® Postepy Hig Med Dosw (online), 2017; 71: 975-987
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 2016.05.18
Accepted: 2017.07.11
Published: 2017.12.07

Perspective in optimization of stem cell therapies for heart regeneration*

Perspektywy optymalizacji terapii komórkowej z użyciem komórek macierzystych dla regeneracji serca mięśniowego

Paulina Gapska, Maciej Kurpisz

Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

Summary

There is a variety of mechanisms(s) factor(s) that may influence stem cell therapies for heart regeneration. Among the best candidates for stem cell source are: mesenchymal stem cells (also those isolated from adipose tissue), cardiac cell progenitors (CPC) and descendants of iPSC cells. iPSC/s can be potentially beneficial although their pluripotent induction has been still in question due to: low propagation efficacy, danger of genomic integration/instability, biological risk of current vector system teratoma formation etc. which have been discussed in this review. Optimization protocols are required in order to enhance stem cells resistance to pathological conditions that they may encounter in pathological organ and to increase their retention. Combination between gene transfer and stem cell therapy is now more often used in pre-clinical studies with the prospect of subsequent clinical trials. Complementary substances have been contemplated to support stem cell viability (mainly anti-inflammatory and anti- apoptotic agents), which have been tested in animal models with promising results. Integration of nanotechnology both for efficient stem cell imaging as well as with the aim to provide cell supporting scaffolds seem to be inevitable for further development of cellular therapies. The whole organ (heart) reconstruction as well as biodegradable scaffolds and scaffold-free cell sheets have been also outlined.

Key words: **heart regeneration • stem cells • cellular therapies • scaffolds • tissue engineering • whole heart reconstruction**

GICID: 01.3001.0010.6665
DOI: 10.5604/01.3001.0010.6665
Word count: 5682
Tables: –
Figures: –
References: 120

* An article has been funded by: The National Centre for Research and Development, Poland, grant number STRATEGMED1/233624/5/NCBR/2014 and grant number PBS3/A7/27/2015.

Author 's address: Maciej Kurpisz, MD, PhD, Institute of Human Genetics, Polish Academy of Sciences, Department of Reproductive Biology and Stem Cells, Strzeszynska 32;60-479 Poznan, Poland; e-mail: kurpimac@man.poznan.pl

Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality, especially in highly developed countries, and is commonly associated with myocardial infarction (MI). According to the World Health Organization, myocardial infarction and coronary artery disease lead to 29% of deaths worldwide [64]. The prevalence of MI has been continuously increasing due to the changes in lifestyle and aging of the population. It has been known that a large myocardial infarction eliminates even 20- 30% of the total heart mass (approximately 300g), containing about 1.8- 2.7 billion cardiomyocytes.

Myocardial infarction is associated with the left ventricular (LV) dilatation and interstitial fibrosis in the infarcted myocardium, which cause depressed cardiac performance and can be independent determinants of morbidity and mortality after MI. LV remodeling is the result of the overexpression of various factors, including proinflammatory cytokines, angiotensin II, and norepinephrine, which have direct pathophysiological effects on cardiac myocytes, noncardiac myocytes, and the extracellular matrix [58].

Current therapeutic approaches to MI (pharmacological therapies and interventional strategies) put emphasis on the improvement of symptoms and prolonging life, and they do not address the fundamental problem, which is the loss of cardiac tissue.

Heart transplantation is considered to be the most effective therapy but it is associated with many problems such as: immunological rejection, age restrictions, surgical complications, and insufficient number of donors or medical costs. For this reason, the ongoing research worldwide has turned towards stem cell-based therapies, which aim to reconstruct the loss of myocardium with new functional cardiac cells. Nevertheless, the effects of cardiac cell therapy are limited due to the problems with low survival rate after stem cell transplantations and insufficient ability of cell homing and engraftment.

The experimental studies and early clinical trials conducted to overcome these limitations focused on identifying perfect cell stem candidates for cellular therapy, the best method of cell delivery, optimal cell dose and timing of its administration. There are two major strategies applied to improve cardiac cell therapy. The first one focuses on the pretreatment of the cells in order to stimulate their adhesion or directed migration, survival and differentiation. Whereas the second strategy aims to provide the appropriate environment for cell recruitment, long- term engraftment and possibly differentiation/function [19]. In this review, we will summarize recent advances on cardiac cell therapy and methods used to enhance its efficacy in clinical applications.

Optimal stem cells for cardiac regenerative therapy

Multiple types of stem cells have been included in numerous preclinical and clinical trials to establish their regenerative potential that could be used for either direct or indirect (paracrine) effects of cell therapy to infarcted myocardium. The ideal cell type should meet the following characteristics: safety (no tumor formation or arrhythmias), improvement of heart function, ability to regenerate cardiac muscle, easy accessibility with no immune system rejection or ethical issues involved.

Embryonic stem cells (ESCs)

Embryonic stem cells were considered to be promising candidates for cardiac regenerative therapy due to their strong proliferation potential and the ability to differentiate into the cells from three germ layers: ectoderm, endoderm and mesoderm. However, for a long time, clinical trials in humans have not been conducted due to bioethical controversies related to their origin. Other ESCs-related obstacles involved immune rejection and teratoma formation [24]. Quite recently, however, a pioneering Menasche group at Georges Pompidou European Hospital in Paris opened a clinical trial (ESCORT- transplantation of human Embryonic Stem Cells- derived prOgenitors in severe heaRT failure) using pericardial flaps seeded with cardiomyocytes made out of hESCs [5].

Adult Stem Cells (ASCs)

Another group of candidates for cardiac cell transplantation therapy is represented by the members of adult stem cells (ASCs) including: bone marrow cells (BMCs), skeletal myoblasts (SKMs), endogenous cardiac stem cells (CSCs) and adipose- derived stem cells (ADSCs).

Although limited in numbers, somatic stem cells, their proliferation and differentiation potential when compared to ESCs, the easiness and efficiency of their isolation from the patient caused that ASCs have been taken under consideration as an optimal candidate for cardiac cell therapy. Furthermore, transplantation of autologous patient-derived cells avoids immune rejection and ethical controversies.

Skeletal Myoblasts (SKMs)

It has been known that mature skeletal muscle fibers contain a pool of undifferentiated and inactive satellite

cells (myoblasts), which exhibit proliferative activity and differentiate into muscle fibers to regenerate injured or dying skeletal muscle cells. Nevertheless, transplanted skeletal myoblasts are electrically and mechanically uncoupled from the host myocardium, which is a major setback for their application in cardiac regenerative cell therapy. SKMs were ones of the first cell types to enter the clinical trials for heart regeneration. Small non-randomized phase I trials demonstrated functional benefits including improved left ventricular ejection fraction (LVEF) and increased tissue viability after myoblasts engraftment; however, high loss rate of skeletal myoblasts has been observed and ventricular arrhythmias have occurred in the myoblast-treated patients [92]. The first prospective randomized placebo-controlled phase II SKM trial (MAGIC), revealed no benefits from transplanted autologous skeletal myoblasts to postinfarction scar [63]. Nevertheless, other experiments conducted to test skeletal myoblasts as potential candidates for cardiac regenerative therapy are promising. The results of a recent study indicate that mechanical preconditioning of transplanted skeletal myoblasts enables their interaction with cardiomyocytes *in vivo* [103].

