REVIEW ARTICLE

Cardiac regeneration: current therapies—future concepts

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ABSTRACT

Cardiovascular disease (CVD) continues to be one of the main causes of death in the western world. A high burden of disease and the high costs for the healthcare systems claim for novel therapeutic strategies besides current conventional medical care. One decade ago first clinical trials addressed stem cell based therapies as a potential alternative therapeutic strategy for myocardial regeneration and repair. Besides bone marrow derived stem cells (BMCs), adult stem cells from adipose or cardiac tissue have been used in current clinical studies with inconsistent results. Although outcomes in terms of safety and feasibility are generally encouraging, functional improvements were mostly disappointingly low and have failed to reach expectations. In the future, new concepts for myocardial regeneration, especially concerning recovery of cardiomyocyte loss, have to be developed. Transplantation of novel stem or progenitor cell populations with "true" regenerative potential, direct reprogramming of scar tissue into functional myocardium, tissue engineering or stimulation of endogenous cardiac repair by pharmacological agents are conceivable. This review summarizes current evidence of stem cell based regenerative therapies and discusses future strategies to improve functional outcomes.

KEYWORDS

Myocardial infarction; regenerative medicine; stem cells; tissue engineering; reprogramming

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Introduction

In 2009 cardiovascular disease (CVD) still accounted for 32.3% of all deaths in the United States and therefore continues to be one of the main causes of death (1). From 1999 to 2009, the rate of death due to CVD has declined, but nevertheless the burden of disease remains high. Although improved medical care and acute management of myocardial infarction have led to a considerable reduction of early mortality rate survivors are susceptible to an increased prevalence of chronic heart failure as they develop scarring followed by ventricular remodeling despite optimum medical care (2,3).

Interestingly, cardiovascular operations and interventional procedures increased by 28% from 2000 to 2010 implicating an enormous cost factor for the healthcare system (1). For 2009, it was estimated that the direct and indirect costs of CVD and stroke add up to about $312.6 billion in the United States, which was more than for any other diagnostic group (1).

The main issue of current pharmacological, interventional or operative therapies is their disability to compensate the irreversible loss of functional cardiomyocytes (4). Hence, the future challenge of cardiovascular therapies will be the functional regeneration of myocardial contractility by novel concepts, like cell based therapy, tissue engineering or reprogramming of scar fibroblasts (5,6).

Current therapies—adult stem and precursor cells

After promising preclinical results using adult stem and precursor cells for cardiac regeneration a rapid clinical translation using autologous bone marrow cells (BMCs) in patients was initiated (7,8). In the last few years numerous clinical trials addressing the transplantation of various adult stem cell populations for cardiac regeneration have been performed. Essential characteristics for the selected adult stem cell populations are the potential to proliferate, migrate and the ability to transdifferentiate into various mature cell types (9). Today, different adult stem cell sources like BMCs, myocardium or adipose tissue derived cells were already used in clinical trials. Beside direct intracoronary or intramyocardial transplantation of adult stem cells into the heart mobilization of autologous progenitor cells by administration of different cytokines [i.e., erythropoietin (EPO) or granulocyte

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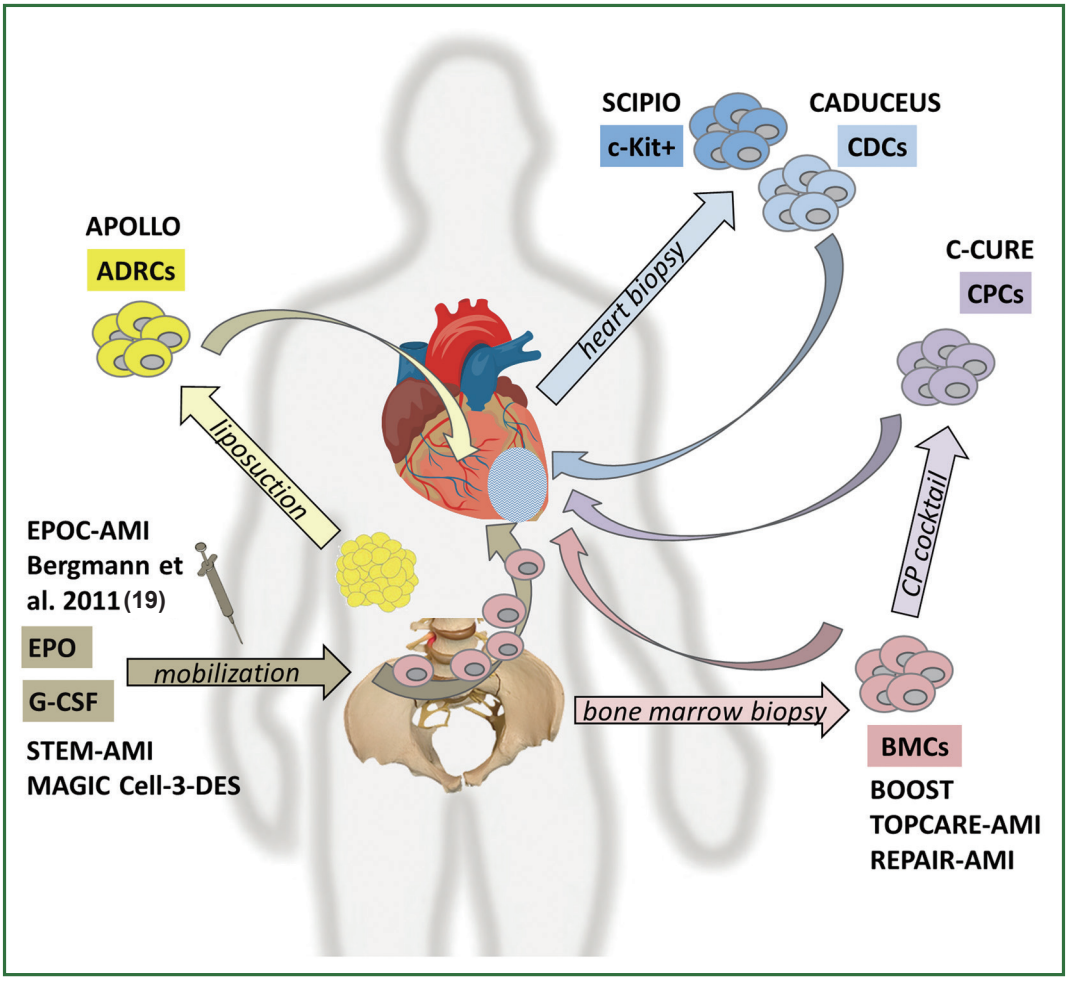

