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REVIEW

Stem cells and cardiac regeneration

Alfred A. Kocher,[1] Bernhard Schlechta,[2] Aneta Gasparovicova,[2] Ernst Wolner,[2] Nikolaos Bonaros[1] and Günther Laufer[1]

1 Department of Cardiac Surgery, Innsbruck Medical University, Innsbruck, Austria
2 Department of Cardiac Surgery, Vienna Medical University, Vienna, Austria

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Correspondence
Alfred A. Kocher MD, Department of Cardiac Surgery, Medical University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria. Tel.: ++43 512 504 23806; fax: ++43 512 504 22528; e-mail: alfred.kocher@i-med.ac.at

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Summary

Despite many advances in cardiovascular medicine, heart failure (HF) remains the leading cause of death in developed countries affecting at least 10 million people in Western Europe alone. The poor long-term prognosis of HF patients, and immense public health implications has fuelled interest in finding new therapeutic modalities. Recent observations of the beneficial effect of stem cells on the damaged heart in animal experiments have generated tremendous excitement and stimulated clinical studies suggesting that this approach is feasible, safe, and potentially effective in humans. Cell-based myocardial regeneration is currently explored for a wide range of cardiac disease states, including acute and chronic ischemic myocardial damage, cardiomyopathy and as biological heart pacemakers. The aim of the present manuscript is to review the work that has been done to establish the role of stem cells in cardiac repair, give an update on the clinical trials performed so far, as well as to discuss critically the controversies, challenges and future surrounding this novel therapeutic concept.

Scope of the problem

Heart disease, including coronary atherosclerosis and myocardial infarction were known in remote antiquity, with the oldest documentation in Egyptian mummies. Description of the clinical syndrome of angor pectoris can be found in the Ebers' papyrus, the oldest preserved medical document from about 1552 BC, and clinical and prognostic data regarding cases of angina, infarction and sudden death are documented in the Corpus Hippocraticum [1]. While there are no data on the incidence in those days, cardiovascular disease has become the biggest healthcare burden of our times. Nearly 5 million Americans have heart failure (HF) today, with an incidence approaching 10 per 1000 population among persons older than 65 years of age [2]. Cardiovascular disease is the leading cause of death in Europe, of which nearly half is attributable to coronary artery disease (CAD) [3]. This situation is expected to become worse, with a sharp increase in CVD in developing countries and a predicted 25 million CVD deaths worldwide by 2020. The aging world population faces a pandemic of CAD as projected by the WHO [4].

Therapeutic options

Although there are a number of investigative therapeutic modalities for treating end-stage HF, the two primary treatment options available are pharmacologic therapy and cardiac transplantation [5]. Advances in medical therapy have had an important impact on symptom status and short-term survival of patients with moderate to severe HF. The mainstay life-saving drugs are angiotensin-converting enzyme (ACE) inhibitors and β-blockers. Additional benefits are obtained when angiotensin-receptor blockers or aldosterone antagonists are added [6]. Existing pharmacologic agents have met with only moderate success in patients with class IV HF, and the 1-year survival rate is only 40–50% [7]. Heart transplantation remains the treatment modality with the best outcome

with 5-year survival rates of around 65%, however, due to the current serious shortage of donor organs only 3000 cardiac transplants are performed each year worldwide with 15 000 patients on waiting lists for transplants [8]. The success of cardiac transplantation remains further limited by the complications of long-term immunosuppression and the development of allograft CAD. Since the inception of the artificial heart program at the National Institutes of Health in 1964, a variety of circulatory support devices have been developed and are being used as bridge to transplant and more recently for destination therapy in patients experiencing end-stage HF who are ineligible for transplantation. The randomized evaluation of mechanical assistance for treatment of congestive HF (REMATCH) trial explored the use of left ventricular (LV) assist devices (LVADs) as permanent implants and showed they can increase median survival by 7.4 months and improve functional status in comparison with medical management in end-stage HF patients. The clinically meaningful survival and quality-of-life benefit for patients with LVADs comes at a high device failure rate and numerous complications, mainly infections and bleeding as a result of necessary anticoagulant therapy, while raising the cost of end-of-life care considerably [9,10].

Cellular therapy

Functional restoration of the damaged heart presents a formidable challenge with none of the current treatment modalities leading to a reduction in scare size after myocardial infarction or significant improvement of an impaired cardiac pumping ability in HF. Conversely, cell-based cardiac repair offers the promise of regenerating damaged myocardium by rebuilding the injured heart from its component parts. Ideally, transplanted cells would mimic the lost myocytes morphologically and functionally, with the ability to contract and to establish electrical connectivity with the native myocardial cells. Exploration of stem cell transplantation as a potential means of treating patients with cardiac disorders has attracted immense scientific and public interest only recently. The concept of stem cells, however, is old and originates from attempts to understand the mechanisms of tissue homeostasis and renewal in adults, particularly in the hematopoietic system. The existence of a blood stem cell was proposed as long ago as 1909 [11]. The legend of Prometheus, who transgressed the law of the ancient gods and stole fire for humankind and was chained to the Caucasus for punishment, where a vulture preyed daily on his liver, which was renewed as quickly as it was devoured indicates that the remarkable potential of the body to rebuild itself – a key feature of stemness – was known in the distant past [12]. Meanwhile, stem cells have been identified in many adult human organs and tissues, not only in those that undergo frequent renewal, but also in others like the nervous system, which until recently was believed to be incapable of renewal during adult life [13]. The dogma of the heart as a postmitotic organ that is terminally differentiated with approximately 5 billion cells at birth which would only decrease with age was established in the 1970s and preserved for almost three decades. In the early 1990s, Anversa *et al.* described that cardiomyocytes undergo apoptosis at a significant rate and hereby the traditional view of the heart as an organ incapable of regeneration has been challenged [14]. Seminal studies and observations by the very same researchers have led to a paradigm shift in cardiac biology. They demonstrated that myocyte mitosis occurs not only in the fetal but also the adult heart and that myocyte turnover is markedly enhanced in pathologic states, such as HF and myocardial infarction [15]. Cell proliferation contributes to the homeostasis of the normal myocardium and the increase in muscle mass after myocardial infarction. The same group of investigators were the first to show dividing cells of extracardiac origin in sex-mismatched heart transplant patients, indicating that extracardiac progenitor cells are capable of repopulating most major cell types in the transplanted human heart including not only cardiomyocytes, but also endothelial, smooth muscle and Schwann cells [16]. And finally it was Anversa again who succeeded in identifying and characterizing lineage committed cardiac stem cells (CSCs) residing in the myocardium, which ultimately give rise to small developing myocytes that further evolve into the adult phenotype [17]. Thus a new conceptual framework of the heart has emerged. The heart is now viewed as a self-renewing organ in which myocyte regeneration occurs throughout the organism lifespan with CSCs preserving organ homeostasis and cell turnover. In the circumstance of a devastating, acute muscle cell death from myocardial infarction myocyte replacement by endogenous repair mechanisms to offset the extent of tissue loss is insufficient, although it could be sufficient to repair subclinical lesions after the blockage of small capillaries. As a result, the concept of cardiac regeneration by exogenous cellular elements has gained increasing attention. Initially the goal was to replace lost myocardial tissue by contractile elements. Therefore cell-based cardiac repair began with the transplantation of autologous skeletal satellite cells, progenitor cells that normally mediate regeneration of skeletal muscle [18]. However, in addition to myocardial loss, cardiomyocytes in the immediate vicinity of the scar tissue become hibernating because of insufficient myocardial perfusion. Hence, promotion of blood vessel formation is another important pillar of cardiac regeneration by stem cells. Stem cells are reported to differentiate into