Bone marrow cells (BMCs)

Autologous bone marrow cells (BMCs) were among the most widely used for clinical therapy. The BMCs consist of mixture of hematopoietic stem cells and endothelial progenitor cells (HSCs and EPCs- constitute approximately 2–4% of BMCs), mesenchymal stem cells (MSC < 0.1%) and rarely some numbers of side cell population [22]. Both undifferentiated subpopulations: HSCs and MSCs can be induced to become components of myocardium [26]. MSCs when injected into the murine myocardium, showed the ability to differentiate into cardiomyocytes [102]. Moreover, MSCs lack major histocompatibility complex antigens of class II (HLA-DR), which opens a possibility for their allogeneic usage. The effect of MSCs in heart regeneration is not only due to their ability to differentiate into cardiomyocytes but mostly due to their paracrine activity [21].

Mesenchymal stem cells are thought to secrete cytoprotective cytokines, chemokines, and growth factors that are considered to inhibit apoptosis and fibrosis (scar formation), to promote proliferation, differentiation and vascularization [13]. The mechanism of MSCs action is based on their ability to inhibit the activation of NF-κB, thus reducing the expression of TNF-α and IL-6, and increasing anti-inflammatory cytokine IL-10 production [23].

Autologous MSCs transplantation has been shown to reduce infarct size, improve left ventricular ejection fraction (LVEF) and reverse remodeling after myocardial infarction [88]. Nevertheless, a meta-analysis of 49 trials performed by Nowbar and colleagues in 2014, showed modest myocardial recovery after BMCs transplantation [70]. By now, cardiac regenerative therapy with BMCs showed mild to modest benefits in left ventricular (LV) function, which was additionally often short-lived. The recent study discovered that the combination of human MSCs and cardiac stem cells have a better impact on the reduction of infarct size and the improvement of cardiac functions than MSCs alone. Nevertheless, the myocardial regeneration with new cardiomyocytes and new blood vessel production, after BMCs transplantation, has not been documented.

Adipose- derived stem cells (ADSCs)

Another population of stem cells that have been shown to present therapeutic potential in cardiac regenerative therapy is adipose-derived stem cells (ADSCs). The adipose tissues were demonstrated to contain multipotent stem cells and mainly consist of adult mesenchymal stem cells and endothelial progenitor cells. These populations of adult stem cells have been shown to differentiate into multiple cell lineages including cardiomyocytes [80].

It has been reported that the administration of ADSCs can effectively improve left ventricular function in animal models of myocardial infarction [46]. For instance, in a rat MI model, transplantation of ADSCs improved LVEF, increased angiogenesis and decreased fibrosis [62]. Recent reports have also shown that human ADSCs exhibit perivascular characteristics by showing increased migration in response to vascular endothelial growth factor (VEGF)-165 and platelet-derived growth factor (PDGF)-BB, thus contributing to increased microvascular density (during angiogenesis) migrating towards blood vessels [2]. Furthermore, ADSCs can improve heart function by the recruitment of native cardiac resident stem or progenitor cells by paracrine mechanisms. Several studies provided evidence that ADSCs contain a population of adult multipotent mesenchymal stem cells that improve left ventricular function but mainly by growth factor-mediated paracrine effects. Since the presence of cardiomyocytes within the MSC grafts appeared to be rare, MSCs are thought to trigger angiogenesis mainly through paracrine pathways [67].

The preliminary results of clinical trials suggest that ADSCs may provide stabilization of infarct size and improve maximal oxygen consumption [79]. The beneficial effects of ADSCs with enhancement of angiogenesis and cardiovascular protection appear to be related to their multipotency and ability to secrete growth factors. Taking into consideration the large amount of adipose tissue in general and the fact that ADSCs can be safely obtained, this group of adult stem cells can be a potential new source of cells for cardiovascular therapy.

Cardiac stem cells (CSCs)

Since 2003, it has been known that the adult heart contains a group of stem cells supporting its own regeneration. These cells are multipotent, clonogenic and

self-renewing, and give rise to cardiomyocytes, smooth muscle cells and endothelial cells. Population of cardiac stem cells was initially isolated from the adult rat heart and characterized as expressing the tyrosine kinase receptor c-kit, and lacking any markers of hematopoietic lineage [11]. CSCs have also been identified in humans, in which a new wave of clinical trials have been begun. Several different populations of CSCs have been identified and characterized including c-kit+, cardiospheres, sca-1+, side cell population, Is11+, epicardial and SSEA-1+ progenitor cells. However, a class of human c-kit-positive cardiac cells is the best characterized and the most studied CSC population.

Several studies documented the ability of CSCs to promote regeneration and improvement in both LV structure and function, and heart remodeling prevention in various animal models of post-MI cardiomyopathy [52]. The results of the first clinical trial (SCIPIO) are consistent with the preclinical studies and suggest that intracoronary infusion of autologous CSCs results in an improvement of LV systolic function and in a reduction of infarct size [12].

Cardiosphere cardiac stem cells are also characterized as a multipotent, self-renewal and clonogenic cell subpopulation with the ability to regenerate the cardiomyocyte *in vivo*. In the first clinical trial conducted with cardiosphere-derived cells (CADUCEUS), a therapeutic (beneficial) effect has been observed [57].

The problem with CSCs is mainly related to the cell engraftment and retention issue. Less than 1% of transplantated cells can be identified 4 weeks after transplantation, whereas the majority of successfully retained cells die due to apoptosis, inflammatory factors or ischemia.

Some studies focused on the comparison of the myocardial repair potency of different adult stem cell types and the highest rated were CSCs due to the greatest functional benefits, balanced production of paracrine factors and the lowest dose to gain therapeutic effects [49]. However, as previously mentioned, the combined therapy with both CSCs and MSCs provided better effects than CSCs alone.

Summarizing, cell-based clinical trials to treat MI with BMSCs, CSCs and ADSCs have led to an improvement in heart function, also a left ventricular ejection fraction, or extended life expectancy, mostly as a result of paracrine effects instead of direct differentiation into cardiomyocytes. The latest strategies, however, indicate another line of research when using cardiopoietic driven cell populations out of bone marrow-derived stem cells [8]. This procedure could convert traditional "paracrine procedures" into more structurally-oriented cells of cardiac provenience (CHART-1 design). However, a definitive documentation of such cell conversion is lacking. First, phenotypic and genotypic cell characterization was based solely on MEFc nuclear/cytosol ratio, and long-term cell structural conversion has not been yet confirmed. Clinical endpoints were measured at a 6-month follow-up scheme, clearly classical for "paracrine procedures" heart improvement. Further documentation is urgently required for implanted cells imaging in order to document the proof of concept.

Novel strategies in cardiac cell therapy with the use of Adult Stem Cells

The general problem with the transplantation of stem cells is the very low survival rate in the host organ and a small amount of cells which migrate (retain) to regions of myocardial infarction. It has been shown that more than 90% of the stem cells die within 24 hours after transplantation, which is related to an ischemia, hypoxia, and proapoptotic conditions [101]. Moreover, it has been documented that only 1.3% to 2.6% of intracoronary injected stem cells migrated to the myocardium two hours after injection and about 1.49 % was reported after the time of 20 hours [37].