Figure 1. Regenerative therapies and cell sources currently administered in clinical trials. Current clinical trials use BMCs, ADRCs or CPCs to regenerate impaired myocardium after ischemic events. Alternatively cytokines like EPO or G-CSF are employed to mobilize resident progenitor cells from the bone marrow. BMCs, bone marrow cells; ADRCs, adipose-tissue derived regenerative cells; CPCs, cardiopoietic stem cells; CP-cocktail, cardiopoietic cocktail; CDCs, cardiosphere derived cells; G-CSF, granulocyte colony stimulating factor; EPO, erythropoietin.

colony stimulating factor (G-CSF)] were also evaluated in first clinical trials (summarized in Figure 1 and Tables 1,2).

Bone marrow derived stem cells (BMCs)

The bone marrow as an autologous source for adult stem cells is easily accessible and renewable. Significant improvement in left ventricular function after transplantation of BMCs has been reported in animal models of myocardial infarction (22,23). The BMCs consist of different subpopulations like BM-hematopoietic stem cells (BM-HSCs, ~2-4%), mesenchymal stem cells (also termed BM-mesenchymal stromal cells, BM-MSCs, ~0.1%), BM-endothelial progenitor cells (BM-EPCs) or side population cells (9). BM-HSCs give rise to lymphoid and myeloid lineages during embryonic development, whereas BM-MSCs develop into osteoblasts, chondrocytes and skeletal myocytes (24). BM-MSCs are physiologically situated in the stromal adherent fraction of the bone marrow where they maintain homeostatic turnover of BM-HSCs (25). Both multipotent precursor cell populations are self-renewing, grow clonogenic, and can be induced to differentiate into a cardiomyocyte phenotype *in vitro* (26-28). BM-HSCs are typically characterized by surface receptors like CD133, CD34 or CD117 (c-KIT) whereas BM-MSCs express CD105, CD73 and CD90 but lack CD34, CD45 and others (28). BM-EPCs have embryonic angioblastic properties and are able to promote the repair of damaged endothelium (29).

Multiple clinical trials with various results have been performed with BM-HSCs, BM-MSCs and BM-EPCs (9,30-33). In a recently published metastudy (3) combining the results of 50 studies from 2003 to 2011 BMC transplantation improved the left ventricular ejection fraction (LVEF) by an average of

Table 1. Transplantation of adult stem cells-clinical trials mentioned in the text.

Trial	Year/ reference	Study design	Patients (number)	Patients (diagnosis)	Cell type	Application	Follow up (months)	Follow up (method)	Functional outcome (LVEF)
BOOST	2009 (10)	r, c	60	STEMI (PCI)	BMCs	ic	~60	MRI	No significant improvement (BMC group *vs.* control)
REPAIR-AMI	2010 (11)	r, db, pc	204	STEMI (PCI) (LVEF <45%)	BMCs	ic	24	MRI (only 59 patients)	No significant improvement (BMC group *vs.* control)
TOPCARE-AMI	2011 (12)	r	55	STEMI (PCI)	CPCs or BMCs	ic	60	MRI	Improvement by 11% (BMC/CPC group: baseline *vs.* 5-year follow up, P<0.001), no control group
C-CURE	2013 (2)	r, c	48	CHF (LVEF 15-40%)	CPSCs (derived from MSCs)	ic	6	echo	Improvement by 7% (CPSC group: baseline *vs.* 6 months follow up, P<0.0001), no change for control group
CELLWAVE	2013 (13)	r, pc	103	CHF post MI (LVEF <50%)	Shock wave pretreatment + BMCs	ic	4	LVA	Improvement by 3.2% (shock wave + BMC), improvement by 1% (shock wave + placebo) (P=0.02)
APOLLO	2012 (14)	r, db, pc	14	STEMI (LVEF 30-50%)	ADRCs	ic	6	SPECT	No significant improvement (ADRC group *vs.* placebo)
CADUCEUS	2012 (15)	r, c	25	Recent MI (LVEF <45%)	CDCs	ic	6,12	MRI	LVEF: no difference
SCIPIO	2012 (16,17)	r, c	33	MI (CABG) (LVEF <40%)	CDCs (c-kit+)	ic	4,12	MRI	Improvement by 7.6% (CDC group baseline *vs.* 4 months follow up; P=0.004, n=8); improvement by 13.7% (CDC group baseline *vs.* 4 months follow up; P=0.013, n=5)

Abbreviations: LVEF, left ventricular ejection fraction; r, randomized; db, double blinded; c, controlled; pc, placebo-controlled; STEMI, ST-segment elevation acute myocardial infarction; CHF, chronic heart failure; MI, myocardial infarction; HF, heart failure; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; BMCs, bone marrow derived stem cells; CPCs, circulating progenitor cells; CPSCs, cardiopoietic stem cells; MSCs, mesenchymal stem cells; CDCs, cardiosphere derived cells; ic, intracoronary; echo, echocardiography; LVA, left ventricular angiography; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography.

Table 2. Mobilization of adult stem cells-clinical trials mentioned in the text.