cardiomyocytes, endothelial cells, and smooth muscle cells, and partially restore cardiac function suggesting new avenues for treatment of heart disease [19].

Stem cell definition

Stem cells are undifferentiated tissue progenitor cells that can proliferate and are defined by their ability to self-renew and to form one or more differentiated cell types [20–22]. They can be categorized anatomically, functionally, or by cell surface markers, transcription factors, and the proteins they express. Different populations of stem cells are distinguished by the types of specialized cells that they generate. One clear division of the stem cell family is between those isolated from the embryo, known as embryonic stem cells (ESCs), and those in adult somatic tissue known as adult stem cells. Within these categories, stem cells can be further divided according to the number of differentiated cell types they can produce. Totipotent stem cells are able to form all fully differentiated cells of the body and trophoblastic cells of the placenta. The embryo, zygote, and the immediate descendants of the first two cell divisions are the only cells considered to be totipotent [23].

Pluripotent cells can differentiate into almost all cells that arise from the three germ layers, but are unable to give rise to the placenta and supporting structures. At around 5 days after fertilization, ESCs that form the inner cell mass of the blastocyst are considered pluripotent. Multipotent stem cells are capable of producing a small range of differentiated cell lineages appropriate to their location and are usually found in adult tissues. However, the use of the term multipotent might be somewhat redundant, since some adult stem cells, once removed from their usual location seem to transdifferentiate into cells that reflect their new environment. Stem cells with the least potential for differentiation are termed unipotent; for example, the epidermal stem cell found in the basal skin layer that only produces keratinized squames [23].

Potential donor cell types

Conceptually, a variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings. Studies comparing distinct cell types are scarce. The first clinically relevant cells to be proposed as a surrogate for cardiomyocytes were skeletal muscle myoblasts. Bone marrow which is easily accessible is, at present, the most frequent source of cells used for clinical cardiac repair [24]. It contains a complex assortment of progenitor cells, including hematopoietic stem cells (HSCs), so-called side population (SP) cells, which account for most long-term self renewal [25] (of hematopoietic lineages after single-cell grafting [26]; mesenchymal stem cells (MSCs) or stromal cells [27]; and multipotential adult progenitor cells (MAPCs), a subset of MSCs [28]. Peripheral blood-derived progenitor cells are isolated from mononuclear blood cells and selected *ex vivo* by culturing in 'endothelium-specific' medium prior to reinjection into the heart [24,29,30].

Further progenitor/stem cell populations investigated include: fat tissue-derived multipotent stem cells [31] multipotential cells from bone marrow or skeletal muscle [32] somatic stem cells from placental cord blood [33], amniotic fluid-derived stem (AFS) cells [34], and cardiac-resident progenitor cells that have a heightened predisposition to adopt the cardiac muscle fate [35,36]. In each of these newer cases, techniques to isolate and purify the numerically minor population of potent cells will need to be optimized for clinical use. Most of the cells undergoing clinical evaluation are used in an autologous way, so that tissue rejection is obviated. MSCs are thought to be immune-privileged, have been successfully transplanted experimentally in an allogeneic setting without immunosuppression and are currently evaluated in a clinical trial allogeneicly [24].

Modes of cell delivery

Cells for cardiac repair can be administered in various ways. The goal of any cell delivery strategy is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area. The success of cell delivery is further determined by the local milieu, as it will influence short-term cell survival and cell properties with regard to cell adhesion, transmigration through the vascular wall, and tissue invasion. The three most frequently used routes are intracoronary, percutaneous endocardial or direct intramyocardial injection during surgery. Intracoronary infusion requires migration through the vessel wall into the damaged tissue. Some cell type like bone marrow-derived and blood-derived progenitor cells are known to extravasate and migrate to ischemic areas [37], whereas skeletal myoblasts do not. Satellite cells and mesenchymal cells have been shown to even obstruct the microcirculation after intra-arterial administration, leading to embolic myocardial damage [38]. By contrast, direct delivery of progenitor cells into scar tissue or areas of hibernating myocardium by catheter-based needle injection, direct injection during open-heart surgery, and minimally invasive thoracoscopic procedures are not limited by cell uptake from the circulation or by embolic risk. An

offsetting consideration is the risk of ventricular perforation, which may limit the use of direct needle injection into freshly infarcted hearts. In addition, it is hard to envisage that progenitor cells injected into uniformly necrotic tissue – lacking the syncytium of live muscle cells that may furnish instructive signals and lacking blood flow for the delivery of oxygen and nutrients – would receive the necessary cues and environment to engraft and differentiate. Most cells, if injected directly, simply die [39]. Finally, in diffuse diseases such as dilated nonischemic cardiomyopathy, focal deposits of directly injected cells might be poorly matched to the underlying anatomy and physiology. In experimental models, intravenous delivery of endothelial progenitor cells (EPCs) has been shown to improve cardiac function after AMI [40,41]. However, homing of cells to noncardiac organs limits the clinical applicability of this approach. Thus, it already appears likely that patients' individual pathobiology – the specific underpinnings of their HF – will ultimately influence, if not dictate, the source and route chosen among potential progenitor cell therapies. Given such variations in the underlying clinical context, it is not yet possible on the basis of existing pilot clinical trials, whose design and findings are detailed below, to assert an 'optimal' cell type or 'best' mode of delivery [24].

