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Regenerating the heart

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Cell-based cardiac repair offers the promise of rebuilding the injured heart from its component parts. Work began with committed cells such as skeletal myoblasts, but recently the field has expanded to explore an array of cell types, including bone marrow cells, endothelial progenitors, mesenchymal stem cells, resident cardiac stem cells, and both mouse and human embryonic stem cells. A related strategy for cardiac repair involves cell mobilization with factors such as cytokines. Translation of cell-based approaches to the clinic has progressed rapidly, and clinical trials using autologous skeletal myoblasts and bone marrow cells are under way. Many challenges remain before the vision of healing an infarct by muscle regeneration can be realized. Future research is likely to focus on improving our ability to guide the differentiation of stem cells, control their survival and proliferation, identify factors that mediate their homing and modulate the heart's innate inflammatory and fibrotic responses.

Human beings have a longstanding fascination with regeneration. We marvel at the ability of fish or newts to regenerate body parts while bemoaning our own inability to do the same. Until recently, this fascination was restricted to myths—such as that of Prometheus—and works of science fiction—for example, Gibson's *Neuromancer*. In the last decade, however, major advances in stem cell biology created genuine hope for the emerging discipline of 'regenerative medicine'.

As one of the least regenerative organs in the body, the heart would benefit greatly from regenerative therapy; by the same token, it also poses one of the greatest challenges. Myocardial infarction results in large-scale loss of cardiac muscle (often a billion or more myocytes; **Box 1** and **Fig. 1**), whereas other heart diseases, such as hypertension, valve disease and genetic disorders (for example, cardiomyopathies), cause more sporadic myocyte loss. Heart failure ensues when contractile reserve is depleted below a critical threshold. Heart failure is already the most common cause of hospitalization in US citizens over 65, and, as our population ages, some have predicted epidemic proportions of this disease.

We and other groups hypothesize that heart failure could be reversed or prevented if new myocardium could be grown in diseased hearts. This idea has gained widespread attention recently, leading to numerous basic-science reports and multiple early-stage clinical trials. In this review, we will cover recent developments using the most extensively studied cell types for cardiac repair. We will also review the basic biology of infarct repair, discuss experimental artifacts that can sometimes mimic regeneration and summarize highlights in myocardial tissue engineering. Although reinduction of cell cycle activity in cardiomyocytes is another important cardiac repair strategy, space limitations prevent us from including this topic. The interested reader is referred to several recent reviews on this topic[1–3].

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Transplantation of committed skeletal myoblasts

Cell-based cardiac repair began with the transplantation of autologous skeletal muscle satellite cells (commonly referred to as myoblasts), progenitor cells that normally mediate regeneration of skeletal muscle[4–7]. Although some investigators originally hoped that engrafted myoblasts would transdifferentiate into cardiomyocytes, multiple lines of evidence now indicate that these cells remain committed to a skeletal muscle fate[8] (with the exception of rare fusion events at the graft-host interface[9]). Mature skeletal muscle cells do not express the adhesion or gap junction proteins required to electromechanically couple with one another or with host myocardium[10], and available physiological data suggest that these grafts do not beat in synchrony with the rest of the heart[11,12]. Despite this apparent shortcoming, studies in small and large animal models have reported beneficial effects of myoblast grafting on ventricular contractile function after myocardial infarction[13]. Part of the protection seems to result from reduced ventricular dilation[14], although the complete basis for improved mechanical function is currently unknown.

Despite this gap in understanding, myoblasts were the first cell type to be used clinically for cardiac repair owing to their preclinical efficacy, autologous availability, ability to be amplified *in vitro* and relatively good survival after implantation[15]. Clinical trials of myoblast grafting have recently been reviewed[16] and will be summarized only briefly here. Phase 1 studies have shown the feasibility of growing more than one billion myoblasts from a few grams of muscle tissue, as well as the ability to implant these cells either under direct visualization at the time of cardiac surgery or through catheter-based delivery devices. Pathological studies have documented mature skeletal myofibers in the infarcts months later, although the relatively small graft sizes indicate that improvements in cell retention and survival are needed. A potential safety concern is that four of ten patients in one trial experienced ventricular arrhythmias, necessitating implantable defibrillators[15]. It is important to note, however, that the inherently arrhythmogenic substrate of the failing heart requires randomized, controlled trials to determine a causal relationship. Finally, the uncontrolled nature of these studies precludes any definitive statements about efficacy,

Box 1 Myocardial infarct repair

Myocardial infarctions most commonly result from coronary occlusions, due to a thrombus overlying an atherosclerotic plaque. Because of its high metabolic rate, myocardium (cardiac muscle) (**Fig. 1a**) begins to undergo irreversible injury within 20 minutes of ischemia, and a wavefront of cell death subsequently sweeps from the inner layers toward the outer layers of myocardium over a three- to six-hour period. Although cardiomyocytes are the most vulnerable population, ischemia also kills vascular cells, fibroblasts and nerves in the tissue. Myocardial necrosis (**Fig. 1b**) elicits a vigorous inflammatory response. Hundreds of millions of marrow-derived leukocytes, initially composed of neutrophils and later of macrophages, enter the infarct. The macrophages phagocytose the necrotic cell debris and likely direct subsequent phases of wound healing. Concomitant with removal of the dead tissue, a hydrophilic provisional wound repair tissue rich in proliferating fibroblasts and endothelial cells—termed granulation tissue (**Fig. 1c**)—invades the infarct zone from the surrounding tissue. Over time, granulation tissue remodels to form a densely collagenous scar tissue (**Fig. 1d**). In most human infarcts, this repair process requires two months to complete. Infarcts in smaller experimental animals such as mice or rats heal substantially faster.

At the organ level, myocardial infarction results in thinning of the injured wall and dilation of the ventricular cavity, a process termed ventricular remodeling (**Fig. 1e**). These structural changes markedly increase mechanical stress on the ventricular wall and promote progressive contractile dysfunction. The extent of heart failure after a myocardial infarction is directly related to the amount of myocardium lost. Conversely, patients whose ventricles dilate more extensively suffer more severe heart failure, irrespective of the original infarct size.

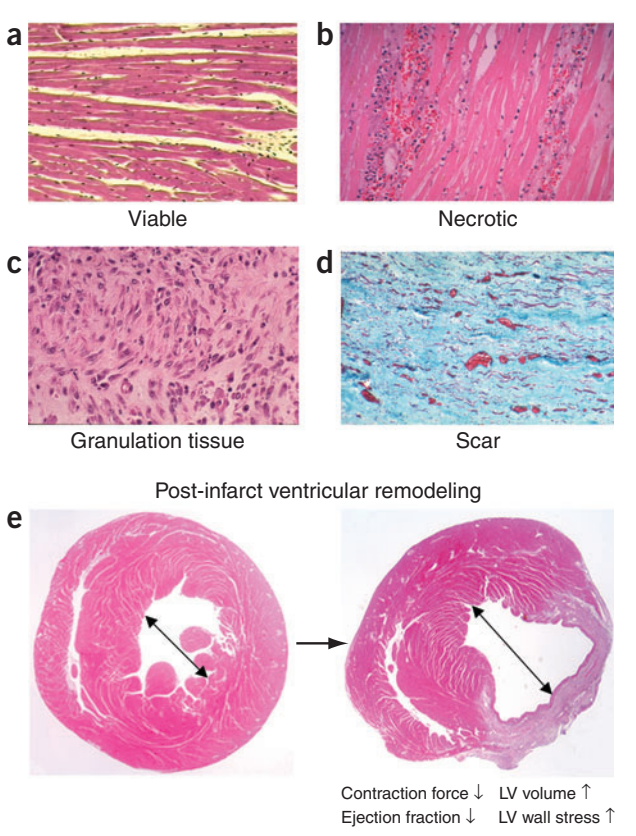

Figure 1 Histological stages of myocardial infarction.

although there are hints of improved function. Larger-scale phase 2 trials are currently enrolling patients, and more definitive results are expected in a few years.

