2001;71:844–850
 33. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S: Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7:430–436
 34. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T: Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001;104:1046–1052