Possible mechanisms of action

The mechanisms by which stem cells repair damaged myocardium or lead to improvement in cardiac function are largely unknown, however, the two fundamental activities of stem cells are, respectively (i) the use of cell therapies to directly or indirectly improve neovascularization, i.e., vasculogenesis, angiogenesis and arteriogenesis; and (ii) differentiation into cardiomyocytes and formation of myocardial tissue. Functional benefits may also be mediated through paracrine secretion of growth factors or cytokines which could indirectly promote survival of cardiomyocytes by inhibition of cardiac apoptosis, may lead to mobilization of endogenous progenitor cells and affect remodeling. Stem cells may also fuse with the native dysfunctional myocytes to augment function [42,43]. A wide range of cell population have been tested and almost all appear to confer benefit which hints at a possible involvement of various mechanisms. The extent to which these different mechanisms are active may critically depend on the cell type and setting, such as acute or chronic injury. Because fundamentally different pathophysiologic processes are targeted and yield some improvement in both experimental and clinical trials, the mechanisms of action are not predetermined but depend also on the host environment. For example, in patients with acute myocardial infarction (AMI), progenitor cell transplantation is predicted to significantly modify postinfarction LV remodeling through enhanced neovascularization and reduced cardiomyocyte apoptosis, irrespective of long-term engraftment and transdifferentiation. Conversely, these mechanisms may have little or no benefit in patients with long-established scars, apart from the functional rescue of hibernating myocytes. Those distinctions might not matter, since patients benefited from many established therapies – including aspirin – before the underlying mechanisms were elucidated. The ultimate success of cell therapy will rest on its ability to show clinical efficacy rather than on the imputed mechanism [24].

Tissue regeneration – goal of cellular transplantation

A promethean goal of medicine is to repair damaged organs. Regeneration is an essential function of the human body, with the relatively long-life span predicated on processes that mend damaged muscles, repair broken bones, replenish blood, and restructure vessels. Most human tissues can rebuild themselves, recapturing their original shape over and over. The heart is less well equipped to deal with injury. An alternative strategy to repair a damaged heart is to stimulate it to regenerate or heal itself. While the sudden interruption of the blood supply caused by occlusion of an artery in mammals and amphibians typically leads quickly to cell death, loss of tissue, and fibrous scar formation, Poss *et al.* [44] demonstrated that zebrafish fully regenerate hearts within 2 months of 20% ventricular resection (Fig. 1). Regeneration occurs through robust proliferation of cardiomyocytes localized at the leading epicardial edge of the new myocardium. They went on to demonstrate that inhibition of regeneration would lead to scarring, since the hearts of zebrafish with mutations in the Mps1 mitotic checkpoint kinase, a critical cell cycle regulator, failed to regenerate and formed scars. Thus, injury-induced cardiomyocyte proliferation in zebrafish can overcome scar formation, allowing cardiac muscle regeneration. Zebrafish will be useful for genetically dissecting the molecular mechanisms of cardiac regeneration and might guide the way for cardiac regeneration in humans [44].

Indications for stem cells and the heart

Cellular cardiomyoplasty is intriguing, novel and complex. While its full potential remains to be seen, attempts to treat a variety of different cardiac pathologies have been reported with encouraging results. Stem cells have been shown not only to improve myocardial function after infarction, but also to yield beneficial effects in chronic ischemia [45], dilated cardiomyopathy, [46–49]

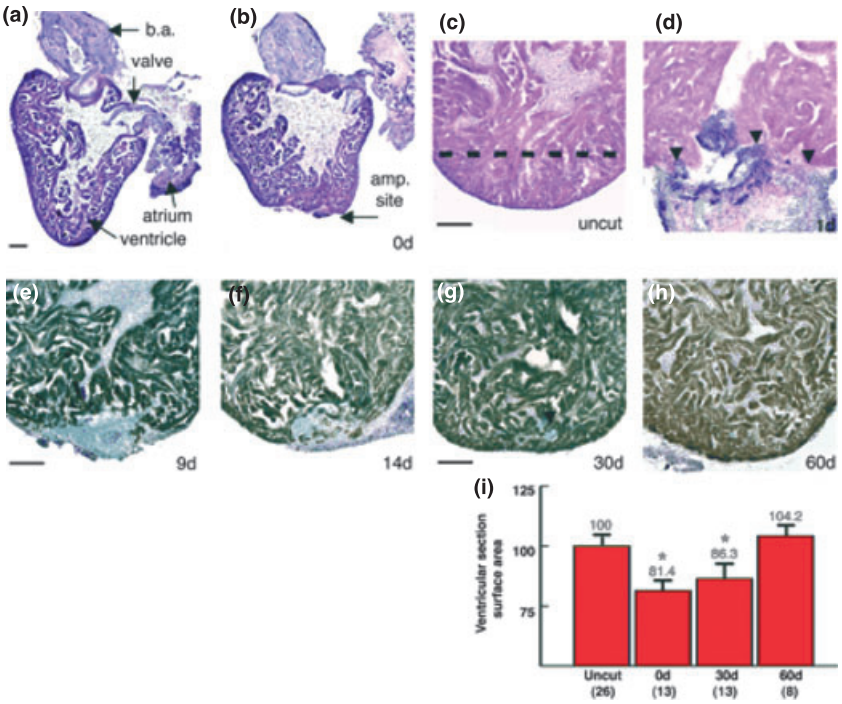

Figure 1 Regeneration of ventricular myocardium in the resected zebrafish heart. Hematoxylin and eosin stain of the intact zebrafish heart before (a) and after about 20% ventricular resection (b). (c) An intact ventricular apex at higher magnification, indicating the approximate amputation plane (dashed line). All images display longitudinal ventricular sections of the amputation plane. (d) The large clot is filled with nucleated erythrocytes (arrowheads). (e) The heart section is stained for the presence of myosin heavy chain to identify cardiac muscle (brown) and with aniline blue to identify fibrin (blue). The apex is sealed with a large amount of mature fibrin. (f) The fibrin has diminished, and the heart muscle has reconstituted. (g) A new cardiac wall has been created, and only a small amount of internal fibrin remains (arrowhead). (h) This ventricle shows no sign of injury. (i) Quantification of healing at 0, 30, and 60 dpa. Values represent the size of the largest ventricular section (mean ± SEM; $*P < 0.05$); parentheses indicate the number of hearts examined. Scale bars, 100 μm. Figure adapted from Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science* 2002: 298: 2188. Reprinted with permission from AAAS.

rhythm disturbances, and more recently in acute myocarditis [50].