Transplantation of multipotent adult stem cells

Lessons from clinical transplantation studies. Transplantation of solid organs or bone marrow in patients has provided important insights into the ability of circulating cells to repopulate adult tissues. In such studies, the circulating cells have a different genotype from the solid tissue, allowing one to track their fate after the transplant. (Similar processes likely take place outside the transplant setting, but the transplant makes the events easier to detect.) The most common examples involve transgender transplants, for example, male patients who receive female hearts (**Fig. 2**). In such cases, the Y chromosome identifies a cell as extracardiac in origin, whereas immunostaining with specific cell markers identifies the cell type. These studies have provided strong evidence for circulating endothelial progenitors in patients. In our own work, we have observed that ~25% of microvascular endothelium in transplanted hearts originates from extracardiac sources (E. Minami, M.A.L. & C.E.M., unpublished data). Other investigators have seen similarly high endothelial chimerism, either after heart transplantation[17] or in the coronary circulation after bone marrow transplantation[18]. There is also evidence for smooth muscle repopulation of both arteries and veins[19,20], as well as perineural Schwann cell repopulation in the transplanted heart (E. Minami, M.A.L. & C.E.M., manuscript submitted). Interestingly, one group has reported that a significant fraction of the smooth muscle cells in coronary atherosclerotic plaques are derived from circulating progenitor cells in bone marrow transplant patients[21]. If this important observation is confirmed, it has profound implications for the pathogenesis and treatment of atherosclerosis.

More controversial is the extent to which circulating progenitors can repopulate the cardiomyocyte compartment. There is general agreement that Y+ cardiomyocytes can be found in female donor hearts. Most investigators have found cardiomyocyte chimerism to be a very rare phenomenon, with a frequency in the range of one in 10^4 to 10^3 total cardiomyocytes[17,18,22,23]. Quaini *et al.*[19] reported a much higher frequency, however, averaging 18% of total cardiomyocytes. Because this study included several patients who died within 30 days of transplantation, when T-cell and macrophage burden is highest in the allograft, it is possible that nuclei from Y+ leukocytes contributed to this elevated count (**Box 2** and **Fig. 3**).

In sum, the data from clinical transplantation studies strongly support a role for endogenous, circulating progenitor cells in neovascularization, with high contributions to endothelium and lesser contributions to smooth muscle. The preponderance of evidence suggests that circulating progenitors make only a very limited contribution to cardiomyocyte repopulation. Our interpretation of these data is that, from a therapeutic standpoint, it will likely be easier to induce myocardial revascularization from circulating progenitor cells than it will be to induce remuscularization.

Bone marrow. Since the groundbreaking 1998 report of Ferrari *et al.*[24] that cells from bone marrow contribute to skeletal muscle regeneration

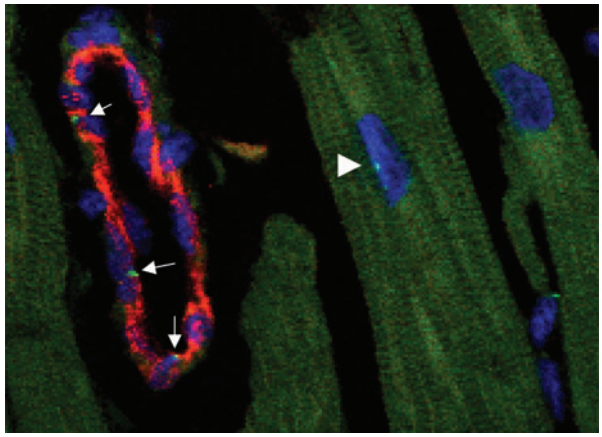

Figure 2 Chimerism in a transplanted human heart. Hearts from male patients receiving female hearts were studied by confocal microscopy at least one year after transplantation. *Ulex europaeus* lectin staining (red) was used to identify endothelium, and Y chromosome fluorescence *in situ* hybridization (green) was used to identify cells derived from extracardiac sources. Cardiomyocytes were visible owing to autofluorescence of myofibrils. Three endothelial cells within a single venule are Y+ (arrows). A single Y+ cardiomyocyte is shown (arrowhead). As described in the text, endothelial chimerism was common, whereas cardiomyocyte chimerism was rare. Photograph by E. Minami.

in mice, multiple groups have studied the potential of marrow cells to effect myocardial regeneration. The first evidence suggesting that this might be possible came from Bittner *et al.*[25], who transplanted marrow from wild-type male mice into dystrophic *Dmd*$^{mdx/mdx}$ females. They reported rare Y+ nuclei in cardiomyocytes, although they did not specifically quantify the frequency. A subsequent study examined the ability of a marrow population highly enriched for hematopoietic stem (HS) cells (the Hoechst dye–effluxing "side population") to contribute to myocardial regeneration after infarction[26]. Lethally irradiated wild-type mice had their hematopoietic systems reconstituted with side population cells from Rosa26 mice, which constitutively expressed β-galactosidase. Several months after establishing high levels of donor-derived cells in the circulation, these mice were subjected to myocardial infarction, and their hearts were studied histologically two and four weeks later. Rare cardiomyocytes expressing β-galactosidase in the peri-infarct zone, estimated at 0.02% of total myocytes, were found. Interestingly, donor-derived endothelial cells were 1,000-fold more common, averaging ~3% of total endothelium. These studies suggested that endogenous cells from marrow (derived from the hematopoietic compartment) contribute to cardiac repair. As in the human transplantation studies[17,18,22,23], the coronary endothelium was repopulated to a far greater extent than were cardiomyocytes. Interestingly, Alvarez-Dolado *et al.*[27] demonstrated that

Box 2 Potential microscopic artifacts

We have identified two common and potentially misleading artifacts in our own studies of circulating progenitors: myocardial leukocytes and the high intrinsic autofluorescence of infarcted tissue. Bone marrow–derived leukocytes are associated with most types of myocardial injury and can be mistaken for stem cell–derived myocytes. A human heart transplant, with immunofluorescent staining for myosin light 2V (red) and nuclei (blue), is shown in **Fig. 3a**. The arrow indicates a cardiomyocyte with what seems to be a myocyte nucleus. When the image in **Fig. 3a** is stained for markers for human leukocytes (CD45 plus CD68, green), the apparent myocyte nucleus is clearly seen to belong to an intracytoplasmic leukocyte (**Fig. 3b**). A transmission electron micrograph from a patient with a myocardial infarction shows a necrotic cardiomyocyte with clearly recognizable myofibrils (mf) (**Fig. 3c**). Its basal lamina is outlined by arrows. A macrophage (Mϕ) residing in the myocyte cytoplasm has engulfed some of the myofibrils. An enlargement of the boxed region in **Fig. 3c** shows that the macrophage nucleus (Nu) is in close proximity to cardiac myofibrils, highlighting the danger of mistaking this for a myosin-positive, marrow-derived cell (**Fig. 3d**).