Many studies have shown that stem cell therapy of heart failure depends on the mechanisms associated with the promotion of angiogenesis and suppression of myocardial apoptosis, and/or immune regulation [105]. To address these problems, novel approaches to improve the existing strategies have been developed, e.g. various methods of cells preconditioning before transplantation into damaged myocardium.

Cell preconditioning

It has been reported that mesenchymal stem cells, when hypoxically preconditioned, showed increased expression of pro-survival and pro-angiogenic factors, e.g., hypoxia-inducible factor 1, angiopoietin-1, vascular endothelial growth factor and its receptor, Flk-1, as well as erythropoietin, Bcl-2, and Bcl-xL [31].

Since paracrine factors have been known to promote myocyte proliferation, angiogenesis and to inhibit apoptosis, they are commonly used to enhance the therapeutic effect of stem cells transplanted into infarcted myocardium. Positive effects have been obtained after MSCs preconditioning with some growth factors and cytokines (e.g., SDF-1α), which suppressed cells apoptosis, enhanced their survival, engraftment, vascular density, and, while mediated by SDF/CXCR4 signaling pathway, improved myocardial function [78].

Recent findings have indicated that preconditioning of BMSCs using the Hypoxia- inducible factor 1α (HIF-1α) prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) enhanced their survival and paracrine activity [55], while pretreatment of adipose-derived stem cells (ADSCs) using 5-azacytidine induced cardiogenic differentiation [81].

Likewise, preconditioning using physical methods (mechanical stress, magnetic field and low O2 pre-culture) has been employed for cardiac stem cells. Mechanical stress suppressed the proliferation of CSCs, but promoted their production of inflammatory cytokines and angiogenic factors [42]. Magnetic field was used to drive cardiac-specific differentiation into adult cardiac progenitor cells [27], whereas low O2 conditions improved cells yield and quality [48].

Genetic engineering

An additional strategy to enhance the therapeutic efficacy of stem cells by improving cell survival, homing, engraftment and pro- regenerative capacity of transplanted cells may be the combination of the cell and gene therapy. Thus, in order to attenuate the apoptotic effect after transplantation, genetically modified stem cells have been engineered. The survival rate of MCSs has been increased after cell modification with anti-apoptotic gene Bcl-2. In respect to that, the results obtained have shown a reduction in infarct size and an improvement in cardiac function [50]. Pro-survival effects have also been achieved with MSCs transduced with Akt, known as protein kinase B (PKB) [51], heme oxygenase (HO-1) [101] and Cx43 (connexin43) [106]. Similarly, the transplantation of autologous ADSCs combined with HO-1 transduction showed improved function and remodeling prevention of infarcted myocardium [47]. Genetic engineering methods have also been used to enhance CPCs cell proliferation and survival. For example, overexpression of previously mentioned, Pim-1 kinase, resulted in rejuvenation of phenotypic and functional properties likely by increasing telomerase activity and telomere lengths [68].

In addition to anti-apoptotic genes, stem cells used in cardiac therapy have also been modified with genes responsible for the promotion of angiogenesis, e.g. the vascular endothelial growth factor (VEGF) [32], angiopoietin-1 (Ang-1) [54], granulocyte chemotactic protein (GCP-2) [40]. The results obtained with MSCs in a mouse/rat models showed significant improvement in angiogenesis, cell survival rate, cardiac function, and reduction of infarct size [32,40,54]. Similarly, enhanced pro-angiogenic effects have been reported in experiments with CPCs after VEGF introduction [118] or with those over- expressing Ang-1 [117]. Subsequently, human ADSCs transduced with the gene for VEGF and hepatocyte growth factor (HGF) resulted in greater angiogenesis and improved cardiac function.

The overexpression of protein kinase G1 α (PKG1α) has also been reported to improve both MSCs survival and their angiomyogenic potential in infarcted heart. The survival rate and heart function were improved; moreover, the anti-apoptotic proteins (Akt, GSK3β, and Bcl-2) and paracrine factors were increased [109].

There is accumulating evidence that gene modified stem cells have also attenuated the problems related to hypoxia, migration and immune response. Genetic modification of MSCs by hypoxia inducible factor-1α (HIF-1α) overexpression has been shown to improve the angiogenesis process under a hypoxic condition by paracrine and autocrine mechanisms [83]. Likewise, intramyocardial transfection of HIF-1α and co-transplantation of MSCs in an experimental model of MI increased cells survival, engraftment, and angiogenesis; however, it reduced apoptosis [33]. Stromal derived factor-1α (SDF-1α), a pro-angiogenic and cardiomyocyte protective protein is known to reduce the problem related to the migration of the transplanted MSCs from the damaged site [4]. Furthermore, SDF-1α inhibited hypoxia/SD (SD- serum deprivation) induced MSCs apoptosis through PI3K/Akt and ERK1/2 signaling pathways [114]. It has been also documented that the mesenchymal stem cells modified with tumor necrosis factor receptor (TNFR) gene have shown the anti-apoptotic and anti-inflammatory effects after transplantation into infarcted heart [7].

Moreover, it has been reported that the promotion of CSCs recruitment, engraftment and significant reduction of infarct size occur via CXCR4/PI3K signaling pathway [107]. Likewise, some studies have also proved that CSCs engraftment and differentiation were improved after basic fibroblast growth factor (bFGF) introduction [100].

MicroRNAs/Exosomes

It is suggested that both normal and disordered cardiac functions, e.g., myocyte hypertrophy, ventricular dilation, apoptosis and myocardial fibrosis are controlled by microRNAs. Recent findings have implied that miR-133, and miR-1 are the key regulators of cardiac hypertrophy [14], whereas miR-499 promoted differentiation of CSCs into mechanically integrated cardiomyocytes [30], suggesting their therapeutic application in heart disease.

Another novel molecular mechanism using the therapeutic potential of MSCs includes the function of exosomes. They are considered as vectors for miRNAs communication among cells. After being transferred by exosomes, microRNA can silence corresponding mRNA in target cells [96]. MSCs-derived exosomes play an essential role in MSCs-based therapy by multiple anti-apoptotic miRNAs (e.g., miR-221), which activate cell survival signaling pathway. Recent studies have clearly clarified that exosomal miR-221 reduce the expression of p53 upregulated modulator of apoptosis (PUMA). Since PUMA was shown to interplay with BCL-xL and p53 and activate pro-apoptotic proteins, its inhibition by miR-221 effects in CMCs survival [116]. Moreover, recent studies have revealed that the administration of exosomes from MSCs benefits in a few pro-regenerative processes such as: anti-cardiac remodeling, anti-inflammatory effects [3] and neovascularization [87].

Drug administration

In order to improve cells viability, implementation of drug therapy concomitant to stem cell transplantation has also been studied. The combination therapy with both MSCs and drugs have been reported to improve the therapeutic effects after cell transplantation to infarcted heart in animal models. Statins, such as lovastatin and rosuvastain, are thought to play a cytoprotective role against hypoxia- and serum deprivation- induced apoptosis via PI3K/Akt and MEK/ERK1/2 signaling pathways [112,119]. Moreover, rosuvastatin administration decreased the pro-apoptotic proteins Bim and Bax and increased the anti-apoptotic proteins Bcl-xL, Bcl-2, as well as the paracrine effects of MSCs [119]. In addition to statins, trimetazidine (TMZ) has also been reported to have a protective impact on MSCs H/SD-induced apoptosis via Akt pathway, and increased the paracrine functions of MSCs [29]. A recent study has also revealed that the effect of the ASCs treatment for myocardial infarction has been enhanced when the therapy was combined with cyclosporine A- nanoparticles (CsA-NP) [115]. Likewise, treatment with 17β- estradiol (E2)- enhanced CSCs therapeutic potential [108].