Stem cell therapy in nonischemic dilated cardiomyopathy

The role of stem cell therapy in nonischemic dilated cardiomyopathy has only been explored in single patients [48] pilot studies [47] and clinical trials published in Chinese with no abstract in English available. While stem cell injection in those patients resulted in significant improved NYHA functional class and increased ejection fraction further studies are required to elucidate possible mechanisms and the true potential of this treatment approach in this setting.

Stem cells as biological heart pacemakers

Abnormalities in the pacemaker function of the heart or in cardiac impulse conduction may result in the appearance of a slow heart rate, traditionally requiring the implantation of a permanent electronic pacemaker [51]. Grafting stem cells as peacemaking cells, either derived directly during the differentiation of human ESCs (hESCs) or engineered from MSCs in an attempt to generate biological alternatives to implantable devices into the myocardium has been reported by several groups. Back in 1993, Maltsev *et al.* [52] reported of ESCs differentiated *in vitro*, into cardiomyocytes (CMs) representing phenotypes corresponding to sinus node, atrium or ventricle of the heart. Their action potential revealed shapes, pharmacological characteristics and hormonal regulation inherent to adult sinus nodal, atrial or ventricular cells. Xue *et al.* [53] demonstrated that electrically active, donor CMs derived from hESCs could be stably genetically engineered by a recombinant lentivirus to functionally integrate with otherwise-quiescent, recipient, ventricular CMs to induce rhythmic electrical and contractile activities *in vitro*. The integrated syncytium was responsive to the β-adrenergic agonist isoproterenol as well as to other pharmacologic agents, such as lidocaine. Similarly, a functional hESC-derived pacemaker could be

implanted in the left ventricle *in vivo*. Detailed optical mapping of the epicardial surface of guinea pig hearts transplanted with hESC-derived CMs confirmed the successful spread of membrane depolarization from the site of injection to the surrounding myocardium.

Co-workers generated CM cell grafts from hESCs *in vitro*, using the embryoid body differentiating system [54,55]. This tissue formed structural and electromechanical connections with cultured rat CMs. After transplantation into the hearts of swine with complete atrioventricular block these hESC-derived CMs showed integration and pacing function, as assessed by detailed three-dimensional electrophysiologic mapping and histopathologic examination.

Still the field is very much in its infancy. Little is known of the longevity of the constructs used and the challenges with regard to teratogenicity of hESCs and rejection.

Stem cells in ischemic heart disease

Ischemic heart disease is by far the most prevalent pathology of the heart, the most important field of stem cell research for the heart and thus the focus of the current review. Despite medical therapy most patients will develop HF or major LV systolic dysfunction at some time after an MI. While mortality may be decreasing, the morbidity associated with coronary heart disease is increasing as more people survive acute MI and grow old. Therefore, a fundamental shift in the underlying etiology of HF is becoming evident, in which the most common cause is no longer hypertension or valvular disease, but long-term survival after AMI. The molecular, cellular, biochemical, and structural changes occurring in the myocardium, often referred to as remodeling, have been studied extensively in patients with HF [56]. After MI, a series of progressive adverse effects takes place, including: (i) noncontractile and potentially expanding scar tissue forming in the infarcted zone; (ii) the volume load induced by such expansion; and (iii) the pressure load induced by the increased volume load. The mixed pressure and volume load [57,58] leads to a remodeling of the entire left ventricle in proportion to infarct size [59] with a fall in ejection fraction. However, the increase in the left-ventricular volume augments the stroke volume by the Starling mechanism so that cardiac output is relatively normal. Figure 2 summarizes the three patterns of remodeling. Early postinfarct remodeling could be beneficial and promote survival, but with deleterious hemodynamic consequences in the long-term. The increase in wall stress in the scar area results in lengthening of the remaining contractile tissue, and can occur up to 2 years postinfarct with increased cardiovascular death. In the postinfarct period, enhanced activity of metalloproteinases breaks down the existing collagen while promoting the formation of new collagen that is poorly cross-linked, resulting in a side-to-side slippage of myocytes that contributes to ventricular remodeling [60,61] with thinning of the left-ventricular wall. Some manifestations of remodeling can occasionally be reversed by load reduction aiming to lessen the distending or deforming forces. ACE-inhibitor therapy helps to attenuate the increase in wall stress and to reduce dilation of the left-ventricle. If left-ventricular dilation is avoided, then the pure hypertrophic response of surviving myocytes gives hemodynamic benefit. Such early recovery could be explained by postreperfusion stunning or intrinsic repair of the surviving left-ventricular myocytes. β-blockade also reduces the afterload, and hence the intracavity systolic pressure, increases the ejection fraction while reducing end-diastolic left-ventricular volumes. There is also evidence that prolonged, near-complete unloading of the left ventricle with the use of a LVAD is associated with structural reverse remodeling accompanied by functional improvement [60,61].