Autofluorescence is a recognized problem in normal striated muscle and can be mistaken for EGFP or a fluorescent immunostain. After injury, autofluorescence increases due to accumulated lipofuscin, blood-derived pigments and other intrinsic fluors such as flavins and reduced nicotinamide adenine dinucleotide (NADH). **Fig. 3e** shows a section from a two-week-old myocardial infarct in a wild-type control mouse, stained only with a blue DNA dye to identify nuclei by conventional fluorescence microscopy. The apparent green signal is entirely due to autofluorescence; this animal never received EGFP+ cells. Subendocardial sparing can be seen in the trabecular regions. The boxed area from **e**, with intense autofluorescence, is shown in **Fig. 3f**.

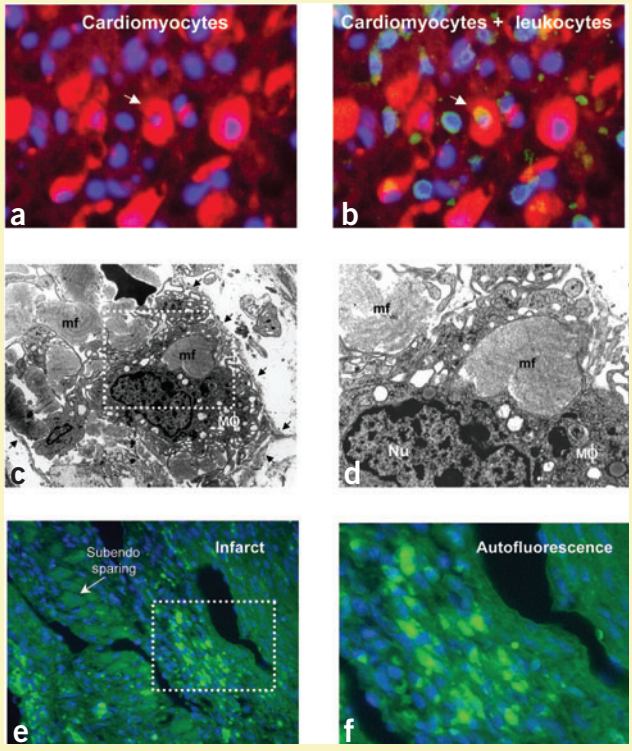

Figure 3 Examples of potentially misidentified cardiomyocytes: confusion with infiltrating leukocytes and autofluorescence.

marrow-derived cells occasionally fused with cardiomyocytes in the absence of injury, giving rise to hybrid cells. Their frequency of fusion is roughly comparable to the frequency of progenitor-derived cardiomyocytes in both mouse and human transplantation studies, raising the possibility that fusion is the principal mechanism through which such cells arise.

To test the ability of directly delivered HS cells to promote myocardial regeneration in a therapeutic model, Anversa and collaborators[28] isolated these cells from mice constitutively expressing enhanced green fluorescent protein ($EGFP^+$) and injected them into the peri-ischemic region of wild-type mice with acute myocardial infarcts. At one to two weeks postinfarction, mice with successful injections had regenerated ~68% of their infarcts with EGFP+ cells. Evidence for regeneration included colocalization of EGFP fluorescence with immunostaining for cardiomyocyte markers, including sarcomeric actins and myosins, troponin and several transcription factors active in cardiomyocytes. Despite staining for these markers, however, the new myocytes did not resemble normal cardiomyocytes, in that they were small, round to spindle-shaped, and had no sarcomeres. Importantly, echocardiographic and hemodynamic indices of left ventricular function were improved in the HS cell–injected animals. This study energized the field with hope that a readily accessible population of autologous cells might regenerate the heart.

Unfortunately, several other groups could not reproduce this finding. In collaboration with Field's lab, we used genetic methods to track the fate of HS cells injected into infarcts. We reasoned that cardiac-specific transgenes should be activated in HS cells if they became cardiomyocytes. To test this, we isolated HS cells from transgenic mice carrying the α-cardiac myosin heavy chain promoter driving either nuclear-localized β-galactosidase or EGFP, and injected them into acute myocardial infarcts[29]. Despite the use of an assay capable of detecting a single positive cell in the heart, none of the 117 mice studied showed activation of the cardiac-specific transgene. We then engrafted HS cells constitutively expressing EGFP into acute infarcts in an additional 27 mice and, again, found no increase in cardiomyocytes in the infarct. Finally, using bone marrow transplantations, we reproduced findings of others regarding rare marrow-derived cardiomyocytes in infarct border zones. We concluded there was no significant cardiac differentiation after HS cell transplantation.

At the same time, Balsam *et al.*[30] showed that EGFP+ HS cells injected into infarcts did not form cardiomyocytes, but instead differentiated into blood cells, predominantly granulocytes. Despite the absence of transdifferentiation in their study, these authors did note an improvement in ventricular function in the HS cell–engrafted group. Nygren *et al.*[31] also found that hematopoietic cells formed leukocytes almost exclusively in infarcts. Using both cardiac-restricted and constitutively active transgenic reporters, this group tested direct injection of various populations and several mobilization strategies. None of the strategies formed significant new numbers of cardiomyocytes, although endogenously derived circulating cells were noted to fuse with host myocytes at the infarct border.

Thus, there appears to be a general consensus that endogenous, marrow-derived cells can give rise to rare cardiomyocytes through mechanisms that, at least in part, involve cell fusion. The physiological significance of this fusion event is currently unknown and deserves further study. A recent study indicated that marrow-derived cells are not reprogrammed to express cardiac genes when they fuse with cardiomyocytes *in vivo*, suggesting that they may have a hybrid phenotype[32]. Whether marrow-derived cells can give rise to large-scale regeneration of the heart remains controversial. Despite the inability of three independent groups to reproduce the original report of regeneration, no clear explanation for the discrepancy has emerged. Anversa's group[33] recently reported that they did reproduce their original findings, using a less-purified population of marrow cells. Additionally, while this review was in preparation, another group reported large-scale regeneration of the heart using a nonhematopoietic population of cells derived from the marrow[34]. At present, we can only advise the reader to stay tuned to this rapidly moving area of research. A clearer picture is bound to emerge, and it will do so more quickly if researchers in the field collaborate and sort out the critical variables among different laboratories.

Clinical trials involving bone marrow for myocardial repair have recently been reviewed in detail[16,35], so only a brief summary will be provided here. Most studies have focused on bone marrow mononuclear cells, a heterogeneous population of hematopoietic and mesenchymal cells containing <0.1% stem cells, although one study also tested peripheral blood–derived progenitor cells[36]. Two general designs have been used: intracoronary delivery to patients with recent myocardial infarctions and catheter-based intramyocardial injection into patients with chronic ischemic disease and old infarcts. These studies have generally shown that catheter-based delivery of bone marrow cells to the heart is feasible. Encouragingly, no significant complications have been reported. Like the skeletal myoblast trials, most of the bone marrow trials have been uncontrolled or used nonrandomized controls for comparison, limiting assessment of efficacy. Still, most of the studies suggested enhanced myocardial contractile function, enhanced perfusion or both.