Since NF- κB is a transcription factor thought to have a potential role in the pathogenesis of heart failure by promoting inflammatory and fibrotic responses [93], this factor has also been involved in the recent studies. The results obtained indicated that the blockade of NF-κB activity with its inhibitor significantly inhibited mortality and suppressed the LV remodeling, providing beneficial effects in a rat model. Moreover, using the phosphorylation inhibitor of the IκB (the inhibitor of the NF-κB), IMD-0354 (IMD), the amount of accumulated inflammatory cells in the infarcted heart have been reduced and the expression of proinflammatory cytokines and chemokines have been suppressed, contributing to the reduction of myocardial fibrosis [74].

iPSCs

In 2006, Takahashi and Yamanaka generated a pluripotential stem cell population (iPSCs) transducing mouse adult fibroblasts with four, defined transcription factors (TFs), OCT3/4, Sox2, c-Myc, and Klf4 (so-called "Yamanaka factors"). The cells that they obtained express ESCs cell marker genes and have characteristics similar to embryonic stem cells in respect to their morphology and growth properties [99], which make them another attractive candidates for cardiac regenerative medicine. It has been also reported that iPSCs differ from ESCs with regard to gene expression and DNA methylation pattern [73].

The aim of somatic reprogramming is to generate autologous pluripotent stem cells that are easy to create in the lab, or at any conditions with no difficulties to obtain individually-tailored specific precursors in order to avoid rejection by the patient's immune system in transplantation therapy.

iPSCs have been reported to differentiate into several types of cardiomyocytes (atrial, nodal and ventricular), with similar characteristics to 'native' cardiomyocytes. Moreover, no significant difference has been observed between cardiomyocytes derived from either ESCs or iPSCs [110]. Several signal proteins associated with heart development have also been reported to boost the efficiency of ESCs and iPSCs driven cardiogenesis through Wnt/beta- catenin [69], activin/nodal [77], TGF-beta/BMP2 access [10].

The majority of iPSCs has been produced by forced expression of defined transcription factors using retroviral and lentiviral origin vectors [98], with the accompanying insertion of transgenes into the host genome. Moreover, the transcription factors used to generate iPSCs (c-Myc and Klf4) are known for their oncogenic activity. In order to resolve induced pluripotent stem cells- related problems, which among others include the low efficiency of iPSC generation (0.001- 2%) [86], insufficient kinetics, and safety at all- reprogramming, many methodological improvements have been pursued. For instance, the reprogramming factors can be now delivered using Sendai viral system (SeV), based on a negative--strand RNA virus with no ability to integrate into the host genome, thus ameliorating the danger for human application [71].

In addition to reprogramming approaches, other methods have been developed in order to meet the clinical application requirements through, e.g. virus-free iPSCs generation [36], piggyBac transposons, episomes or minicircle systems. More recent approaches to generate iPSCs include the use of recombinant proteins, synthetic mRNAs and microRNAs. Reprogramming fibroblasts to iPSCs with modified mRNA has been shown to reach up to 1.1%, whereas the combination of lentiviral transduction with miR302/367 demonstrated 10% efficacy. Moreover, a chemical approach is considered as a promising strategy to improve the iPSC generation (CiPSCs). Dozens of small molecules have been reported to replace reprogramming factors and to improve the efficacy of iPSC reprogramming. Such an approach may eliminate the drawbacks of standard used methods (e.g., the risk of tumorigenesis from genomic integration of exogenous sequences or overexpression of oncogenes).

It has been reported that iPSCs epigenetic characteristics, such as CG methylation and histone modification, may affect the function of iPSC- derived cells [53]. Since the generation of iPSCs by somatic cell reprogramming involves global epigenetic remodeling, the effectiveness of reprogramming depends on chromatin-modifying enzymes, e.g. DNA methyltransferase inhibitors (eg. 5-aza-cytidine) and histone deacetylase (HDAC) inhibitors [34]. For instance, Valproic acid (VPA), an HDAC inhibitor, improve reprogramming efficiency by more than 100-fold [34]. Moreover, VPA enables efficient induction of pluripotent stem cells with only Oct4 and Sox2, without introduction of the oncogene *c-Myc* [35].

Novel strategies to genetically modify human iPS cells at 'safe harbor' sites in the genome have been developed in order to avoid perturbing neighboring gene expression [16,76]. Safety criteria established to evaluate potential safe-harbors include: a distance of at least 50kb from the 5' end of any gene, at least 300kb from any cancer-related gene and microRNA, and a location outside of transcriptional units and ultraconserved regions [76]. Cerbini et al. have just published the results of the recent experiments, in which they constructed transcription activator-like effector nucleases (TALENs) targeting the safe-harbor like gene CLYBL. Based on the recent study, it has been shown that a novel target for TALEN–enhanced integrative gene--transfer, located in intron 2 of the Citrate Lyase Beta-Like (CLYBL) gene, provided up to 10-fold higher transgene expression compared to widely used AAVS1 [16].

Nanotechnology

Another problem that cardiac cell therapy has to face is that stem cells injected directly into the myocardium migrate to remote organs. It has been documented that the majority of cells delivered through intracoronary infusion accumulate not in the infarction region, but in the border zone, whereas the retention of cells in the myocardium is an important determinant of their therapeutic effect.

The aim of tissue engineering is to replace or support infarcted areas by the implantation of compounds comprising the cells with degradable biomaterial scaffolds. The selection of the appropriate biomaterial of a scaffold that best suits to the requirements of the 'native' microenvironment of cardiac tissue is crucial. It should assume suitable cell- material interactions in order to ensure proper cell adhesion, proliferation, differentiation, and maturation [45].

It can be noted that there is enormous progress in the biomaterials generation, which exhibits both structural and functional characteristics similar to extracellular matrices [18]. Generally, there are two categories of tissue constructs produced for cardiac therapy purpose: scaffolds (based on collagen, fibrin, matrix synthetic polymers and decellularized heart) and scaffold- free (cell- sheets and cell- aggregation technologies).

There is accumulating evidence that nano tissue engineering approach seems to improve the efficiency of cellular cardiomyoplasty [9,20,61,75,97].

Biodegradable 3D scaffolds

Various types of 3D myocardial tissues have been engineered by seeding cardiomyocytes into alginate, collagen, fibrin or synthetic polymers, e.g. poly(glycolic acid) scaffolds.

The implantation of previously fabricated 3-dimensional myocardial tissue using cardiomyocytes and 3D porous alginate scaffolds have shown the encouraging results. Leor and colleagues have reported almost complete disappearance of the scaffold and good integration into the host with significant improvement of heart functions, such as the attenuation of LV dilatation, the recovery of LV contractility, and intense neovascularization [44].