Conceptually, replacement of akinetic scar tissue by viable myocardium should improve cardiac function and impede progressive LV remodeling. It is important to emphasize that the basic treatment modalities of CAD remain coronary artery bypass grafting (CABG), percutaneous coronary intervention (PCI), and medical therapy. All of which can only delay, but not reverse remodeling. Stem cell treatment of the heart has not been shown to lead to the development of large calibre coronary vessels but rather to capillaries and arterioles by both angiogenesis and vasculogenesis. Therefore, stem cells are either used as adjunct to PCI or CABG or in patients with angiographically proven coronary CAD without viable percutaneous or surgical treatment options. These include patients with diffuse small vessel disease, in-stent restenosis, and chronic total occlusion. It has been estimated that over 100 000 patients may be in this 'no-option' group in the USA each year [62]. Many alternative approaches have been tested in the past, including transmyocardial laser revascularization, active and passive cardiomyoplasty, gene therapy, surgical ventricular remodeling, coronary endarterectomy and growth factor application, most of which yielded no or very little improvement at best.

While zebrafish fully regenerate hearts within 2 months of 20% ventricular resection, cardiac injury in mammal typically leads to scarring, with minimal regeneration of heart muscle. Potential ways to gain the regenerative capacities of a zebrafish include overriding cell cycle checkpoints controlling myocyte proliferation, inhibiting pro-death pathways (apoptosis), supplementing angiogenic mechanisms via growth factors, inducing mobilization of precursors of cardiac muscle or providing exogenous cells

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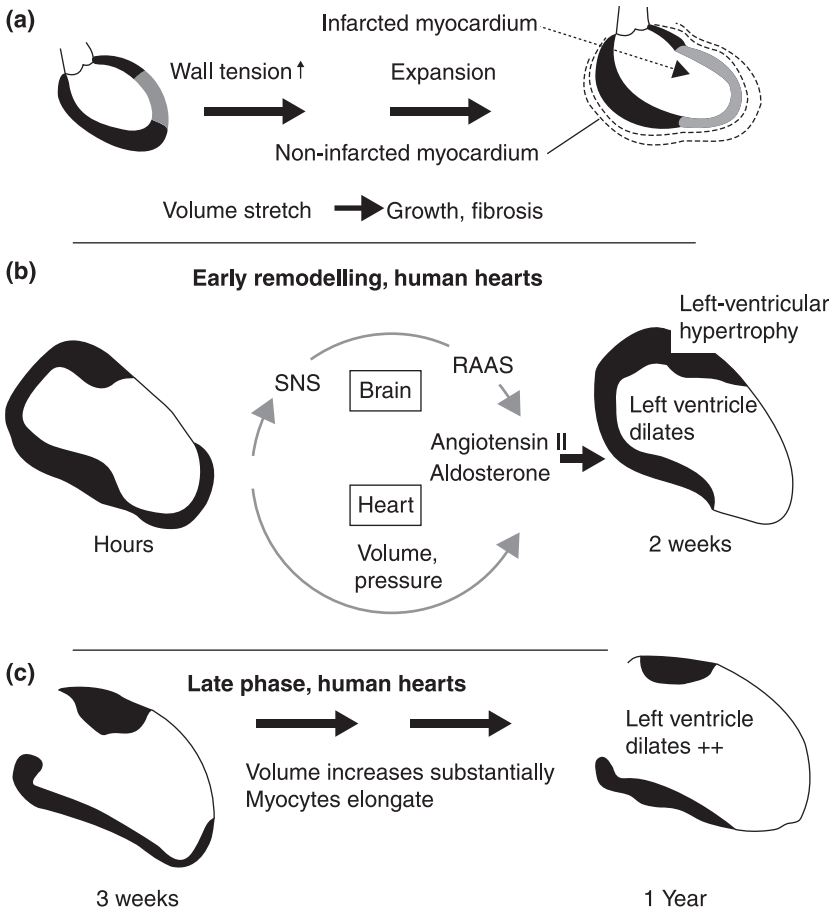

Figure 2 Postinfarct left-ventricular remodeling patterns. (a) Simplified overall pattern based on animal models. There is potential for substantial remodeling of infarct zone and increased volume of non-infarcted zone. Endocardial wall motion of human hearts in (b) early postinfarct phase and (c) late postinfarct phase derived from contrast ventriculography. Black, extent of preserved movement of endocardial surface in noninfarcted zone. SNS, sympathetic nervous system. Figure adapted from McKay RG, Pfeffer MA, Pasternak RC, *et al*. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation* 1986; 74: 693. Reprinted with permission from Lippincott Williams & Wilkins.

as surrogate or precursors of cardiac muscle. Inducing mobilization of precursors of blood vessels and cardiac muscle may be one way to enhance cardiac repair. Indeed, vascular endothelial growth factor (VEGF) [63] and granulocyte-macrophage colony stimulating factor (GM-CSF) [64] were found to augment EPC levels and improve neovascularization, and subsequent studies documented EPC mobilization by numerous other proangiogenic growth factors – stromal cell-derived factor-1 (SDF-1), angiopoietin-1, placental growth factor, and erythropoietin [65–67]. The number of circulating EPCs in adults can be enhanced by treatment with statins and estrogens as well as exercise. In these first small trials, an increase in restenosis was observed, which may be partially explained by the rise in leukocyte number to leukemic levels via plaque growth or destabilization. Adverse vascular events have also been attributed to granulocyte colony stimulating factor (G-CSF) in patients with intractable angina who were not candidates for revascularization and even in patients without cardiac disease [68,69]. It may be preferable to use strategies that augment circulating progenitor cells without causing massive inflammation. A second open question regarding systemic mobilization is whether enough progenitor cells will home where needed, to the sites of cardiac injury, since systemically administered human progenitor cells were predominantly trapped by the spleen [70]. Stem cell mobilization might be most worthwhile combined with selective enhancements of progenitor cell homing or as a prelude to isolating cells for local delivery.

Clinical trials

The experimental evidence that administration of stem cells leads to restoration of myocardial function in models of ischemic cardiac damage is overwhelming and exciting [24,71]. Because of this success in animal studies, translation into clinical trials started early [72]. Of note, the most frequently tested cell types in clinical trials are skeletal myoblasts and bone-marrow- or blood-derived progenitor cells. One major pitfall of using autologous cells is that the number of functional stem cells is generally depleted with a markedly reduced proliferation potential in the elderly and patients with cardiovascular disease.