At present, there are two published randomized, controlled studies involving bone marrow cells for cardiac repair. Wollert *et al.*[37] carried out the first trial, in which 30 patients received intracoronary injections of unfractionated mononuclear cells (2.4 billion cells/patient), and 30 patients in the control group did not get sham marrow aspiration or cell infusion. Patients receiving marrow cells had a 6% increase in ejection fraction compared with controls. In the second study, Chen *et al.*[38] described intracoronary delivery of bone marrow–derived mesenchymal cells in 34 patients (48–60 billion cells/patient!), whereas 35 control patients underwent a bone marrow harvest but received only saline infusion. Mesenchymal cell infusion resulted in increased fluoro-deoxyglucose uptake, improved wall motion, reduced ventricular dilation and a net increase of 14% in ejection fraction versus controls.

Most recently, at the 2005 American College of Cardiology meeting, late-breaking results were announced for a double-blind, randomized, placebo-controlled trial from Belgium (S. Janssens *et al.*, unpublished data). Thirty-two patients received intracoronary unfractionated bone marrow cells within 24 hours of acute infarction, compared with 34 patients who underwent marrow harvest but placebo infusion. Notably, the groups did not differ in ejection fraction after treatment. Nevertheless, magnetic resonance imaging showed that marrow cell infusion was associated with greater infarct shrinkage (wound contraction), suggesting that enhanced infarct repair might be one mechanism by which marrow cells exert an effect on the heart.

These studies indicate that transplanting bone marrow cells into the heart is feasible and seems to be safe. Initial studies suggest improved ventricular function and perfusion. In our opinion, additional randomized, controlled clinical trials to explore efficacy and mechanism of action are warranted.

Endothelial progenitors. In 1997, Asahara and colleagues[39] reported evidence for circulating endothelial progenitor cells in humans. Using magnetic beads with antibodies to CD34, they separated a population from human peripheral blood that attached to fibronectin and grew

well under conditions known to favor endothelium. The proliferating cells displayed endothelial gene expression patterns *in vitro* and incorporated into neovascularization in ischemic hind-limb models in mice and rabbits. Multiple laboratories have since reproduced these basic findings, showing that the cells can be mobilized with growth factors and/or cytokines[40], will home to areas of injury (in part mediated by SDF-1/CXCR4 interaction[41,42]) and often will integrate into growing vessels[43]. There is evidence that patients with chronic diseases such as heart failure or diabetes have dysfunctional endothelial progenitors[44]. Their numbers are reduced in the circulation, they do not home and migrate as well, and they do not divide as robustly. Interestingly, their vigor can be largely restored by growing the cells in the presence of various statins (hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitors). The mechanism underlying this effect of statins is currently unknown, but may involve preventing senescence by the loss of telomere capping proteins[45]. The endothelial progenitor field continues to branch out (!) and is being intensively studied by tumor biologists, who are interested in either preventing tumor angiogenesis or targeting therapeutics to vascularized tumors.

An important study by Kocher *et al.*[46] provided evidence that human endothelial progenitors could home to and revascularize a myocardial infarct. They isolated granulocyte colony–stimulating factor (G-CSF)-mobilized CD34+ cells from human volunteers and injected them intravenously into athymic rats, two days after myocardial infarction. They found that the human cells were incorporated into the infarct's neovasculature, forming a chimeric coronary circulation. Cells that did not express CD34, as well as mature endothelium from human saphenous vein, failed to home to the infarct. Animals receiving CD34+ cells showed partial recovery of left ventricular function (by echocardiography), whereas sham-injected animals or those receiving CD34-negative cells showed deteriorating ventricular function. Treatment with CD34+ cells was also associated with reduced late cardiomyocyte death at the infarct border zone.

One interpretation of these data is that CD34+ cells facilitated angiogenesis, increased perfusion and prevented the chronically ischemic cells at the border zone from dropping out. Another possibility is that paracrine factors produced by the CD34+ cells were responsible, independent of their incorporation in the coronary circulation. In a provocative report, Rehman *et al.*[47] used a different, but widely accepted, method to derive EPCs[48] and characterized the resultant cell population as largely composed of monocyte and/or macrophage-derived cells and including relatively few cells with an endothelial or stem cell phenotype. These authors nonetheless proposed renaming this cell preparation "circulating angiogenic cells" because the cells did secrete multiple proangiogenic cytokines, including vascular endothelial growth factor, hepatocyte growth factor, G-CSF, and granulocyte-macrophage colony–stimulating factor. Working with a different cell preparation that has been implicated as containing vascular progenitor cells, Kinnaird *et al.*[49] showed that cultured human bone marrow–derived stromal cells also express arteriogenic cytokines and that this expression was increased under hypoxic conditions. (Intriguingly, medium conditioned by these cells enhanced collateral flow, attenuated muscle atrophy and improved limb function in a murine hind-limb ischemia model compared with controls.) The latter study is intriguing in light of an earlier study by Schaper's group[50] that failed to reproduce previous findings that bone marrow–derived cells were directly incorporated into growing vessels—even in the context of ischemia or tumor-related angiogenesis. These authors did find genetically tagged marrow-derived cells in close proximity to developing collateral vessels, however, and many of these cells immunostained for arteriogenic growth factors.

These three reports[47,49,50] suggest an alternative hypothesis for the contribution of 'precursor' cells to postnatal growth: one centered on proarteriogenic paracrine signaling rather than direct incorporation. Given the observation that at least some of these cells exhibit leukocytic markers, this hypothesis also recalls a view long championed by the Schaper group that the macrophage has a central role in controlling postnatal vascular growth[51]. It is possible that stem cell researchers are rediscovering this process from another angle. In any event, although we believe it is highly likely that such cell preparations will find eventual widespread clinical application in ameliorating cardiac ischemia, it would behoove the field to first unambiguously determine their mechanisms of action.

Cytokine mobilization. Once a role for endogenous marrow-derived progenitor cells in infarct repair was discovered, a logical next step was to attempt to induce their mobilization with cytokines. G-CSF is the best-studied cytokine in this regard, with published data in small animals and primates and early human trials under way. Several groups have reported that G-CSF treatment in mice improves ventricular function postinfarction in mice[52], rats[53] and pigs[54]. On the other hand, Deten *et al.*[55] found no beneficial effect of G-CSF in mice, and Norol *et al.*[56] found no beneficial effect in baboons. Furthermore, when Orlic *et al.*[57] extended their successful mouse protocol to rhesus monkeys, they observed higher mortality with cytokine treatment and no benefit to infarct structure or function. The reason for the discrepancy is unfortunately unknown.

Putting aside the question of effects on contractile function for the moment, it is worth asking whether G-CSF has other effects on the myocardium that may help explain a mechanism of action (or lack thereof). Although G-CSF was originally thought to mobilize progenitors that contributed to myocardial regeneration, Nygren *et al.*[31] provided strong evidence that no significant regeneration occurs after G-CSF treatment. G-CSF unquestionably mobilizes progenitor cells, but it is worth keeping in mind that >99% of G-CSF-mobilized cells are committed granulocytes and monocytes. As these leukocytes are the major effectors of infarct repair, it seems plausible that a marked increase in their availability could modulate infarct repair, independent of regeneration. In support of this notion, Minatoguchi *et al.*[58] recently showed in a rabbit model that G-CSF increased macrophage influx into the infarct, accelerated infarct repair and reduced the size of the final scar.