Trial	Year/ reference	Study design	Patients (number)	Patients (diagnosis)	Drug type	Drug dose/ application	Follow up (months)	Follow up (method)	Functional outcome (LVEF)
EPOC-AMI	2010 (18)	r, ol, be, c	35	STEMI (PCI)	EPO (low dose)	One dose of 6,000 IU during PCI	6	SPECT	Significantly improved by 6.5% (EPO group baseline *vs.* 6 months follow-up, P=0.003), no significant improvement in controls
Bergmann *et al.*	2011 (19)	r, db, pc	28	HF (PCI)	EPO (low dose)	35 IU/kg body weight (weekly for 6 months)	6	echo, MRI	Significantly improved by 4.9% (echo, P=0.019) and 5.0% (MRI, P=0.042) (EPO group *vs.* placebo)
STEM-AMI	2010 (20)	p, r, sb, pc	60	STEMI (PCI) (LVEF <45%)	G-CSF	G-CSF 5 μg/kg subcutaneously (daily for 5 days)	6	MRI	No significant improvement (G-CSF group *vs.* placebo)
MAGIC Cell-3-DES	2012 (21)	r, c	117	MI (PCI)	G-CSF + subsequent injection of PBMCs	G-CSF 10 μg/kg subcutaneously (daily for 3 days) + PBMC injection (im)	24	MRI	No significant improvement (G-CSF/PBMC group *vs.* control)

Abbreviations: LVEF, left ventricular ejection fraction; r, randomized; ol, open label; be, blinded endpoints; sb, single blinded; db, double blinded; c, controlled; pc, placebo-controlled; STEMI, ST-segment elevation acute myocardial infarction; MI, myocardial infarction; HF, heart failure; PCI, percutaneous coronary intervention; EPO, erythropoietin; G-CSF, granulocyte colony stimulating factor; PBSCs, peripheral blood stem cells; im, intramyocardial; echo, echocardiography; MRI, magnetic resonance imaging; SPECT, single-photon emission computed tomography.

3.96% and infarct size was reduced by 4.03% compared with the standard treatment groups (P=0.00001). These benefits were found in acute myocardial infarction as well as in chronic ischemic heart disease patients.

A few years ago first long term results of stem cell transplantation clinical trials were published (10-12). The key question was whether short-term improvements were persistent over the long term. Two trials analyzing BMC transplantation for cardiac repair, TOPCARE-AMI and BOOST, have published their five year follow-up results. For the BOOST trial no significant difference in LVEF between the control and the BMC-treated group was detectable (10). The five year results of TOPCARE-AMI, however, confirmed a persistence of the beneficial effects on LV function (12). LVEF was improved by 11% (P<0.001) at 5 years. Though, it has to be mentioned that the trial design lacks a placebo-controlled treatment group. The REPAIR-AMI trial, as the largest double-blind, placebo-controlled trial with 204 patients, presented its 2-year follow-up data and found no ameliorated LVEF in the BMC- compared to the placebo treated group (11).

Owing to inconsistent functional outcomes of BMC transplantation further methodological refinements to improve the efficiency of BMC delivery were undertaken. The C-CURE trial advanced the paradigm of lineage specification in stem cell therapy (2). BM-MSCs were harvested from the bone marrow and then driven into cardiopoietic stem cells (CPCs) under addition of a cardiopoietic cytokine cocktail during extra-corporal cultivation. The CPCs were then endomyocardially delivered into 48 patients with a history of myocardial infarction. After six months an increase of LVEF by 7% was observed in the cell therapy group as determined by echocardiography whereas the standard care group only showed an improvement of 0.2% (P<0.0001). The study is limited since it is neither blinded nor placebo-controlled. However, the paradigm of pretransplant lineage specification might proof as an interesting rationale for further clinical validation.

The CELLWAVE study combined the delivery of BM-MSCs with a cardiac shock wave pretreatment (low-energy shock wave under continuous electrocardiographic trigger) in order to improve homing of the delivered cells while increasing

the local expression of chemoattractants such as stromal cell-derived factor 1 (SDF-1) and vascular endothelial growth factor (VEGF) (13). The shock wave BMC group showed an absolute change in LVEF of +3.2% after four months whereas the shock wave placebo infusion group only displayed a 1% improvement (P=0.02).

In summary, clinical trials have documented that BMC transplantation is safe and feasible but the ambitious goal of cardiac functional recovery after myocardial infarction remains elusive (3). Outcomes in terms of efficacy are inconsistent and in most series only transient (15). However, several clinical trials are announced and ongoing (34).

Adipose tissue derived regenerative cells (ADRCs)

More than a decade ago, it was shown that human lipoaspirates contain multipotent cells with a differentiation potential beyond that of the adipocytic lineage (26). These ADRCs share many properties with BM-MSCs (35). Adipose tissue as an autologous source for therapeutic regenerative cells is particularly advantageous since it can be harvested in relatively large quantities by liposuction (35) and contains up to 2,500 times more MSC-like cells than freshly isolated bone marrow (14). The considerably higher amount of MSC-like cells in adipose tissue would obviate a time consuming expansion prior to application for therapeutic assignment as it is necessary for BM-MSCs. A preclinical study in pig analyzing the effects of ADRC administration after acute myocardial infarction demonstrated functional improvement (36). The APOLLO trial is a randomized, double-blind and placebo-controlled study to investigate the safety and feasibility of intracoronary infusion of ADRCs (20 million cells) in 14 acute phase patients with large ST-segment elevation acute myocardial infarction (STEMI) (14). SPECT analysis demonstrated a trend but no significant improvement of LVEF compared to the placebo group. Nevertheless, a randomized, placebo-controlled phase IIb/III trial (ADVANCE) with an intracoronary infusion of two doses of ADRCs in up to 375 patients with STEMI is planned (14).

Mobilization of progenitor cells

A different strategy for cardiac regeneration by adult stem cells is the mobilization of progenitor cells from the bone marrow by administration of growth factors or cytokines such as fibroblast growth factor (FGF), VEGF, EPO or G-CSF (37).

EPO is a glycoprotein hormone that controls erythropoiesis and is known to increase the oxygen-carrying capacity of the blood (38). Hypoxic ischemic cardiomyocytes and vascular endothelial cells have surface EPO receptors which make them potential targets for EPO treatment (39). In animal models high-dose EPO administration led to reduced infarct size and functional preservation (40,41). However, clinical studies of single high doses of EPO following acute myocardial infarction were disappointing (42). Current clinical trials, like EPOC-AMI or the study of Bergmann *et al.*, were sought to investigate the low-dose application of EPO (either short- or long-term) (18,19). The EPOC-AMI trial (18) showed a significantly improved LVEF in the short-term low-dose EPO group of 6.5% (P=0.003). Bergmann *et al.* (19) reported an enhanced LVEF for the low-dose long-term EPO group [echo: (5.2±2.0)%, P=0.013; MRI: (3.1±1.6)%, P=0.124]. No significant improvements were found for the placebo groups.