Skeletal myoblasts

Skeletal myoblasts, or satellite cells, are progenitor cells that normally lie in a quiescent state under the basal membrane of mature muscular fibers and normally mediate regeneration of skeletal muscle. They represent an autologous source of cells that demonstrate a contractile phenotype. These cells can be expanded in culture and up to 10^9 cells have been grown from a few grams of muscle tissue. Myoblasts differentiate into myotubes and multiple lines of evidence now indicate that these cells retain skeletal muscle properties when transplanted into an infarct scar with the exception of rare fusion events between skeletal muscle cells and cardiomyocytes. Although myotubes remain functionally isolated as they do not couple with resident cardiomyocytes electromechanically and therefore do not beat in synchrony with the rest of the heart, studies in small and large animal models of myocardial infarction have reported beneficial effects of myoblast grafting on both systolic and diastolic performance [73–75]. Part of the protection seems to result from reduced ventricular dilatation, although the complete basis for improved mechanical function is currently unknown. Concern exists about the possible occurrence of serious arrhythmias, a complication which has been shown only in case of skeletal myoblast transplantation [76]. SMs might induce arrhythmias by several mechanisms, such as electrotonic stimulation of cardiac cells, electrical heterogeneity of action potentials, increased nerve sprouting, and local tissue injury induced by intramyocardial injection. Since cardiac rhythm disturbances have not been seen with other cell types the latter mechanism are somewhat unlikely.

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. The use of SMs in humans was first reported by Menasche *et al.* in a single patient with severe ischemic HF [77]. Autologous skeletal myoblasts were implanted into the postinfarction scar during CABG of remote myocardial areas. Five months later, there was an evidence of contraction and viability in the grafted scar on echocardiography and positron emission tomography and symptomatic improvement. Subsequent nonrandomized studies showed an improvement in symptoms and LV ejection fraction (LVEF) [78–80]. A potential safety concern is that four of 10 patients in one trial experienced ventricular arrhythmias, necessitating implantable defibrillators [81]. The results of the phase II, first randomized, placebo-controlled trial Myoblast Autologous Grafting in Ischemic Cardiomyopathy (Magic) were presented at the Annual Meeting of the American Heart Association in November 2006 in Chicago. The study included 97 patients at 24 medical centers in Europe undergoing CABG after myocardial infarction with moderate to severe LV systolic dysfunction. All patients received an implanted cardioverter defibrillator. The study was ended early, since the treatment was not superior to placebo on the primary endpoints of improvement in regional contractility or global function. A significant decrease was documented of LV volumes, a finding which might be clinically relevant since ventricular dimensions are predictors of outcome. Long-term follow-up data are awaited and the rather not encouraging results presented so far should be considered as preliminary data, as analyses may change in the final publication [82].

Progenitor cells

A number of clinical studies employing bone-marrow derived stem cells (BMCs) have been performed to date with only few randomized, controlled trials [40,71,83]. The largest study of cardiac cell therapy reported by Schächinger *et al.* [84], the reinfusion of enriched progenitor cells and infarct remodeling in AMI (REPAIR-AMI) trial, is a multicenter trial of intracoronary infusion of BMC after successful PCI for AMI. At 4 months, the absolute improvement in LVEF, measured by angiography, was greater among patients treated with BMC than among those given placebo (5.5% vs. 3.0%, $P = 0.01$). Subgroup analysis suggested that the benefit was greatest in patients with the worst LVEF at baseline. This double-blind and fully controlled trial provides the best evidence yet for beneficial effects of BMC after AMI. Enthusiasm is tempered somewhat by the modest size of the effect and by a recent report from the bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial that the relative improvement in LVEF after infusion of BMC at 6 months, as compared with no infusion, was no longer significant at 18 months, suggesting that the main effect was an acceleration of recovery [85]. While data on ventricular function at 1 year are not available for REPAIR-AMI trial, it could be demonstrated that intracoronary administration of BMCs is associated with a significant reduction of the occurrence of major adverse cardiovascular events after AMI including death, myocardial infarction, or necessity for revascularization and rehospitalization for HF compared to patients receiving placebo. In contrast, in the smaller autologous stem-cell transplantation in AMI (ASTAMI) trial involving three noninvasive imaging methods, Lunde *et al.* [86] did not find a significant improvement in LVEF at 6 months in the mononuclear BMC group, as compared with the control group. The study was powered to have an 80% chance of detecting a change of 5% points in LVEF; thus,

a smaller effect could have been missed. However, the change closest to achieving significance – the change in LVEF as measured by magnetic resonance imaging ($P = 0.054$) – actually favors the control group, arguing against this explanation. Technical differences in the characteristics or handling of the infused BMC might explain the different outcome. Of note, the median number of mononuclear cells injected in this trial was 68×10^6 and the median number of CD34+ cells was a mere 0.7×10^6. Janssens *et al.* [87] also did not detect an improvement in global ventricular function at 4 months in the BMC group as compared with the control group, although infarct size was reduced and regional wall motion was improved in the BMC group. The identification of features of BMC preparations and of patients that are predictive of a favorable response should help to resolve these discrepancies and to focus future trials. Given the relatively small number of events, this result will require replication in larger cohorts. However, it reinforces the message that BMC infusion is not only feasible but also safe, and it raises the possibility that clinical benefits may exceed the modest improvement seen in ventricular function. These studies provide a realistic perspective on this approach while leaving room for cautious optimism and underscoring the need for further studies.

The transplantation of progenitor cells and recovery of LV Function in Patients with chronic ischemic heart disease (TOPCARE-CHD) trial by Assmus *et al.* [88] evaluated the effects of BMC or progenitor cells derived from circulating blood (CPC) in patients with chronic ventricular dysfunction. In this randomized, crossover trial, the absolute change in LVEF was significantly greater among patients receiving BMC than among those receiving CPC. The groups received the other type of cell in the next phase of the trial, but the result was independent of the order in which the cells were given, suggesting that the BMC effect is somewhat specific. Which quantitative or qualitative differences in the cell populations account for their different effects is currently unknown. Although the benefit observed after BMC infusion was modest (an increase in LVEF by 2.9% points), it is remarkable that any benefit was seen in these patients, who were studied on average more than 6 years after infarction and who were already receiving optimal medical care. The TOPCARE-CHD trial suggests that BMC can have effects beyond simple acceleration of healing after infarction. Whether repeated infusions would yield additive benefits and whether these benefits would persist will be important questions for future trials. Assmus herby confirms the data by Willerson *et al.*, who described for the first time that injection of bone marrow cells is not only safe but also increases exercise capacity in patients with ischemic cardiomyopathy who were heart transplant candidates [45].