A second possibility was raised in an intriguing study by Harada *et al.*[59]. These authors demonstrated that cardiomyocytes expressed the G-CSF receptor, and that G-CSF treatment protected against oxidant-induced death *in vitro*. In their hands, G-CSF treatment of mice did not affect initial infarct size, but significantly influenced infarct repair, resulting in smaller final infarcts, increased vascular density, reduced cardiomyocyte and endothelial apoptosis, and improved ventricular function. They then blocked G-CSF signaling specifically in cardiomyocytes (through a cardiac-restricted, dominant-negative STAT3 transgene) and repeated the experiments. Although comparable mobilization of marrow-derived cells was observed, all the protective effects of G-CSF were lost in the transgenic hearts. These data provide strong evidence that G-CSF acts directly on cardiomyocytes and suggest that there must be cross talk between cardiomyocytes and other cell types such as endothelium.

This large body of preclinical data suggests that G-CSF treatment can significantly influence myocardial infarct repair. The mechanism probably does not involve myocardial regeneration, but may involve accelerated inflammation and/or direct cytoprotective signaling through cardiomyocytes. It is somewhat troubling that not all groups find G-CSF effective, particularly the two negative studies in

nonhuman primates. Despite these discrepancies, G-CSF treatment has already entered the clinical arena. Kang *et al.*[60] administered G-CSF or G-CSF plus intracoronary mononuclear cells to patients with recent myocardial infarctions. Although patients receiving the cell infusion showed improved ventricular perfusion and function, the study was terminated because of an unexpectedly high in-stent restenosis rate (five of seven patients receiving G-CSF+ cells, two of three patients receiving G-CSF only). Additional studies of G-CSF are currently underway in the US and Europe. One preliminary study from Kuethe *et al.*[61] did not report significant restenosis. Once again, we can only advise the reader to stay tuned to this rapidly evolving area of basic and clinical research.

Mesenchymal stem cells. Mesenchymal stem cells reside in the bone marrow's stromal compartment, the connective tissue–rich, nonhematopoietic region of the marrow. This compartment received little attention in the early days of stem cell research because it was thought to serve only a simple structural role. Interest in marrow stromal cells picked up, however, when it was discovered that these cells produced growth factors and cytokines that supported hematopoiesis, both *in vivo* and *in vitro*[62]. It was work from Caplan's group[63], however, that identified a multipotent population within the stromal compartment. They showed that a subset of cells in marrow readily gave rise to osteoblasts and adipocytes, and, with a bit more coaxing, could be induced to form chondrocytes or skeletal muscle cells. They termed these progenitors mesenchymal stem cells (MSCs). Subsequent studies demonstrated multilineage differentiation from cloned MSCs[64].

As potential applications of MSCs for cardiac repair have been recently reviewed[65], we will only touch on highlights here. Initial interest was stimulated by studies suggesting that these cells could become cardiomyocytes *in vitro*[66,67], although the necessity of using inducing regimens such as 5-azacytidine limits the clinical applicability of such strategies. Several lines of evidence indicate that direct injection of noninduced MSCs into the heart improves ventricular function postinfarction in rats[68] and pigs[69]. In exploring the potential of MSCs to become cardiomyocytes, Toma *et al.*[70] injected LacZ-labeled human MSCs into the ventricular cavity of immunocompromised mice, where a fraction was delivered to the heart through the coronary circulation. They found sporadic LacZ+ cells within the ventricular myocardium that expressed cardiac genes and were morphologically consistent with cardiomyocytes. The authors did not investigate possible fusion events with host cardiomyocytes. On the other hand, Martin *et al.*[69] directly injected MSCs into infarcted pig hearts and found that, although they stained with several muscle markers, their morphology resembled fibroblasts more than cardiomyocytes, and no electromechanical junctions with other graft cells or with host cells were observed. The MSC-engrafted hearts had much thicker infarct scars and reduced ventricular dilation, indicating that one mechanism of benefit involves attenuation of pathological ventricular remodeling.

One particularly useful property of MSCs is that they appear to have local immunosuppressive properties that permit them to survive transplantation in an allogeneic setting. Although beyond the scope of the present review, the potential mechanisms of this immunomodulatory effect of MSCs have been explored by several groups (and detailed in recent reviews[71–73]). Importantly, if the allotolerance of the MSCs is borne out in clinical trials, this will greatly reduce the cost and effort required for cell production and quality control (thereby easing regulatory burdens), and it could allow frozen cells to be shipped to the point of use when needed.

A final surprise came with the discovery that MSCs home to areas of injury. Bittira *et al.*[74] labeled rat MSCs with LacZ and delivered them to normal rats by a tail vein injection. As had been previously reported, these cells homed to the bone marrow and were rare in other locations. When treated rats were later subjected to a myocardial infarction, LacZ+ cells were found within the healing infarct. This indicates a previously unsuspected role for endogenous MSCs in homing to sites of injury. Following up on this observation, others have shown that intravenous administration of exogenous MSCs improves ventricular function postinfarction[68]. MSCs seem to home best on the first day postinfarction, a time when levels of SDF-1 are greatest in the infarct[68]. The ability of intravenously delivered MSCs to enhance ventricular function, coupled with evidence for their allotolerance, has led investigators from Johns Hopkins University and Osiris Therapeutics to launch a Phase 1 clinical trial of intravenously delivered, allogeneic MSCs in patients with recent myocardial infarction.

Resident myocardial progenitors. Until recently, the adult heart was one of the last organs in which a definitive progenitor population had not been identified. Work from several groups now suggests that the heart, like the brain, may contain a resident population of progenitor cells with cardiomyogenic potential. Beltrami *et al.*[75] isolated cells from the adult rat heart that expressed c-kit and, after expanding the cells under limiting dilution, injected them into acutely ischemic myocardium. They reported that these cells differentiated into cardiomyocytes, smooth muscle cells and vascular endothelium, replacing the majority of the infarcted tissue. Although the EGFP-tagged cells stained positively for cardiac myosin, they were much smaller than typical cardiomyocytes and had no discernible sarcomeres (appearing morphologically consistent with fibroblasts). Nevertheless, ventricular function was significantly improved in the cell-engrafted hearts. Dawn *et al.*[76] recently reported that after intracoronary administration, these cells traversed the vascular barrier and improved ventricular function in a rat infarct model.

A second progenitor population was described by Oh *et al.*[77], who isolated cells from the adult mouse heart based on Sca-1 expression. When these cells were subjected to DNA demethylation with 5-azacytidine treatment, they activated several cardiac-specific genes *in vitro*. Sca-1+ cells were then injected intravenously into mice six hours after myocardial infarction. After two weeks, engrafted donor cells expressing cardiac markers (sarcomeric actin and troponin I) were found in the host heart. Cre-Lox recombination studies indicated that approximately one half of the donor-derived cells had fused with host cardiomyocytes and the other half had differentiated without fusion. One challenge to an eventual clinical application of these cells is the necessity of treating them with 5-azacytidine to activate cardiac gene expression, as it would be preferable not to induce widespread DNA demethylation before cell transplantation.