In the infarcted area of myocardium, there are some structural changes within the extracellular myocardial matrix. The amount of collagen type I decreases from 80% to 40% [18]. It has been observed that collagen matrix demonstrates some features common with cardiac tissue [120], and, when used as a delivery vehicle, significantly reduces the relocation of transplanted MSCs [20]. Many reports have documented that a collagen tissue patch coated with MSCs, playing a role in a reverse remodeling process, impacts functional myocardial improvement [17].

The results obtained in a rat MI model using autologous mesenchymal stem cells (MSCs) seeded on collagen-1 scaffold showed an improvement in the perfusion and the reduction of infarct size with an increase of ventricular wall thickness and angiogenesis [61]. Another collagen-based strategy included vascular endothelial growth factor (VEGF) delivery using genetically modified skeletal myoblasts, which promoted the vascularisation of the infarcted myocardium [56]. Human embryonic stem cell and human induced pluripotent stem cell-derived cardiomyocytes have also been used in 3D collagen matrix [104].

Fibrin patches generated using both fibrinogen and thrombin with either MSCs or hESC-VCs (human embryonic stem cell- derived vascular cells) have been tested in a swine MI model [111]. Sun and colleagues conducted an experiment in a rat myocardial infarction model, showing that fibrin embedded adipose-derived mesenchymal stem cells (ADMSCs) exhibited increased activity in improving left ventricular (LV) performance and reducing LV remodeling when compared with the ADMSCs alone [97].

Synthetic polymers, such as polygycolic acid (PGA) [15], epsilon-caprolactone/L-lactide (PCLA) [60], poly(glycerol- sebacate) (PGS) [59], and polyglycolic acid cloth (PGAC) [25], have also been tested in cardiac tissue engineering. The results obtained with various types of cells (BMCs, MSCs, vascular smooth muscle cells (SMCs), cardiomyocytes) examined in numerous animal models were promising for future cardiac applications (favourable cardiac features, improved LV function, and increased angiogenesis).

The recent results obtained in a mouse MI model, with both a poly(ethylene glycol)–fibrinogen (PF) scaffold and two types of iPSCs: MiPS (iPS cells engineered to secrete matrix metalloproteinase 9- MMP9) and PiPS (iPS cells engineered to secrete placental growth fac-

tor- PlGF), showed significant improvement in revascularization and hemodynamic parameters compared with non- engineered cells or PF alone [9].

The major advantage of 3D scaffolds is the easiness of engineering, but scientists also put emphasis on the correlation between the stiffness of the material and the contractile function of the engineered construct [59]. It has also been reported that cells in stretched constructs had a more mature phenotype, e.g., greater cell elongation, increased gap junction expression, and better contractile elements. Enhanced survival and engraftment of transplanted cells within a stretched construct have been reported in a rat MI model [65].

Whole Heart Reconstruction

Another type of highly promising application in the cardiac regenerative medicine constitutes the three-dimensional extracellular matrix scaffolds. This approach is based on the use of decellularized animal-donor organs with vascular network and extracellular matrix [17], e.g. 3D myocardial tissue was generated by reseeding cardiomyocytes into decellularized rat whole heart [75].

It has been known that structural and functional molecules in the extracellular matrix include glycosaminoglycans and the collagens, elastin, fibronectin, laminin, and vitronectin [6]. Moreover, it has been demonstrated that cardiac extracellular matrix (ECM) properties, which vary in time after MI, may have an impact on cardiac differentiation of delivered stem cells [113]. Three-dimensional extracellular matrix scaffolds as a template for organ reconstruction by recellularization is thought to promote the phenotype and function of the incorporated cells [84]. Scaffold material generated by decellularization is documented to preserve its characteristics including its integrity, bioactivity as well as the vascular, lymphatic and neuronal network [6]. Since it is also biodegradable and does not elicit an immune response from the host, ECM meets the requirements of the perfect biomaterial for the tissue engineering purpose [39]. It has been documented that the degradation products of ECM, including growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), impact the process of recruitment and proliferation of the cells used in the recellularization of the bioscaffold [84].

Based on recent reports, it seems possible that the three-dimensional extracellular matrix scaffolds may allow heart muscles to restore normal cardiac function [6,75]. Various methods of ECM scaffold preparation (including the process of decellularization and implantation) have been tested in order to increase the release of growth factors, to reduce the immune rejection and, as a result, to improve the effect of cell implantation [39]. The results of the recent study have shown that the therapeutic effect was significantly improved when mesenchymal stem cells have been previously preconditioned with transforming growth factor-β (TGF- β) [28].

Tissue engineering

Injectable systems

The major disadvantage of cell seeding is related to the irregular distribution of cells in the scaffold. Moreover, it has also been reported that rigid elements of the matrix isolate cardiomyocytes from each other. Thus, semi-liquid matrix exhibit advantages over the rigid materials, which have been shown to display problems with the continuity of the myocardial architecture, synchronization of the contraction, vascularization and signal transfer [41].

This problem has been resolved by Zimmermann et al., who used a mix of cells with a soluble hydrogel of collagen type I and extracellular matrix protein (Matrigel). As a result, electrical coupling with the host myocardium and improvement of heart function without arrythmia have been observed [120].

Other authors have also documented improved heart geometry and function after the injection of both stem cells and liquid Matrigel into the myocardium. Injectable scaffolds have been thought to exhibit some additional favorable features, e.g., easy and minimal invasive delivery directly to the infarct zone [41].

Since Matrigel is derived from mouse sarcoma cells. The limitations of its usage in the clinical applications include the fact that Matrigel is not tissue-specific and it is also associated with the increased risk of tumor formation [1].

Other injectable materials tested for cardiac regenerative therapy include: hydrogels based on N-isopropylacrylamide [95] or poly(ethylene glycol) (PEG) [85].

Several possible obstacles of injectable materials have been noted and they include insufficient mechanical support for the infarcted myocardium and the risk of blocking the flow of blood in the host [82].

Cell sheet engineering

Scaffold-related problems, such as the inflammatory response due to their biodegradation in the host and undesired cell migration, have resulted in new 3D technology development. In contrast to 3-D biodegradable scaffolds, a novel technology without scaffolds, called cell sheet engineering, has been initiated due to poly (N-isopropyl acrylamide) (PIPAAm) invention [72].

Shimizu et al. reported that the layered cardiomyocyte sheets *in vivo* demonstrate a long survival, macroscopic pulsation and characteristic structures of native heart tissue [91]. The transplantation method of layered cardiomyocytes has been successfully tested with cardiomyocytes of various origin (ESCs, iPSCs) [43,94].

Sekiya and colleagues have demonstrated that cardiac cell sheets express angiogenesis-related genes and thus their transplantation results in the neovascularization of the host myocardium [89]. Based on a rat model, it has been shown that the survival and growth of implanted myocardial cell sheets were preserved for at least one year. Their impact on the cardiac stem cell therapy improvement include the increase in contractility, angiogenesis, and the reduction of fibrosis [90].

Increased LV wall thickness and inhibition of fibrosis and necrosis in the scar was observed as a result of cardiomyocyte sheets transplantation in a rat MI model [66]. Similar results have been obtained for various cells types (adipose- derived MSCs, mESC-derived cardiac cells, endothelial and skeletal muscle cells) [67]. Additionally, using a rat MI model, it has been further demonstrated that the role of MSCs sheets in the improvement of the cardiac function is also related to enhanced paracrine activity [67].