Future strategies

Stem cell types that might have the most potential for future applications for treating damaged heart muscle are ESCs, amniotic stem cells and MSCs, since experimental studies using these cells show the most promising results so far. Another realistic road to success is cardiac tissue engineering with the true potential of full myocardial regeneration.

Embryonic stem cells

Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos, they grow indefinitely in an undifferentiated state whilst retaining the ability to differentiate to all cell types in the adult body including cardiomyocytes [89]. In culture these cells contract rhythmically [90]. ES require special protocols for optimal maintenance of self-renewal and efficient differentiation [91]. Methods for upscaling have been described for mESCs and pure populations of up to 10^9 cardiomyocytes have thus been generated [92,93]. Limited experience with ESC-CM [94–96] indicates that the potential for cardiac repair is higher compared to bone marrow cells. It has been postulated that ESCs lack MHC protein expression and therefore do not evoke an immune response in the host. However recent studies showed that hESCs do express MHC class I molecules [97] albeit at low levels and expression increased upon differentiation *in vitro*. Hence ES appear not to be immune privileged. A possible solution for graft rejection is banking of hECSs with a range of HLA profiles. Because of unresolved ethical and legal issues, concerns about tumorogenicity and arrhythmogenecity of the cells, and the need to use allogeneic cells for transplantation ESCs have not been investigated broadly and will not be used clinically in the near future.

Amniotic stem cells

In a manuscript published in January 2007 in Nature Biotechnology, DeCoppi *et al.* [34] reported the isolation of a new type of stem cell from amniotic fluid that has many characteristics of ESCs without the ethical baggage. AFS cells seem to represent an intermediate stage between embryonic and adult stem cells in terms of their versatility. They are fully undifferentiated and pluripotent, i.e., having the potential to give rise to multiple lineages including representatives of all three embryonic germ layers. The full range of adult somatic cells to which amniotic stem cells can give rise remains to be determined. AFS grow rapidly, doubling every 36 h, and the cell lines are capable of extensive self-renewal without dif-

ferentiation. Unlike ES cells, they can be readily obtained from routine clinical amniocentesis specimens without harm to the donor, and they multiply indefinitely without forming tumors. AFS may provide a convenient source of stem cells, as they can be propagated easily, maintain genetic stability and can be induced to differentiate into myogenic cells. Banking of cell lines from 100 000 pregnancies could offer reasonably good tissue matches to 99% of the population [98].

Mesenchymal stem cells

Compared with other cells types considered for cardiomyoplasty, MSCs appear to possess unique properties that may allow for convenient and highly effective cell therapy. MSCs are found in many tissues and participate in adult growth as well as damaged tissue repair and regeneration [99]. Bone marrow provides the best accessible and renewable source of adult MSCs. MSCs have been tested for their ability to differentiate readily into several lineages *in vitro*, including chondrocytes, adipocytes, osteocytes and their potential to supply growth factors and cytokines to repairing tissue [100]. hMSC from a modest bone marrow aspirate can be expanded *in vitro*, ≥1 million-fold and retain the ability to differentiate to several mesenchymal lineages. MSCs are thought to be immune-privileged, which may be related to their secretion of immunosuppressive factors or the cell surface phenotype that is of low immunogenicity. Long-term allo-MSC engraftment in infarcted myocardium in rats and in swine in the absence of immunosuppression without evidence of immunologic rejection was demontrated [101,102]. MSC can be delivered systemically, and differentiate into a cardiomyocyte-like phenotype when implanted in healthy myocardium [103]. As opposed to the muscle precursor cells, allo-MSCs have the ability to be used immediately after acute injury. Furthermore, MSCs can be readily transduced by a variety of vectors and maintain transgene expression after *in vivo*, differentiation. The ability to treat MI patients with allo-MSCs in an emergent setting at the time of coronary reperfusion may constitute a distinct clinical advantage over autologous cellular cardiomyoplasty and is currently being tested in a study sponsored by Osiris Therapeutics. The current results from many labs and early cardiac clinical studies suggest important therapeutic approaches will be forthcoming through MSC use [104–107].

Cardiac tissue engineering

A different concept in cardiac regeneration is grafting *ex vivo* engineered heart muscle. This approach may theoretically allow complete replacement of diseased myocardium or reconstitution of cardiac malformations. Large myocardial patches depend critically on metabolic supply, thus vascularization is crucial. Not only structural but also electrical integration into the host myocardium is necessary. Zimmermann *et al.* [108] have developed a methodology to create engineered heart tissue (EHT) from neonatal rat heart cells, liquid collagen I and Matrigel as well as growth supplements, reconstituted in circular molds and subjected to mechanical strain. Under these conditions, cardiac organoids developed spontaneously and showed contractile as well as electrophysiologic properties of working myocardium. Implantation experiments in healthy rats showed survival, and signs of terminal differentiation of EHT grafts.

In a rodent model of myocardial ischemia EHTs integrate and electrically couple to host myocardium display strong vascularization and exert beneficial effects on systolic and diastolic LV function without inducing arrhythmias. This observation is not trivial given the fact that EHTs are not homogeneous heart muscles but organoids consisting of muscle strands, primitive capillaries, fibroblasts, smooth muscle cells and macrophages in a collagen matrix. Although complete reversal of myocardial dysfunction after EHT engraftment was not observed, this study can serve as a proof of principle for a tissue engineering approach in repair of cardiac muscle. However, cardiac tissue engineering is still in its infancy with several important questions that remain in terms of potential clinical applications [108].