A third progenitor population was described by Martin *et al.*[78], who isolated Hoechst dye–effluxing side population cells from the adult mouse heart. They reported that, when cocultured with unfractionated cardiac cells, some of the cardiac side population cells began to express the sarcomeric protein α-actinin. Published information on cardiac side population cells remains limited. The frequency of cardiac differentiation has not been reported, nor is it known to what extent this represents *de novo* differentiation or fusion. Potential differentiation-inducing factors produced by the cardiomyocytes have not been identified. To our knowledge, *in vivo* transplantation studies with cardiac side population cells have not been reported.

The most recent candidate progenitor population comprises cells expressing the LIM-homeodomain transcription factor islet-1 (isl1). Evans *et al.*[79] had shown that isl1+ cells contributed to a second wave of cardiomyocyte formation during development, where they contribute

substantially to the right ventricle, atria, outflow tracks and part of the left ventricle. Building on this work, Laugwitz *et al.*[80] demonstrated that cells expressing isl1 were present in the neonatal and, to a lesser extent, adult heart. They developed an elegant genetic system to inducibly mark isl1+ cells and their differentiated progeny and then studied the potential of isl1+ cells from neonatal hearts. In whole-heart cultures, the isl1+ cells initially expressed no cardiac markers, but over time most differentiated into cardiomyocytes (evidenced by gene expression, sarcomeric organization, action potentials and calcium transients). Purified isl1+ cells could be expanded in culture without differentiating, but when mixed with cardiomyocytes, they showed rapid differentiation into cardiomyocytes. Interestingly, cardiomyocyte cultures fixed in formaldehyde still induced cardiac differentiation of the isl1+ cells, effectively ruling out fusion as an explanation for activation of the cardiac program. As a cautionary note, it should be emphasized that these studies were all carried out with cells from approximately two-day-old rat pups. A challenge for this cell population will be to test whether the much rarer isl1+ cells in the adult heart can be isolated, expanded and induced to form cardiomyocytes. If so, they will be a promising population to test in cardiac repair applications.

At present, all of these resident cardiac progenitor cells are claimed to be distinct from one another: for example, the Sca-1+ cells do not express c-kit; the c-kit+ cells do not express Sca-1; and isl1+ cells express neither Sca-1 nor c-kit. It seems paradoxical that an organ known for its lack of regenerative capacity would harbor multiple nonoverlapping sets of cardiomyocyte progenitors. Clearly, these cells do not function as robust progenitors after a major insult *in vivo* (unlike, for example, skeletal muscle satellite cells). If they have an *in vivo* progenitor function, it seems more likely to involve a slow turnover process. Alternatively, their progenitor properties may be an artifact associated with *in vitro* isolation. If such a property could be reproducibly achieved and controlled, however, it would be a very useful artifact.

Embryonic stem cells

Ethical and political considerations aside, embryonic stem (ES) cells possess several features that make them an attractive source for cell-based cardiac therapies. Derived from the inner cell mass of preimplantation stage mammalian embryos, ES cells have unlimited or nearly unlimited capacity for self-renewal[81] and well-established protocols for their derivation, propagation and differentiation. ES cells have unquestioned cardiac potential, and ES cell–derived cardiomyocytes express the molecular elements required for successful electromechanical coupling with host myocardium[82–85]. Nonetheless, it has been only seven years since the isolation of human ES cells was first reported[86], so this promising cell source undoubtedly will lag behind the previously discussed adult stem cell sources in reaching clinical application.

Mouse embryonic stem cells. In contrast with their human counterparts, mouse ES cells have been available for over two decades, and this model system has taught us important lessons about cardiac development and potential regenerative applications. Doetschman *et al.*[87] were among the first to describe that removal of ES cells from the conditions needed to maintain pluripotency (that is, either primary fibroblast feeders or supplementation with leukemia inhibitory factor) results in the spontaneous formation of cystic three-dimensional aggregates, so-called "embryoid bodies," that include foci of beating myocardium. Subsequently, numerous investigators have shown that these mouse ES cell–derived cardiomyocytes exhibit a developmentally appropriate program of cardiac gene expression[88–91], as well as the expected electrophysiologic and contractile phenotype. Interestingly, even at a comparatively early time point of *in vitro* differentiation, mouse ES cell–derived cardiomyocytes show electrophysiologic specialization into ventricular, atrial and nodal/pacemaker cell subtypes[90,92].

That said, the very property of ES cells that makes them attractive for regenerative medicine applications—their capacity to differentiate into essentially all cell types of the adult organism—also represents the greatest challenge to their use. A defining characteristic of ES cells is their ability to give rise to a teratoma upon implantation, an obviously unacceptable outcome within an injured heart (**Fig. 4a**). Much effort has therefore focused on deriving highly purified preparations of mouse ES cell–derived cardiomyocytes that are free of undifferentiated stem cells.

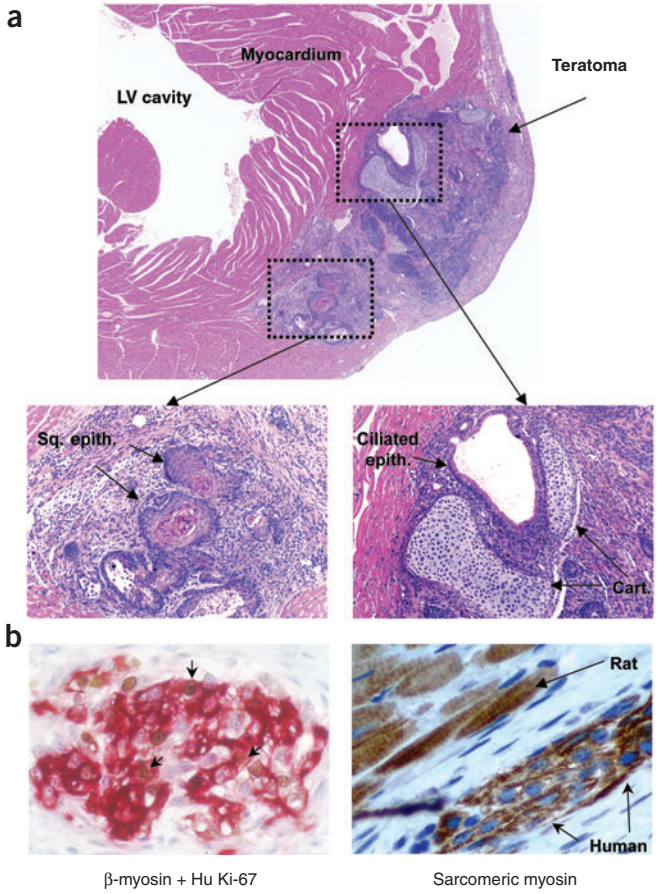

Figure 4 ES cell grafts. (**a**) Grafting of undifferentiated mouse ES cells. Undifferentiated mouse ES cells consistently formed teratomas when implanted into the hearts of immunotolerant hosts. The teratoma replaced much of the left ventricular wall. The boxed insets show ectoderm-derived keratinizing squamous epithelium (Sq. epith., left), mesoderm-derived cartilage (Cart., right) and endoderm-derived ciliated columnar epithelium (Ciliated epith., right). Tissue was stained with hematoxylin and eosin staining. (**b**) Grafting of human ES cell–derived cardiomyocytes. Human ES cells were differentiated as embryoid bodies, and cultures enriched for cardiomyocytes were transplanted into hearts of nude rats. As shown in the left panel, one week later the human cardiomyocytes could be identified by β-myosin heavy chain expression (red) and showed substantial proliferative activity, as evidenced by staining with a human-specific Ki-67 antibody (brown, arrows). As shown in the right panel, by 4 weeks the human cardiomyocytes (identified in serial sections with human-specific genomic probes) showed early sarcomere formation (sarcomeric myosin staining, brown).