Cardiomyocytes derived from pluripotent stem cells have been tested with encouraging results in both cell-sheet [38,43] and cell-aggregation technologies [94]. This suggests that novel scaffold-free human myocardial patches may meet the requirements for successful cardiac tissue engineering.

Concluding remarks

Further critical elements of stem cell therapy have to be taken under consideration in order to improve the pro-regenerative effect of stem cells towards cardiac tissue, such as the timing, cell dose, and delivery techniques not described in the present review. Nevertheless, the other organs benefit already from cellular therapies and medical application of stem cells has been seen as an imminent future of our biotechnological progress.

References

[1] Albini A., Melchiori A., Garofalo A., Noonan D.M., Basolo F., Taraboletti G., Chader G.J., Gavazzi R.: Matrigel promotes retinoblastoma cell growth in vitro and in vivo. Int. J. Cancer, 1992; 52: 234-240

[2] Amos P.J., Shang H., Bailey A.M., Taylor A., Katz A.J., Peirce S.M.: IFATS collection: The role of human adipose-derived stromal cells in inflammatory microvascular remodeling and evidence of a perivascular phenotype. Stem Cells, 2008; 26: 2682-2690

[3] Arslan F., Lai R.C., Smeets M.B., Akeroyd L., Choo A., Aguor E.N., Timmers L., van Rijen H.V., Doevendans P.A., Pasterkamp G., Lim S.K., de Kleijn D.P.: Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res., 2013; 10: 301-312

[4] Askari A.T., Unzek S., Popovic Z.B., Goldman C.K., Forudi F., Kiedrowski M., Rovner A., Ellis S.G., Thomas J.D., DiCorleto P.E., Topol E.J., Penn M.S.: Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003; 362: 697-703

[5] Baas T.: A big heart. SciBX, 2014; 7; 1-2

[6] Badylak S.F., Weiss D.J., Caplan A., Macchiarini P.: Engineered whole organs and complex tissues. Lancet, 2012; 379: 943-952

[7] Bao C., Guo J., Lin G., Hu M., Hu Z.: TNFR gene-modified mesenchymal stem cells attenuate inflammation and cardiac dysfunction following MI. Scand. Cardiovasc. J., 2008; 42: 56-62

[8] Bartunek J., Davison B., Sherman W., Povsic T., Henry T.D., Gersh B., Metra M., Filippatos G., Hajjar R., Behfar A., Homsy C., Cotter G., Wijns W., Tendera M., Terzic A.: Congestive heart failure cardiopoietic regenerative therapy (CHART-1) trial design. Eur. J. Heart. Fail., 2016;18: 160-168

[9] Bearzi C., Gargioli C., Baci D., Fortunato O., Shapira-Schweitzer K., Kossover O., Latronico M.V., Seliktar D., Condorelli G., Rizzi R.: PlGF-MMP9-engineered iPS cells supported on a PEG-fibrinogen hydrogel scaffold possess an enhanced capacity to repair damaged myocardium. Cell Death Dis., 2014; 5: e1053

[10] Behfar A., Zingman L.V., Hodgson D.M., Rauzier J.M., Kane G.C., Terzic A., Pucéat M.: Stem cell differentiation requires a paracrine pathway in the heart. FASEB. J., 2002; 16: 1558-1566

[11] Beltrami A.P., Barlucchi L., Torella D., Baker M., Limana F., Chimenti S., Kasahara H., Rota M., Musso E., Urbanek K., Leri A., Kajstura J., Nadal-Ginard B., Anversa P.: Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell, 2003; 114: 763-776

[12] Bolli R., Chugh A.R., D'Amario D., Loughran J.H., Stoddard M.F., Ikram S., Beache G.M., Wagner S.G., Leri A., Hosoda T., Sanada F., Elmore J.B., Goichberg P., Cappetta D., Solankhi N.K., et al.: Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a andomized phase 1 trial. Lancet, 2011; 378: 1847-1857

[13] Caplan A.I., Dennis J.E.: Mesenchymal stem cells as trophic mediators. J. Cell Biochem., 2006; 98: 1076-1084

[14] Carè A., Catalucci D., Felicetti F., Bonci D., Addario A., Gallo P., Bang M.L., Segnalini P., Gu Y., Dalton N.D., Elia L., Latronico M.V., Høydal M., Autore C., Russo M.A., et al.: MicroRNA-133 controls cardiac hypertrophy. Nat. Med., 2007; 13: 613-618

[15] Carrier R.L., Papadaki M., Rupnick M., Schoen F.J., Bursac N., Langer R., Freed L.E., Vunjak-Novakovic G.: Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol. Bioeng. 1999; 64: 580-589

[16] Cerbini T., Funahashi R., Luo Y., Liu C., Park K., Rao M., Malik N., Zou J.: Transcription activator-like effector nuclease (TALEN)-mediated CLYBL targeting enables enhanced transgene expression and one-step generation of dual reporter human induced pluripotent stem cell (iPSC) and neural stem cell (NSC) lines. PLoS One, 2015; 10: e0116032

[17] Chachques J.C.: Development of bioartificial myocardium using stem cells and nanobiotechnology templates. Cardiol. Res. Pract., 2011; 2011: 806795

[18] Chachques J.C., Trainini J.C., Lago N., Masoli O.H., Barisani J.L., Cortes-Morichetti M., Schussler O., Carpentier A.: Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM clinical trial): one year follow-up. Cell Transplant., 2007; 16: 927-934

[19] Chavakis E., Koyanagi M., Dimmeler S.: Enhancing the outcome of cell therapy for cardiac repair: progress from bench to bedside and back. Circulation, 2010; 121: 325-335

[20] Dai W., Hale S.L., Kay G.L., Jyrala A.J., Kloner R.A.: Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology. Regen.

Med., 2009; 4: 387-395

[21] DeSantiago J., Bare D.J., Semenov I., Minshall R.D., Geenen D.L., Wolska B.M., Banach K.: Excitation- contraction coupling in ventricular myocytes is enhanced by paracrine signaling from mesenchymal stem cells. J. Mol. Cell. Cardiol., 2012; 52: 1249-1256

[22] Dimmeler S., Zeiher A.M.: Cell therapy of acute myocardial infarction: open questions. Cardiology, 2009; 113: 155-160

[23] Du Y.Y., Zhou S.H., Zhou T., Su H., Pan H.W., Du W.H., Liu B., Liu Q.M.: Immuno- inflammatory regulation effect of mesenchymal stem cell transplantation in a rat model of myocardial infarction. Cytotherapy, 2008; 10: 469-478

[24] Freund C., Mummery C.L.: Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models. J. Cell. Biochem., 2009; 107: 592-599

[25] Fukuhara S., Tomita S., Nakatani T., Fujisato T., Ohtsu Y., Ishida M., Yutani C., Kitamura S.: Bone marrow cell-seeded biodegradable polymeric scaffold enhances angiogenesis and improves function of the infarcted heart. Circ. J., 2005; 69: 850-857

[26] Fukuhara S., Tomita S., Yamashiro S., Morisaki T., Yutani C., Kitamura S., Nakatani T.: Direct cell-cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage in vitro. J. Thorac. Cardiovasc. Surg., 2003; 125: 1470-1479