G-CSF is secreted by monocytes, fibroblasts, and endothelial cells and is involved in myeloid development and neutrophil maturation (43). In animal models improved haemodynamic function and infarct remodeling could be shown (44,45). Two meta-analyses regarding the role of G-CSF in cardiac repair after myocardial infarction demonstrated that G-CSF failed to improve LV function (46,47). The STEM-AMI trial also failed to show LVEF improvement six months after G-CSF administration (20).

The ability of BMC mobilization either by EPO or G-CSF to promote cardiac repair remains controversial. Alternative approaches like the MAGIC Cell-3-DES trial combined G-CSF stimulation with an intramyocardial application of peripheral blood stem cells (PBSCs) (21). After two years LVEF was improved by 2.8% (P=0.045) in the G-CSF/cell group but not in the control group. Future efforts are made by combining G-CSF with DPP-IV (CD26/dipeptidylpeptidase IV) inhibitors to increase the homing of stem cells by inhibiting the degradation of SDF1alpha, an important homing related surface receptor (48). Several DPP-IV inhibitors have already been chemically approved for anti-diabetic treatment (49,50). The SITAGRAMI-Trial intends to analyze the impact of G-CSF and Sitagliptin (DPP-IV inhibitor) in patients suffering from acute myocardial infarction (48).

Other cytokines, such as FGF, VEGF, growth hormone (GH) or insulin-like growth factor (IGF) have been applied in various randomized, controlled clinical trials, unfortunately without reproducing the efficacy observed in pre-clinical investigations [summarized in (37)].

Mechanisms of stem cell action in the diseased heart

Different regenerative mechanisms of transplanted cells within the injured myocardium have been described (9). (I) Transplanted stem cells could directly transdifferentiate into cardiomyocytes; (II) Paracrine effects could induce growth of resident cardiomyocytes; (III) Resident endogenous myocardial stem cells may be stimulated; (IV) Cell fusion between transplanted cells and resident cardiomyocytes could be induced. Currently, it is understood that the predominant working

mechanism of BMC or ADRC therapy is mediated through a paracrine release of antiapoptotic, immunomodulatory, and proangiogenic host- and cell-derived factors (51). Hence, the moderate positive effects of stem cell delivery to impaired myocardium are more likely due to enhanced neovascularization rather than a formation of new cardiac muscle. A direct transdifferentiation into cardiomyocytes or a cell fusion between the transplanted stem cells and resident myocytes were considered unlikely.

Cardiac derived stem cells

Current clinical studies also use different populations of autologous cardiac progenitor cells, directly derived from the human adult heart (52). To yield cardiosphere derived cells (CDCs) percutaneous endomyocardial biopsy specimens are grown in primary culture where they develop multicellular clusters known as cardiospheres (53). CDCs are a natural mixture of stromal, mesenchymal, and progenitor cells expressing distinct surface receptors (e.g., c-Kit) (53). *In vitro* CDCs grow clonogenic and have multilineage potential. Safety and efficacy of CDC transplantation has been demonstrated in preclinical studies (53,54). In a murine model of myocardial infarction the functional outcome of different stem cell type transplantations, including BM-MSCs, ADRCs and CDCs were compared (55). CDCs were clearly superior in terms of ischemic tissue preservation, anti-remodelling effects and functional benefits (55).

The results of the CADUCEUS trial, a phase I randomized trial, were recently published (15). CDCs were derived from right ventricular endomyocardial biopsies and expanded for about five weeks before retransplantation. The CDCs were delivered by intracoronary infusion into the infarct related artery in 17 patients with recent myocardial infarction (3-5 months). Outcomes were compared with eight patients who received conventional medical therapy. No difference with respect to LVEF-change could be detected after six months but infarct size was significantly reduced in the cell treated group (by 7.7%, after 6 months and by 12.3% after 12 months, P=0.001) (15). Unfortunately the study could not be performed in a blinded manner because of ethical concerns regarding right ventricular biopsy on the controls.

Cardiac stem cells, positive for the tyrosine kinase receptor c-KIT, were first described in 2003 (56) as a resident population of stem cells in adult mammalian myocardium. These stem cells are capable of differentiating into cardiomyocytes, vascular smooth muscle cells and endothelial cells, respectively (57). As these cells are furthermore self-renewing and grow clonogenic, they fulfill all criteria of a tissue-specific stem cell (57). Several preclinical studies could demonstrate functional improvements after transplantation of c-Kit positive stem cells into ischemic hearts (56,58,59).

As a milestone in the history of cardiac stem cell therapy the first-in-man trial, the randomized, open-label SCIPIO trial (16,17) intended to analyze the feasibility, safety and efficacy of an intracoronary infusion of c-KIT$^+$/(LIN$^-$) CDCs into patients with sustained myocardial infarction. After one year LVEF was analyzed by MRI in only five treated patients and was found to be improved by 12.3% (compared to baseline, P=0.013). Mortality or major adverse cardiac events (MACE) following cell therapy did not emerge in this study.

The benefits of CDC therapy may be mediated through activation of endogenous regenerative pathways or even through a direct transdifferentiation of delivered cells into cardiomyocytes (15) whereas other studies claim that even CDCs exert most of their beneficial effects via indirect paracrine mechanisms (59,60).

Owing the different study designs and patient populations a direct comparison of most clinical studies is generally difficult. However, the functional outcome of CDC-trials appears slightly more encouraging when compared with BMC/ADRC trials.

Pending issues of current stem cell trials

Most clinical trials using different adult stem cell transplantation or mobilization strategies have proofed safety and feasibility. In order to optimize therapy protocols further efforts have to be made to identify underlying mechanisms of stem cell migration, differentiation and myocardial regeneration. Other important issues are the optimal stem cell type and number, timing of their delivery and application route, respectively.

Above all, the most effective stem cell type for cardiac regeneration has to be defined. BMCs and ADRCs both seem to act in a paracrine way to improve cardiac function whereas CDCs are believed to have some capacity to transdifferentiate into cardiomyocytes. Furthermore, it remains open whether the use of allogeneic cells can be of clinical relevance in the future. Not only that autologous cells have to be expanded for extensive time periods excluding their use in the acute setting, the expansion is furthermore expensive and their quality will vary depending on the patient (5).