Guided cardiac differentiation of mouse ES cells is an attractive approach, but progress in this area has been limited by the complexity inherent to cardiogenesis. Elegant developmental studies in avian models have shown that anterior endoderm provides factors required for cardiac induction[93,94], and avian endoderm (or endoderm-conditioned medium) enhances cardiogenesis within mouse embryoid bodies as well[95]. In terms of defined endogenous factors, transforming growth factor β1[96], bone morphogenetic proteins[96,97] or their endogenous antagonists[98], fibroblast growth factors[99], nitric oxide[100] and various members of the Wnt/wingless signaling family have been implicated in cardiac induction of ES cells[101]. An increasing number of small molecules also have been reported to promote cardiogenesis in mouse ES cells, including retinoic acid[101–103], ascorbic acid[104] and dynorphin B[105]. Perhaps not surprisingly, the efficacy of a given exogenous factor in promoting ES cell cardiogenesis is largely dependent on the concentration and timing of application. For example, late application of low concentrations of retinoic acid to mouse ES cells shows a robust procardiogenic effect, whereas earlier application of higher concentrations has been shown to actually suppress cardiac differentiation[102]. In sum, although it is likely that a suitable set of factors and/or culture conditions will be identified to optimally promote ES cell cardiac differentiation, we clearly do not have such a recipe at present. Further, it is uncertain that guided differentiation in isolation will ever produce a sufficiently pure cardiac preparation for human therapeutic applications.

For these reasons, while work toward recapitulating cardiogenesis *in vitro* proceeds, other investigators have focused on enriching mouse ES cell–derived cardiomyocytes from spontaneously differentiating embryoid bodies. Cardiogenesis within embryoid bodies is undeniably inefficient—cardiomyocytes typically constitute a maximum of a few percent of the total cell population[106]. The most successful approach so far has been genetic selection using a selectable marker driven by a suitable cardiac promoter. Field's group[106] pioneered this strategy by creating a transgene where the cardiac-specific α-cardiac myosin heavy chain promoter drove expression of aminoglycoside phosphotransferase, thereby conferring neomycin resistance selectively to cardiomyocytes in the mixed culture. After neomycin treatment of differentiated embryoid bodies for 8 days, the resulting cultures were 99.6% myosin+ cardiomyocytes, versus 0.6% in the untreated embryoid bodies. More recently, Zandstra *et al.*[103] showed that this selection strategy could be adapted to large-scale production of highly purified ES cell-derived myocytes. Other groups have subsequently used analogous genetic selection strategies, including using fluorescent protein expression driven by cardiac promoters (for example, α-cardiac myosin heavy chain, Nkx2.5, or myosin light chain-2v promoters, etc.) followed by fluorescence-activated cell sorting[107–109].

Using their genetically selected preparation, the Field group[106] was also the first to demonstrate that direct implantation of such mouse ES cell–derived cardiomyocytes into an immunocompatible recipient heart resulted in the successful formation of stable intracardiac grafts. By engrafting dystrophin-positive cells into the dystrophin-null *Dmd* mouse, these authors were able to use immunostaining with antibodies to dystrophin to identify the cardiac implants, which showed alignment and tight apposition with host myofibers. Subsequently, other investigators have implanted mouse ES cell progeny into rodent models of myocardial injury, producing a sustained improvement in contractile function by echocardiography[96,110–112]. Interestingly, in two of the previously cited reports, Terzic and coworkers implanted undifferentiated mouse ES cells into hearts of mice across histocompatibility barriers or into hearts of immunocompetent rats and still described functionally beneficial integration by morphologically indistinguishable mouse ES cell–derived cardiomyocytes[96,113]. These reported findings are intriguing for two reasons. First, in order to survive, the transplanted cells must somehow have avoided immune rejection (even across a species barrier), implying that both undifferentiated ES cells and their differentiated progeny enjoy comparative immune privilege. Second, the surviving cells did not give rise to a teratoma (that is, the expected tumor type composed of elements from all three embryonic germ layers). If correct, the latter observation would suggest that the recipient heart acts as an instructive environment, thereby selectively guiding the implanted undifferentiated mES cells down the cardiac pathway.

As depicted in **Figure 4**, results from our own laboratory do not support these provocative findings. In our hands, grafting of undifferentiated mouse ES cells into the hearts of syngeneic or immunotolerant animals produced large intracardiac teratomas. Conversely, grafting undifferentiated mouse ES cells across immune barriers resulted in teratomas that were subsequently rejected.

In analyzing studies reporting improved contractile function with mouse ES cell grafting, one should again keep in mind that the benefits of cell grafting may not be limited to (or even related to) the formation of new myocardium. As previously discussed, functional benefits might arise from secondary effects such as modulation of postinfarct remodeling or increased angiogenesis.

Human embryonic stem cells. The successful isolation of human ES cells by Thomson and coworkers[86] has opened the possibility of similar forays into cardiac regeneration using myocytes derived from a human pluripotent cell source. As with their mouse counterparts, human ES cell–derived cardiomyocytes show the expected molecular, structural, electrophysiologic and contractile properties of nascent embryonic myocardium[84,85,114,115]. The efficiency of spontaneous cardiogenesis in human ES cell–derived embryoid bodies varies with the precise culture conditions and the particular human ES cell line used[84,114,116]. While resembling their mouse counterparts in many ways, human ES cell–derived cardiomyocytes do differ in an important and potentially exploitable property: proliferation. In contrast to the limited proliferative capacity of mouse ES cell-derived cardiomyocytes[117], human ES cell–derived cardiomyocytes show sustained cell cycle activity both *in vitro*[84,118] and after *in vivo* transplantation into the nude rat heart[119].

In vivo studies with human ES cell-derived cardiomyocytes are just beginning, and there are only three published studies describing their successful application[119–121]. Two studies were aimed at demonstrating engraftment and electromechanical integration with host myocardium within the uninjured hearts of immunosuppressed experimental animals. Both used electrical mapping techniques to show that the site of human ES cell–derived cardiomyocyte implantation served as an ectopic pacemaker (that is, that a wave depolarization arose from the implant site and spread throughout the ventricles). These data unambiguously demonstrate host-graft electromechanical coupling and represent exciting proof-of-concept evidence for the potential utility of human ES cell–derived cardiomyocytes in the formation of biological pacemaker. However, several important challenges remain. First, in both studies, myocytes were derived by physically dissecting spontaneous beating foci from embryoid body outgrowth cultures. While perhaps appropriate for pacemaking applications, physical dissection is not readily scalable and may bias the selection away from quiescent, mature ventricular myocytes (as needed for infarct repair) in favor of more excitable, likely nodal precursors. Second, because primitive myocytes have a well-documented tendency to lose intrinsic automaticity with development, it will be important to follow implanted human ES cell–derived grafts to see if they maintain their pacemaking ability over time.