[27] Gaetani R., Ledda M., Barile L., Chimenti I., De Carlo F., Forte E., Ionta V., Giuliani L., D'Emilia E., Frati G., Miraldi F., Pozzi D., Messina E., Grimaldi S., Giacomello A., Lisi A.: Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc. Res., 2009; 82: 411-420

[28] Godier-Furnémont A.F., Martens T.P., Koeckert M.S., Wan L., Parks J., Arai K., Zhang G., Hudson B., Homma S., Vunjak-Novakovic G.: Composite scaffold provides a cell delivery platform for cardiovascular repair. Proc. Natl. Acad. Sci. USA, 2011; 108: 7974-7979

[29] Gong X., Fan G., Wang W., Wang G.: Trimetazidine protects umbilical cord mesenchymal stem cells against hypoxia and serum deprivation induced apoptosis by activation of Akt. Cell Physiol. Biochem., 2014; 34: 2245-2255

[30] Hosoda T., Zheng H., Cabral-da-Silva M., Sanada F., Ide-Iwata N., Ogórek B., Ferreira-Martins J., Arranto C., D'Amario D., del Monte F., Urbanek K., D'Alessandro D.A., Michler R.E., Anversa P., Rota M., et al.: Human cardiac stem cell differentiation is regulated by a mircrine mechanism. Circulation, 2011; 123: 1287-1296

[31] Hu X., Yu S.P., Fraser J.L., Lu Z., Ogle M.E., Wang J.A., Wei L.: Transplantation of hypoxia- preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J. Thorac. Cardiovasc. Surg., 2008; 135: 799-808

[32] Hua P., Tao J., Liu J.Y., Yang S.R.: Cell transplantation into ischemic myocardium using mesenchymal stem cells transfected by vascular endothelial growth factor. Int. J. Clin. Exp. Pathol.: 2014; 7: 7782-7788

[33] Huang B., Qian J., Ma J., Huang Z., Shen Y., Chen X., Sun A., Ge J., Chen H.: Myocardial transfection of hypoxia-inducible factor-1α and co-transplantation of mesenchymal stem cells enhance cardiac repair in rats with experimental myocardial infarction. Stem. Cell. Res. Ther., 2014; 5: 22

[34] Huangfu D., Maehr R., Guo W., Eijkelenboom A., Snitow M., Chen A.E., Melton D.A. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat. Biotechnol., 2008; 26: 795-797

[35] Huangfu D., Osafune K., Maehr R., Guo W., Eijkelenboom A., Chen S., Muhlestein W., Melton D.A.: Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat. Biotechnol., 2008; 26: 1269-1275

[36] Kaji K., Norrby K., Paca A., Mileikovsky M., Mohseni P., Woltjen K.: Virus free induction of pluripotency and subsequent excision of reprogramming factors. Nature, 2009; 458: 771-775

[37] Kang W.J., Kang H.J., Kim H.S., Chung J.K., Lee M.C., Lee D.S.: Tissue distribution of 18F-FDG-labeled peripheral hematopoietic stem cells after intracoronary administration in patients with myocardial infarction. J. Nucl. Med., 2006; 47: 1295-1301

[38] Kawamura M., Miyagawa S., Miki K., Saito A., Fukushima S., Higuchi T., Kawamura T., Kuratani T., Daimon T., Shimizu T., Okano T., Sawa Y.: Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation, 2012; 126: S29-S37

[39] Keane T.J., Badylak S.F.: The host response to allogeneic and xenogeneic biological scaffold materials. J. Tissue. Eng. Regen. Med., 2015; 9: 504-511

[40] Kim S.W., Lee D.W., Yu L.H., Zhang H.Z., Kim C.E., Kim J.M., Park T.H., Cha K.S., Seo S.Y., Roh M.S., Lee K.C., Jung J.S,. Kim M.H.: Mesenchymal stem cells overexpressing GCP-2 improve heart function through enhanced angiogenic properties in a myocardial infarction model. Cardiovasc. Res., 2012; 95: 495-506

[41] Kofidis T., Lebl D.R., Martinez E.C., Hoyt G., Tanaka M., Robbins R.C.: Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. Circulation, 2005; 112: 173-177

[42] Kurazumi H., Kubo M., Ohshima M., Yamamoto Y., Takemoto Y., Suzuki R., Ikenaga S., Mikamo A., Udo K., Hamano K., Li T.S.: The effects of mechanical stress on the growth, differentiation, and paracrine factor production of cardiac stem cells. PLoS One, 2011; 6: e28890

[43] Lee P., Klos M., Bollensdorff C., Hou L., Ewart P., Kamp T.J., Zhang J., Bizy A., Guerrero-Serna G., Kohl P., Jalife J., Herron T.J.: Simultaneous voltage and calcium mapping of genetically purified human induced pluripotent stem cell-derived cardiac myocyte monolayers. Circ. Res., 2012; 110: 1556-1563

[44] Leor J., Aboulafia-Etzion S., Dar A., Shapiro L., Barbash I.M., Battler A., Granot Y., Cohen S.: Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? Circulation, 2000; 102: 56-61

[45] Leor J., Amsalem Y., Cohen S.: Cells, scaffolds, and molecules for myocardial tissue engineering. Pharmacol. Ther., 2005; 105: 151-163

[46] Li B., Zeng Q., Wang H., Shao S., Mao X., Zhang F., Li S., Guo Z.: Adipose tissue stromal cells transplantation in rats of acute myocardial infarction. Coron. Artery. Dis., 2007; 18: 221-227

[47] Li Q., Verma I.M.: NF-κB regulation in the immune system. Nat. Rev. Immunol., 2002; 2: 725-734

[48] Li T.S., Cheng K., Malliaras K., Matsushita N., Sun B., Marbán L., Zhang Y., Marbán E.: Expansion of human cardiac stem cells in physiological oxygen improves cell production efficiency and potency for myocardial repair. Cardiovasc. Res., 2011; 89: 157-165

[49] Li T.S., Cheng K., Malliaras K., Smith R.R., Zhang Y., Sun B., Matsushita N., Blusztajn A., Terrovitis J., Kusuoka H., Marbán L., Marbán E.: Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere- derived cells. J. Am. Coll. Cardiol., 2012; 59: 942-953

[50] Li W., Ma N., Ong L.L., Nesselmann C., Klopsch C., Ladilov Y., Furlani D., Piechaczek C., Moebius J.M., Lützow K., Lendlein A., Stamm C., Li R.K., Steinhoff G.: Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells, 2007; 25: 2118-2127

[51] Lim S.Y., Kim Y.S., Ahn Y., Jeong M.H., Hong M.H., Joo S.Y., Nam K.I., Cho J.G., Kang P.M., Park J.C.: The effects of mesenchymal stem cells transduced with Akt in a porcine myocardial infarction model. Cardiovasc. Res., 2006; 70: 530-542

[52] Linke A., Müller P., Nurzynska D., Casarsa C., Torella D., Nas-

cimbene A., Castaldo C., Cascapera S., Böhm M., Quaini F., Urbanek K., Leri A., Hintze T.H., Kajstura J., Anversa P.: Stem cells in the dog heart are self- renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc. Natl. Acad. Sci. USA, 2005; 102: 8966-8971