The optimal timing of cell delivery after myocardial infarction is potentially one of the main issues in terms of cell homing and survival. Myocardial infarction is an ischemic event directly followed by a significant inflammatory reaction accompanied by the secretion of cytokines and additional growth factors by macrophages or other immune cells (61). A transplantation of unprotected cells into this environment results in significant cell death (62). It was speculated that delivered stem cells even take part in the inflammation cascade than in the formation of new myocardium and vessels (28). At the moment the optimal time point for stem cell application seems to be within the first month

after myocardial infarction (28).

The optimal form and route of cell delivery into the ischemic heart remains another major challenge. Intramyocardial delivery with several injections could be a risk factor for triggering ventricular arrhythmias. Preferring an intracoronary cell administration might reduce the risk for arrhythmias but may foster cell loss.

All these issues motivate future research for improving delivery methods, find more potent cell types and better strategies to boost cell engraftment (63).

Future concepts

Since studies with adult stem/precursor cells and mobilization therapies did by now not lead to the desired results in terms of a full functional restoration further strategies are under investigation—but so far have not been translated into clinical reality. Transplantation of alternative cell sources with "true" regenerative properties, tissue engineering with various scaffold materials and different cells for colonization, stimulation of resident stem or progenitor cell sources or a reprogramming of scar tissue back into functional myocardium are promising approaches.

Novel cell sources for cardiac regeneration with "true" regenerative properties

Embryonic stem cells (ESCs) are undifferentiated cells obtained from the inner cell mass of blastocysts revealing unlimited self-renewal capacity and pluripotency. They have the potential to develop into derivatives of all three germ layers: endoderm, mesoderm and ectoderm (64). These properties made ESCs particularly interesting for cardiac regeneration (33).

A few years ago, a spectacular discovery overcame ethical and presumably immune rejection issues of ESCs. In 2012 the Nobel Prize in Physiology or Medicine was awarded to Sir John B. Gurdon and Shinya Yamanaka for the discovery that mature somatic cells can be reprogrammed to a pluripotent stage (65,66). Takahashi and Yamanaka (65,67) generated so called induced pluripotent stem cells (iPSCs) by retroviral delivery and subsequent overexpression of a four-gene-set (Klf4, c-Myc, Oct4 and Sox2) from murine and human dermal fibroblasts (Figure 2). IPSCs exhibit similar pluripotency features and differentiation capacity like ESCs (64). Although teratoma formation is still of concern for iPSCs this milestone in cell biology opened a new era in regenerative and personalized medicine (68). The risk of teratoma formation can be reduced by predifferentiating pluripotent cells (ESCs, iPSCs) into cardiomyocytes or cardiac progenitor cells before transplantation (Figure 2). Usually only a small fraction of pluripotent stem cells spontaneously differentiates into cardiomyocytes (5-15%) with relative mature structural and functional properties (64). However, the yield can be improved by directing the differentiation into the cardiac lineage by choosing appropriate culture conditions (69).

Finally, questions remain concerning the ideal maturity level for cells to be implanted. In the course of differentiation from the level of pluripotency to maturity a cell traverses several progenitor stages (Figure 2). Different cardiac progenitor cell populations have been characterized by the expression of specific transcription factors like Flk1, Isl1 or Nkx2.5 (70-72). All of them have the potential to differentiate into various cardiac lineages like endothelial cells, smooth muscle cells or myocytes. Current studies try to induce cardiac progenitor cells from fibroblasts for regenerative purposes. It has been shown that a lentiviral overexpression of only two transcription factors, Ets2 and Mesp1, is sufficient to reprogram fibroblasts into cardiac progenitor-like cells (73) (Figure 2). Induced cardiac progenitor cells (iCPCs) would be advantageous for transplantational medicine since they are still able to proliferate. However, they do not show the same risk of teratoma formation like pluripotent cells.

Transplantation of cells with "true" regenerative potential

Several of the above mentioned novel cell sources, like ESCs, iPSCs or their derivatives have been transplanted after cardiac myocardial ischemia in different animal models (Figure 3).

The efficacy of ESC transplantation after myocardial infarction has early been demonstrated in animal studies (74,75). So far, ESCs have not been used clinically since, aside from ethical and political concerns, ESCs would be immunologically incompatible to the patient and entail the risk of teratoma formation (32).

The use of iPSCs in infarct repair has also been shown in different preclinical models (76-78). First of all therapeutic benefits of iPSC-based treatment have been established in small-animal infarction models (76,77). LVEF was improved two weeks after intramyocardial delivery of murine iPSCs compared to fibroblast transplantation (76) or PBS injection (77) after induction of myocardial infarction in mouse models. Functional improvement could also be achieved by intramyocardial transplantation of undifferentiated pig iPSCs in a porcine infarction model (78). Furthermore, infarct size could be decreased by iPSC transplantation in the mentioned animal studies. In contrast to ESCs iPSCs represent a potential autologous source of cells with an ESC-like phenotype and thus would be particularly useful for transplantation medicine (79).

For transplantation of pluripotent stem cell derived cardiomyocytes it is important to efficiently purify and select the developed cardiomyocytes to get a homogenous cell population (64). Cardiac function in infarcted rat hearts could be ameliorated without teratoma formation by transplanting predifferentiated cardiomyocytes (80,81). However, efficient