Challenges and future

There is evidence across species that regeneration of damaged myocardium occurs as part of a natural repair process, even though the degree of repair ranges widely from the limited induction of mitosis and chemoattraction of extracardiac cells in humans to restitutio ad integrum in the zebrafish. There are many controversies and contradictory publications in stem cell research. In particular, the experimental results of Anversa *et al.* concerning stem cell transdifferentiation into cardiac myocytes has created a profound scientific dispute with two articles by Balsam *et al.* and Murry *et al.* published in the scientific journal Nature with the sole aim of proofing Anversa *et al.* wrong [71,109,110]. Despite the fact that both groups could not demonstrate transdifferentiation of a single bone marrow derived stem cell into a cardiomyocyte, Balsam *et al.* [110] still described a significant, sustained improvement in LVEF in the treatment group as compared to saline controls. The observation that stem cells might augment and assist cardiac regeneration has not only caused controversies, but also led to enormous excitement and intense investigations in the rapidly

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advancing field of cellular plasticity have elucidated some aspects of the scientific foundations of cardiac repair. Knowledge created by basic scientists and clinicians, developmental biologists and engineers has led to a better understanding of the molecular signals and cues of cardiac regeneration, cardiopoiesis and cardiomyogenesis and provided us with greater insights into human biology. The use of stem- and progenitor cells for therapeutic intervention in cardiovascular disease holds not only great promise, but also harbors significant controversy. Despite the advances that have been made in this broad area, it is important to emphasize that there are still fundamental questions that need to be addressed both experimentally and clinically regarding potential features of cell repair. The fact that a plurality of cell types, -preparations, -delivery approaches in a small number of patients with different disease states have been used in mostly uncontrolled clinical trials does not allow for clear answers yet. The most eminent unresolved issues are: cell delivery to the heart and optimization of intramyocardial cell retention and distribution, the best route of delivery, time of transplantation, cell type, cell number, viability of grafted cells. The development of new interventional cardiology procedures for myocardial mapping and cell delivery will allow for a more exact deposition of stem cells. Some experiments have indicated that that only 1.3–2.6% of infused BMC are retained in the heart and <3% of MSCs administered by direct injection persist after 2 weeks [37]. This modest extent of engraftment is only slightly augmented by delivery of higher numbers of cells. Successive and repeated cell injection might help to overcome this problem at least partially. Survival of cells has been shown to be very low with few to no cells detected at a follow up of 16 weeks in some studies. Prevascularization of myocardial scars with angiogenic therapy has led to improved local conditions for cell survival [111,112]. The rapid loss of grafted cells is not only biologically caused. Mechanical leakage and washout may account for a major portion of cell loss after cell implantation as experiments by Teng *et al.* [113] revealed a cell retention rate of only 11% in the beating porcine heart versus 67% in the nonbeating heart. Efforts aimed at reducing mechanical loss in the beating heart may yield a greater benefit than those targeting biologic loss alone. Strategies to genetically modify stem cells aimed to improve survival have been employed [114]. Hill *et al.* [115] observed a strong correlation between the number of circulating EPCs and the subjects' combined Framingham cardiovascular risk factor score. Therefore, with the onset of disease (or the presence of risk factors), the relevant cells appear to decrease in number and lose their reparative function, raising the question what's the chicken, what's the egg. Despite the high number of stem cell studies performed, there is still no consensus on the optimal/minimal cell number required to achieve any effect. In fact in a few clinical studies, investigators used a cell number in clinical trial that would barely suffice to treat a mouse heart [88]. While functional improvement of the infarcted heart by stem cells has been recognized even by fierce disbelievers in cellular cardiomyoplasty the way by which stem cells regenerate the heart are not yet elucidated. A surprisingly wide range of nonmyogenic cell types improves ventricular function, suggesting that benefit may result in part from mechanisms that are distinct from true myocardial regeneration [109,116]. Future trials should be randomized, controlled and designed and powered to examine clinical end points and patients should be followed over the long term and for both beneficial and adverse effects. Simultaneously, we must continue to support basic and translational research that

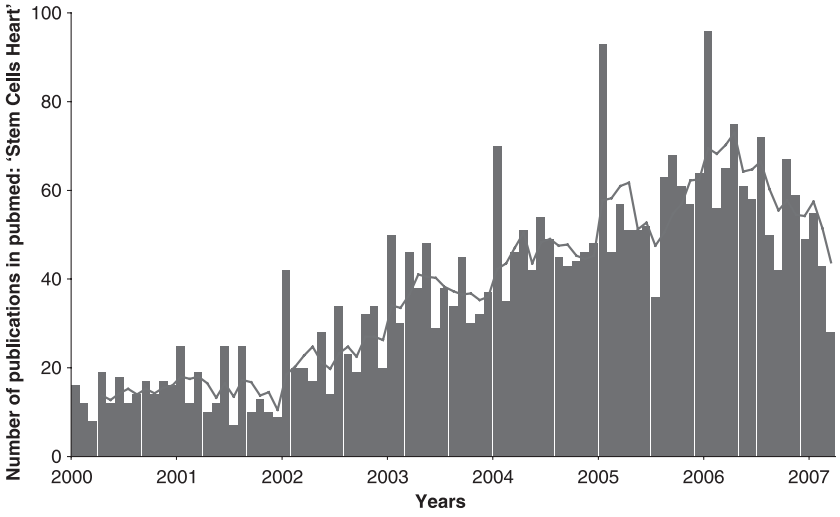

Figure 3 Linear increase in the number of scientific manuscripts on ''stem cells and heart''.

can help guide clinical investigation. More than a decade ago, The concept of cell replacement therapy to treat HF was born more than a decade ago and a flurry of activity ensued (Fig. 3) with an explosion of new reports on human stem cells isolated from a variety of sources including embryonic, fetal, and adult tissues [23].

The recognition that stem cells are found in many adult tissues and that these cells are capable of regenerating damaged organs has been very exciting with a peak in scientific publications in 2006. Meanwhile cell-based rebuilding of the heart is a mainstream experimental concept and preliminary clinical evidence suggests remarkable biologic effects and improvements by stem cell therapy [117–124]. The idea that adult stem cells are capable to fully regenerate the heart has not been proven so far. There is too much at stake for too many patients with cardiac failure to jump to any conclusions or obstruct scientific avenues like exploration of ESCs. Much more work needs to be done before cell based therapy can be used routinely in the clinical setting for people. We are positive, however, that the exciting approach of cellular cardiomypoplasty will lead to an effective clinical therapy and thus has the potential to improve the health of millions of people worldwide each year. The measure of success of this strategy remains the normal anatomy and physiology of the heart.

Authorship

AAK: wrote the paper; BS, AG, EW, NB, GL: analysed data.

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