Recent work from our group has tested the ability of human ES cell–derived cardiomyocytes to form human myocardium after transplantation[119] (**Fig. 4b**). Embryoid body outgrowths were enriched for cardiomyocytes by Percoll fractionation, heat shocked to improve survival and transplanted into the uninjured hearts of athymic (nude) rats. Although cardiomyocyte grafts were initially small, they increased in size sevenfold over a four-week period. This physical expansion was associated with substantial proliferation, evidenced by expression of multiple cell cycle markers (Ki-67, phospho-histone H3), incorporation of bromo-deoxyuridine (BrdU) and frequent mitotic figures. The grafts expressed expected cardiac markers, such as sarcomeric actins and myosins, myosin light chain 2V and atrial natriuretic factor. Sustained proliferation by human ES cell–derived cardiomyocytes may be particularly useful for cardiac repair, because it implies that one may not have to implant the full quantity of cells required for a therapeutic effect and that gradual *in situ* expansion of the graft might also permit angiogenesis to match the increasing metabolic requirements. This important discrepancy between species also highlights the value of studying a human model system where available. Although we find these data encouraging, it remains to be seen whether the ultimate goal of successful engraftment and electromechanical coupling of human ES cell–derived cardiomyocytes can occur within the much more hostile environment of an infarct.

Several other significant obstacles must be overcome if human ES cells are to reach clinical application in ischemic heart disease. First, we will need to develop protocols for the large-scale production of highly purified preparations of cardiomyocytes. Further, given that comparatively early cultures of human ES cell–derived myocytes already show specialization into atrial, ventricular, and nodal cardiac subtypes[115], electrophysiologic considerations may demand highly purified ventricular preparations. Importantly, such preparations must not induce even a single intracardiac teratoma. In our opinion, work with mouse ES cells suggests that genetic selection may be the best means of achieving sufficient cardiac purity. A second important challenge to any therapeutic application with human ES cells will be to prevent immunologic rejection of the graft. In an excellent review, Odorico *et al.*[122] discuss potential strategies for overcoming immune rejection of human ES cells, including traditional allogeneic transplantation with pharmacologic immunosuppression, nuclear reprogramming to generate an autologous human ES cell source, genetic manipulation of major histocompatibility genes to produce a universal donor human ES cell line, and induction of immune tolerance through the transplantation of human ES cell–derived hematopoietic precursors and establishment of bone marrow chimerism.

Tissue engineering

A new discipline closely related to cell-based therapy, tissue engineering couples traditional engineering technologies such as biomaterials, bioreactors, biomechanics and controlled drug release with cell and molecular biology in an attempt to grow new tissues. Several tissue engineering approaches are being explored for cardiac repair, but owing to space limitations, we can provide only a brief overview and refer interested readers to recent reviews[123,124]. The most common approach has been to seed cardiomyocytes onto porous scaffolds, typically constructed from a biodegradable polymer such as poly(lactic-co-glycolic acid) or natural polymers such as collagen. Scaffold seeding has worked well for proliferative, hypoxia-tolerant cells, such as smooth muscle, which expand to fill void spaces after seeding, but it has been more difficult to achieve tissue-like cell densities with nonproliferative rat or mouse cardiomyocytes. Nevertheless, seeded myocardial constructs have been shown to conduct action potentials and beat synchronously[125], as well as hypertrophy in response to electrical stimulation[126]. The discovery that human cardiomyocytes derived from ES cells are proliferative should accelerate work with seeded constructs.

Zimmermann *et al.*[127] developed a novel approach to myocardial tissue engineering wherein cardiomyocytes are cast into collagen gels and mechanically conditioned in a cyclic stretching device. The cardiomyocytes in these constructs have a more natural rod-shaped morphology and well-formed electromechanical junctions, and are aligned into myofibers and capable of considerable force generation. Preliminary studies suggest that some of the cardiomyocytes in these gel-based constructs survive after transplantation into uninjured hearts and undergo further hypertrophy. A different strategy was explored by Shimizu *et al.*[128], who created sheets of cardiomyocytes by plating isolated cells onto a temperature-sensitive polymer surface. At 37 °C, the polymer is hydrophobic and promotes attachment, but when switched to 32 °C it becomes hydrophilic and causes the cell sheet to detach. They showed that, when several sheets were layered atop one another, the sheets fused and beat synchronously, and when implanted into the subcutaneous space of syngeneic rats, the constructs became vascularized and survived for 12 weeks.

One of the biggest challenges facing myocardial tissue engineering (and also much of cell-based cardiac repair) is nutrient delivery. The human left ventricle is typically 1- to 1.5-centimeters thick, yet diffusion can only supply nutrients to a depth of 150 microns. Perfusion bioreactors are being developed to permit *in vitro* growth of constructs thicker than the 150-micron diffusion limit[129]. If centimeter-thick constructs can eventually be grown and maintained *in vitro*, another major challenge will be keeping them alive after implantation until the host coronary circulation can vascularize them. One possible alternative is to engineer sub-millimeter–sized constructs that will be better able to survive on diffusion and allow them to proliferate *in situ* after implantation. This illustrates the need for research in regenerating the vasculature to proceed in parallel to that of regenerating the myocardium.

Challenges to clinical implementation

There are clearly many challenges remaining in the field of cell-based cardiac repair. In addition to those discussed above, several others deserve brief mention. The first is cell delivery. Despite ten years of work in the field, no one has optimized techniques for giving the heart an injection. As a result, ~90% of cells delivered to the heart through a needle are lost to the circulation or leak back out of the injection site[130]. Worse still, retention is extremely variable from study to study, making graft size unpredictable. Development of straightforward approaches such as improved injection media (for example, hydrogels) to increase cell retention or microneedle arrays to improve cell distribution would help move this work toward the clinic.

A second problem is the extensive death of transplanted cells. Regardless of cell type, multiple studies indicate that ~90% of cells successfully delivered to the heart die within the first week[130,131]. Some progress has been made in this area, such as heat shocking cells before engraftment[131] or overexpression of antiapoptotic proteins[131,132], but clearly there is substantial room for improvement.

A third area is proliferation control. Although cell retention or survival correlates linearly with final graft size, cell proliferation exponentially increases graft size. The ability to control the proliferation of cells after transplantation (for example, with a small molecule) would significantly improve the ability to repopulate lost tissue[133]. Finally, virtually all studies involving cell transplantation into the heart have found that scar tissue forms a barrier to proper integration of the implanted cells[134]. In the heart, scar tissue prevents grafted cardiomyocytes

from forming electromechanical junctions with host myocardium required for synchronous contraction, and it might serve as a substrate for arrhythmias. Modulating the host tissue's fibrotic response to cell implantation would move the entire field of cell therapy forward, and we think this is an area deserving of further study.

Closing perspectives

Ten years ago, the concept of regenerating the heart was radical and met with considerable skepticism. Today, using stem cells to rebuild the heart from its component parts is a mainstream experimental concept. Like most researchers in this field, we are optimistic that this approach will eventually lead to an effective clinical therapy. It is also worth sounding a note of caution, however. This field is moving extremely quickly, and expectations are high. The cell therapy community must not follow the trajectory of clinical gene therapy, where a serious clinical complication set the field back many years[135]. The heart is not likely to be regenerated in one fell swoop. More likely, we will repair the heart in small steps, perfecting our interventions over many years. In the meantime, regenerative biology will bring together basic scientists and clinicians, developmental biologists and engineers, compelling us to expand our understanding of cell biology in order to grow new tissues. And if we are successful, cell-based cardiac repair has the potential to improve the health of millions of people worldwide each year.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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