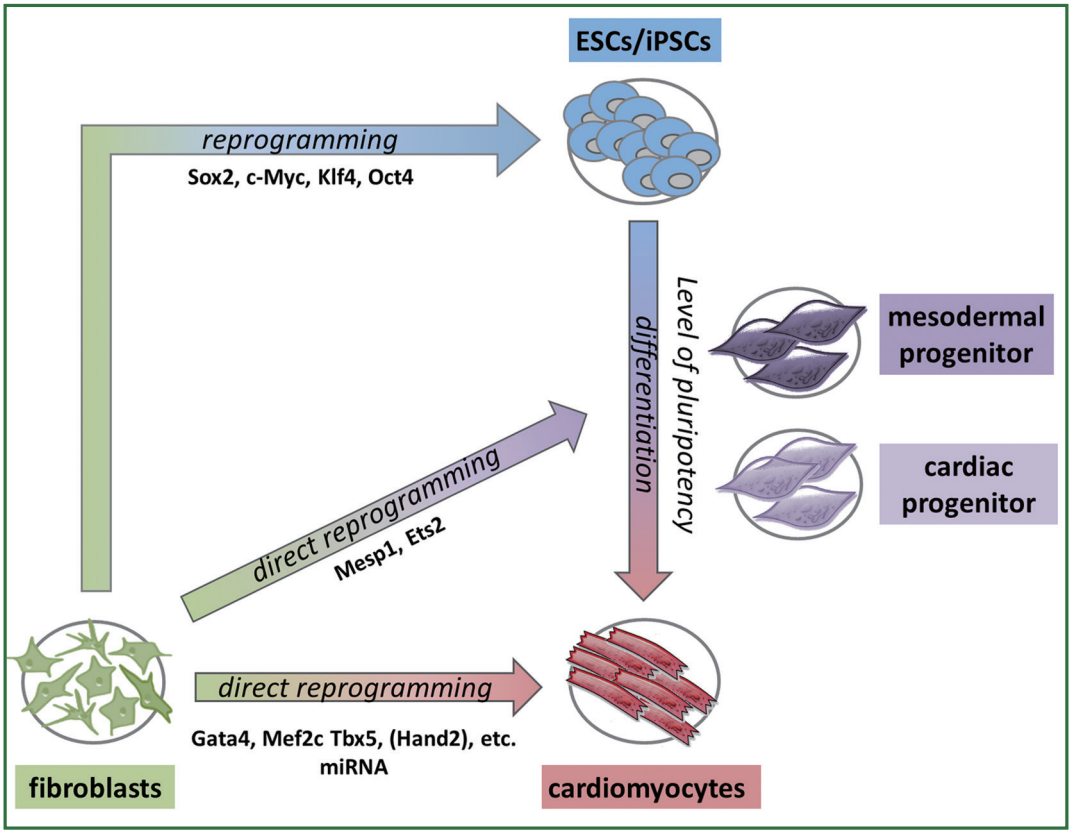

Figure 2. Novel stem cell sources for cardiac regeneration. ESCs (Embryonic stem cells) and iPSCs (induced pluripotent stem cells) generated by reprogramming fibroblasts can be differentiated into cardiac lineages. Cardiac differentiation means the gradual maturation of pluripotent cells (ESCs, iPSCs) over several progenitor stages to functional adult cardiomyocytes. Another approach is to directly convert mature somatic cells like fibroblasts into other mature cell types like cardiomyocytes. It is also imaginable that fibroblasts can be driven to an intermediate stage between full pluripotency and total maturity: a cardiac progenitor stage that is already directed to a cardiac fate but still maintains the ability to proliferate and differentiate in endothelial cells, smooth muscle cells or cardiomyocytes.

engraftment and survival continue to be an issue in preclinical studies (82). Furthermore there is growing evidence that pluripotent stem cell derived cardiomyocytes differentiated from ESCs or iPSCs are rather immature. Whether they can undergo further maturation within the host tissue remains to be determined (83).

A couple of challenges remain before these technologies can be translated successfully from bench to bedside. First of all the high risk of insertional mutagenesis using viral vectors for reprogramming has to be considered (84). Nonintegrating reprogramming techniques by direct protein delivery, miRNA or episomal vectors were already developed but often with lower efficacy (85-87). Moreover, epigenetic features (methylation patterns of the genomic DNA) were observed to be reminiscent of the original somatic cell and therefore indicate incomplete reprogramming (88). Another obstacle is the time needed to derive patient specific iPSCs so that they cannot be used under urgent circumstances (64).

Direct reprogramming-new myocardium from scar tissue

The iPSC technology builds the scientific basis for alternative reprogramming strategies. In 2010 Ieda and colleagues first published the direct reprogramming of fibroblasts into cardiomyocytes without an intermediate pluripotent stage by Gata4, Mef2c, Tbx5 (GMT) (89) (Figure 2). This raises the possibility to directly generate functional myocardium by reprogramming postinfarct scar tissue, which largely consists of fibroblasts (Figure 3). Genetic lineage tracing models were used to demonstrate that resident non-myocytes in the infarcted murine heart could be reprogrammed into induced cardiomyocyte-like cells (iCMs) by retroviral, intramyocardial GMT delivery *in vivo* (90). After three month a reduced infarct size and a modestly attenuated cardiac dysfunction could be documented. In parallel a second group around Eric Olson came up with a more efficient reprogramming strategy combining GMT with Hand2 (91). They succeeded in improving cardiac function and reducing adverse ventricular remodeling after

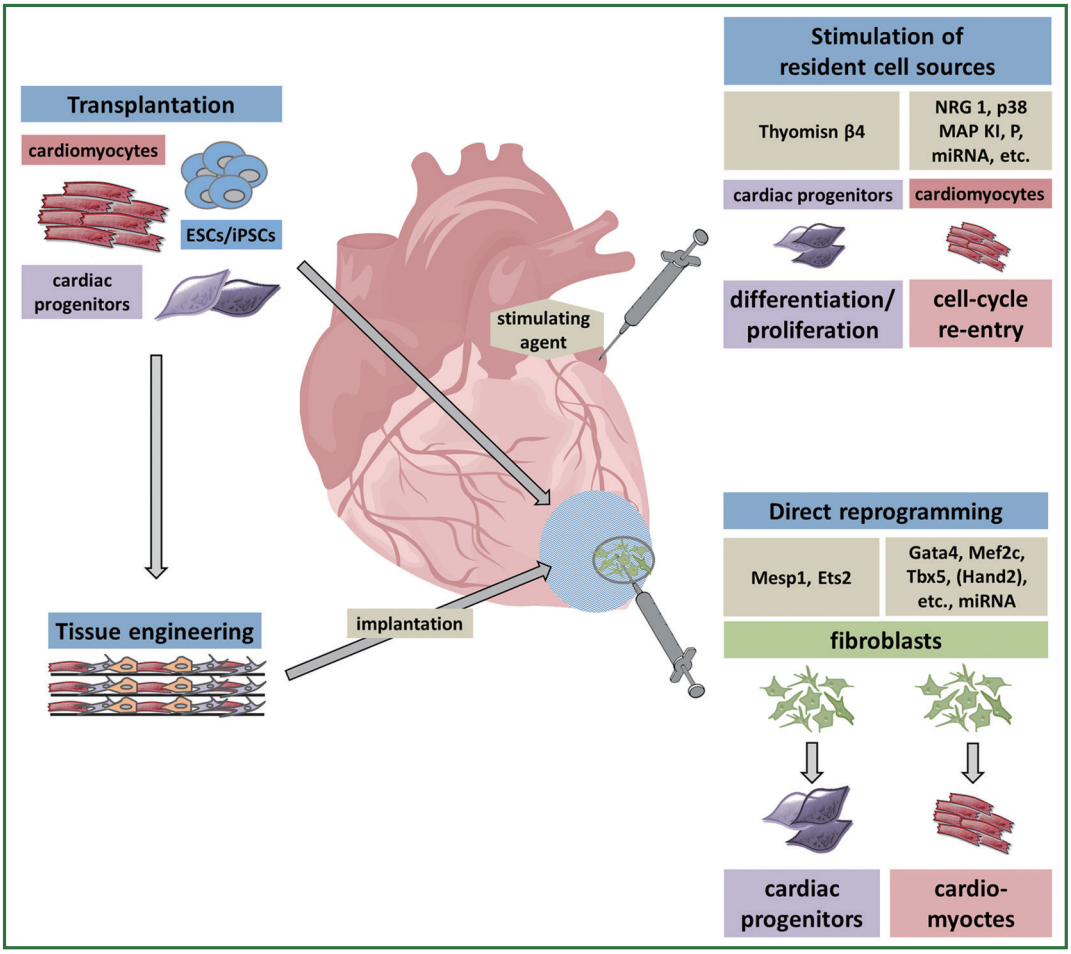

Figure 3. Future concepts for regenerative therapies. Future therapies in terms of myocardial regeneration might be a consolidation of transplantation of cells with "true" regenerative potential, tissue engineering with various scaffolds and cell types, a stimulation of resident cell sources by cytokines or growth factors or a direct reprogramming of scar tissue by delivery of various transcription factors or miRNAs. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; NRG1, neuregulin 1; p38 MAP KI, p38 MAP kinase inhibitor; P, periostin; miRNA, micro RNA.

myocardial infarction by local delivery of GMTH in a mouse model. However, a recent publication (92) indicated that the process of reprogramming fibroblasts to cardiomyocytes by GMT is insufficient. Cardiac and tail tip fibroblasts of multiple transgenic reporter mice were reprogrammed by GMT with varying success. It could be clearly shown that fibroblasts, especially cardiac fibroblasts, were pushed to a cardiac fate (high expression levels of, e.g., Tbx20 and Tnnt2). However, no electrophysiological competent and molecular mature cardiomyocytes could be generated. The authors concluded that GMT induced only a partial cardiac gene program leading to an insufficient reprogramming.

Recently, a combination of microRNAs (miRNAs: 1, 133, 208 and 499) was identified to be capable to reprogram murine fibroblasts to cardiomyocyte-like cells (93) albeit with a low efficiency (1.5-7.7% conversion rate). The effects of miRNA on gene expression can be powerful as a single miRNA may target multiple pathways simultaneously. The most recent study combined a subset of four transcription factors (Gata4, Hand1, Tbx5 and myocardin) with two miRNAs [1, 133] to successfully reprogram human fibroblasts to cardiomyocyte-like cells (94).

The success of direct reprogramming approaches may vary due to the different choice of mouse models and evaluation methods of cardiac phenotypes. Hence, all approaches had significant issues with efficacy that have to be overcome before a therapeutical application becomes feasible.

Tissue engineering-concepts for generation of cardiac tissue

Tissue engineering is actually an extension of cell transplantation in combination with a variety of scaffolds as one is more and more aware that the three-dimensional microenvironment plays an important role in cell differentiation and especially maturation (95) (Figure 3). As single cell approaches are limited

in terms of high cellular wash out and low integration rates tissue engineering approaches may become increasingly important. Zhang and co-workers (95) compared the maturation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) in 2D monolayers and 3D fibrin-based patch cultures. In 3D patches hESC-CMs exhibited significantly higher conduction velocities, longer sarcomeres and an enhanced expression of genes associated with contractile function (like cTNT, αMHC, CASQ2, SERCA2) when compared to 2D cell culture. So far, a variety of scaffolds and cell sources have been used to generate functional cardiac tissue with diverging efficiency *in vitro* [for detailed review see (61,96)].

Furthermore, mechanical and electrical stimulation were found to markedly improve the structural organization and contractile properties of engineered cardiac constructs and resulted in a more mature cardiac muscle structure (61,97,98). Zimmermann and associates have demonstrated that the transplantation of large force-generating engineered heart tissues formed by mechanical pretreatment from neonatal rat heart cells, liquid collagen and matrigel led to undelayed electrical coupling and structural improvement in infarcted rat hearts (99).

For thicker engineered heart tissue constructs *in vitro* it is also necessary to ensure oxygen supply to keep settling cells viable. A study about the penetration depth of oxygen in non-perfused cardiac constructs demonstrated that myocytes could only survive within the first 100 μm of the construct thickness.

Current tissue engineering techniques have mainly been tested for functional improvements in rat models, but whether these approaches can be transferred to large animal models or even to human patients remains elusive (100).

Stimulation of endogenous cardiac repair

It has been demonstrated that the neonatal murine heart holds a remarkable regenerative capacity. Apical excision resulted in a total recovery of the removed tissue by myocyte proliferation without scarring (101). Bergmann and colleagues found that the human adult heart also generates new cardiomyocytes during a life-span with an approximate turnover rate of 1% per year (age 25), declining to 0.45% by the age of 75 (102). The Cold War nuclear bomb tests caused a significant increase of ^{14}C-concentration in the atmosphere that gradually dropped after termination of the tests. Since the genomic DNA of every cell reflects the atmospheric ^{14}C-concentration at the time point of its generation the investigators were able to date the age of cardiac myocytes by the amount of ^{14}C in their nuclei.

Hence, the paradigm of terminally differentiated quiescent myocytes is obsolete since the mammalian adult heart seems to conserve a certain capacity for cardiomyocyte turnover (4). Evidence exists that new cardiomyocytes could be generated by one of the following mechanisms: a division of pre-existing mature cardiomyocytes, a dedifferentiation of cardiomyocytes followed by amplification, or a differentiation of resident progenitor cells (4).

In a transgenic mouse model combined with stable isotope labelling it was recently shown that the genesis of new cardiomyocytes in the adult heart occurs by the division of pre-existing cardiomyocytes and that the same mechanism is engaged in the regenerative processes after myocardial infarction (103). The stimulation of cell-cycle reentry of preexisting cardiomyocytes might therefore be an approach for cardiac regeneration. Pharmacological agents like a p38 MAP kinase inhibitor, the growth factor neuregulin-1 or the extracellular matrix signaling protein periostin showed encouraging results *in vitro* but they failed to replicate their beneficial effects to the full extent *in vivo* (104-106). Slight improvements in cardiac function after infarction could merely be achieved by neuregulin-1 (105) or by combining the inhibition of p38 MAP kinase with FGF1 (104) (Figure 3).

As regulatory pathways that govern heart development and growth are modulated, amongst others, by numerous miRNAs (107), the delivery of miRNAs into the injured heart could be another therapeutic approach to stimulate cardiomyocyte proliferation (Figure 3). Eulalio and coworkers identified several miRNAs inducing cardiac regeneration by a functional screening using a whole genome miRNA library (108). They showed that injection of synthetic miRNAs miR-590-3p and miR-199a-3p into the neonatal mouse heart led to increased cardiomyocyte, but not cardiac fibroblast, proliferation. The adenoviral delivery of these miRNAs into the adult murine heart after myocardial infarction reduced fibrotic scar size and improved cardiac function (108).

Several publications about the regenerative capacity of the hearts of newts, zebrafish or neonatal mice showed that pre-existing cardiomyocytes adjacent to the site of injury undergo a process of dedifferentiation characterized by molecular and/or cytoskeletal changes (101,109,110). Dedifferentiated myocytes can re-express gene markers of immaturity, like Gata4, and disassemble sarcomeric structures (101,110,111). Additionally they pass a proliferative phase evident by the expression of DNA synthesis markers before re-differentiating into mature myocytes.

Furthermore, various populations of endogenous cardiac stem or progenitor cells with the potential to create cardiomyocytes have been identified in the postnatal mammalian heart. They are characterized by different surface receptors like c-Kit (56), Sca-1 (112), the transcription factor Isl1 (113) or by their ability to efflux the fluorescent vital dye Hoechst 33342 (114). Recently, the epicardium, a mesothelial cell layer encapsulating the heart, was presented as another source of progenitor and paracrine cells contributing to heart development and maybe also to regeneration after cardiac injury (115,116). Stimulation of endogenous regeneration may become feasible by using small molecules or growth factors (29) (Figure 3). An activation of

local signaling pathways could further stimulate vasculogenesis and angiogenesis and by this improve local blood flow (29). Thymosin β4 is implicated in the reorganization of the actin cytoskeleton, a process fundamentally required for cell migration and cytokinesis and is regarded as a potential candidate for stimulating resident cardiac progenitors, especially of epicardial origin (117) (Figure 3). Smart and colleagues stimulated adult mice with peritoneal Thymosin β4 injections before induction of myocardial infarction resulting in a significant increased $WT1^+$ epicardial progenitor population two days after the ischemic event (118). Thymosin β4 treatment following myocardial infarction in a mouse model did exhibit overall cardioprotective effects but failed to show differentiation of epicardial cells into cardiomyocytes (119).

However, the question whether myocyte proliferation or resident cardiac progenitor cell stimulation, especially in elderly patients, is sufficient for efficient myocardial regeneration remains open. Further research is needed to identify paracrine signaling pathways involved in activation and recruitment of endogenous cardiomyocyte progenitors (4). A more detailed characterization of the molecular, phenotypic and functional identity of various endogenous cardiac progenitor cell populations in conjunction with the mapping of cell fate by implementing transgene lineage tracing models both *in vitro* and *in vivo* is required to pave the way for "true" myocardial regenerative medicine.

Summary and perspective

In summary, different cell types have been studied in clinical trials with good safety data but heterogeneous results in terms of efficacy. Several short- and long-term challenges like the optimal stem cell type, the optimal timing of stem cell delivery and the optimal application route remain open. Furthermore, integration of the grafts including control of vascularization have to be improved in the future (5). Finally it has to be taken into account, that several legal and practical issues need to be resolved before patients can be treated by stem cell technologies in a consistent and cost-effective manner (68).

Currently, various new cell sources with "true" regenerative potential are on trial. Multipotent ESCs and iPSCs can differentiate into any cell-type but risks of teratoma formation in case of direct transplantation have to be considered. ESCs additionally implicate ethical and immunological concerns. Pluripotent stem cell derived cardiac progenitor cells and cardiomyocytes offer versatile possibilities for regenerative strategies but entail risks concerning sufficient purity or maturity level since it remains to be determined whether further maturation takes place within the host tissue. Functional engraftment and survival of transplanted cells is still an issue that may be overcome by tissue-engineering techniques. A significant obstacle of all these therapies is the time needed to derive sufficient cells or tissue grafts since they cannot be used under urgent circumstances, such as an acute myocardial infarction or a rapidly progressive heart failure (64). Further approaches try to reprogram scar tissue into new functional myocardium by the administration of various transcription factors or miRNA. Unfortunately most of the current reprogramming strategies are realized by viral delivery of the respective factors entailing the risk of severely changing original genomics of the targeted cell. But even by using nonintegrating methods high levels of mutational changes were still observed (84). Finally, scientists intended to stimulate endogenous cell sources like existing cardiomyocytes or resident cardiac progenitor cells by growth factors or small molecules to initiate cardiac regeneration after injury. Unfortunately, significant issues remain with current approaches in regenerative strategies before the shift from bench to bedside can succeed.

In general, all described current and future strategies for cardiac regeneration are restricted by the limited understanding of basic principles, pathways and transcription factor networks that contribute to vertebrate heart development and cardiomyocyte cell differentiation as well as maturation. Since the heart is a most complex organ that requires uninterrupted function and therefore seamless integration of transplanted cells, the creation of functional heart tissue will very likely necessitate a combination of approaches (107). Despite a decade of numerous adult stem cell clinical trials we are still at the beginning of a promising journey in the field of regenerative cardiac medicine.

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