[53] Lister R., Pelizzola M., Kida Y.S., Hawkins R.D., Nery J.R., Hon G., Antosiewicz-Bourget J., O'Malley R., Castanon R., Klugman S., Downes M., Yu R., Stewart R., Ren B., Thomson J.A., Evans R.M., Ecker J.R.: Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature, 2011; 471: 68-73

[54] Liu X.B., Chen H., Chen H.Q., Zhu M.F., Hu X.Y., Wang Y.P., Jiang Z., Xu Y.C., Xiang M.X., Wang J.A.: Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation. J. Zhejiang. Univ. Sci. B., 2012; 13: 616-623

[55] Liu X.B., Wang J.A., Ji X.Y., Yu S.P., Wei L.: Preconditioning of bone marrow mesenchymal stem cells by prolyl hydroxylase inhibition enhances cell survival and angiogenesis in vitro and after transplantation into the ischemic heart of rats. Stem. Cell. Res. Ther., 2014; 5: 111

[56] Lu Y., Shansky J., Del Tatto M., Ferland P., Wang X., Vandenburgh H.: Recombinant vascular endothelial growth factor secreted from tissue-engineered bioartificial muscles promotes localized angiogenesis. Circulation, 2001; 104: 594-599

[57] Makkar R.R., Smith R.R., Cheng K., Malliaras K., Thomson L.E., Berman D., Czer L.S., Marbán L., Mendizabal A., Johnston P.V., Russell S.D., Schuleri K.H., Lardo A.C., Gerstenblith G., Marbán E.: Intracoronary cardiosphere- derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet. 2012; 379: 895-904

[58] Mann D.L.: Mechanisms and models in heart failure: A combinatorial approach. Circulation, 1999; 100: 999-1008

[59] Marsano A., Maidhof R., Wan L.Q., Wang Y., Gao J., Tandon N., Vunjak-Novakovic G.: Scaffold stiffness affects the contractile function of three-dimensional engineered cardiac constructs. Biotechnol. Prog., 2010; 26: 1382-1390

[60] Matsubayashi K., Fedak P.W., Mickle D.A., Weisel R.D., Ozawa T., Li R.K.: Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. Circulation, 2003; 108: 219-225

[61] Maureira P., Marie P.Y., Yu F., Poussier S., Liu Y., Groubatch F., Falanga A., Tran N.: Repairing chronic myocardial infarction with autologous mesenchymal stem cells engineered tissue in rat promotes angiogenesis and limits ventricular remodeling. J. Biomed. Sci., 2012; 19: 93

[62] Mazo M., Planat-Bénard V., Abizanda G., Pelacho B., Léobon B., Gavira J.J., Peñuelas I., Cemborain A., Pénicaud L., Laharrague P., Joffre C., Boisson M., Ecay M., Collantes M., Barba J., Casteilla L., Prósper F.: Tranplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial nfarction. Eur. J. Heart. Fail., 2008; 10: 454-462

[63] Menasché P., Alfieri O., Janssens S., McKenna W., Reichenspurner H., Trinquart L., Vilquin J.T., Marolleau J.P., Seymour B., Larghero J., Lake S., Chatellier G., Solomon S., Desnos M., Hagège A.A.: The Myoblats Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo- controlled study of myoblast trnaplantation. Circulation, 2008; 117: 1189-1200

[64] Mendis S., Puska P., Norrving B. (editors): Global Atlas on Cardiovascular Disease Prevention and Control. World Health Organization, Geneva 2011

[65] Mihic A., Li J., Miyagi Y., Gagliardi M., Li S.H., Zu J., Weisel R.D., Keller G., Li R.K.: The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes. Biomaterials, 2014; 35: 2798-2808

[66] Miyagawa S., Sawa Y., Sakakida S., Taketani S., Kondoh H., Memon I.A., Imanishi Y., Shimizu T., Okano T., Matsuda H.: Tissue cardiomyoplasty using bioengineered contractile cardiomyocyte sheets to repair damaged myocardium: their integration with recipient myocardium. Transplantation, 2005; 80: 1586-1595

[67] Miyahara Y., Nagaya N., Kataoka M., Yanagawa B., Tanaka K., Hao H., Ishino K., Ishida H., Shimizu T., Kangawa K., Sano S., Okano T., Kitamura S., Mori H.: Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat. Med., 2006; 12: 459-465

[68] Mohsin S., Khan M., Nguyen J., Alkatib M., Siddiqi S., Hariharan N., Wallach K., Monsanto M., Gude N., Dembitsky W., Sussman M.A.: Rejuvenation of human cardiac progenitor cells with Pim-1 kinase. Circ. Res., 2013; 113: 1169-1179

[69] Naito A.T., Shiojima I., Akazawa H., Hidaka K., Morisaki T., Kikuchi A., Komuro I.: Developmental stage- specific biphasic roles of Wnt/β-catenin signaling in cardiomyogenesis and hematopoiesis. Proc. Natl. Acad. Sci. USA, 2006; 103: 19812-19817

[70] Nowbar A.N., Mielewczik M., Karavassilis M., Dehbi H.M., Shun-Shin M.J., Jones S., Howard J.P., Cole G.D., Francis D.P.; DAMASCENE writing group: Discrepancies in autologous bone marrow stem cell trails and enhancement of ejection fraction (DAMASCENE): weighted regression and meta- analysis. Br. Med. J., 2014; 348: g2688

[71] Okano S., Yonemitsu Y., Nagata S., Sata S., Onimaru M., Nakagawa K., Tomita Y., Kishihara K., Hashimoto S., Nakashima Y., Sugimachi K., Hasegawa M., Sueishi K.: Recombinant Sendai virus vectors for activated T lymphocytes. Gene. Ther., 2003; 10: 1381-1391

[72] Okano T., Yamada N., Sakai H., Sakurai Y.: A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). J. Biomed. Mater. Res., 1993; 27: 1243-1251

[73] Okita K., Ichisaka T., Yamanaka S.: Generation of germline-competent induced pluripotent stem cells. Nature, 2007; 448: 313-317

[74] Onai Y., Suzuki J., Maejima Y., Haraguchi G., Muto S., Itai A., Isobe M.: Inhibition of NF-kappa B improves left ventricular remodeling and cardiac dysfunction after myocardial infarction. Am. J. Physiol. Heart. Circ. Physiol., 2007; 292: H530-H538

[75] Ott H.C., Matthiesen T.S., Goh S.K., Black L.D., Kren S.M., Netoff T.I., Taylor D.A.: Perfusion-decellularized matrix: using nature›s platform to engineer a bioartificial heart. Nat. Med., 2008; 14: 213-221

[76] Papapetrou E.P., Lee G., Malani N., Setty M., Riviere I., Tirunagari L.M., Kadota K., Roth S.L., Giardina P., Viale A., Leslie C., Bushman F.D., Studer L., Sadelain M.: Genomic safe harbors permit high β-globin transgene expression in thalassemia induced pluripotent stem cells. Nat. Biotechnol., 2011; 29: 73-78

[77] Parisi S., D'Andrea D., Lago C.T., Adamson E.D., Persico M.G., Minchiotti G.: Nodal- dependent Cripto signaling promotes cardiomyogenesis and redirects the neural fate of embryonic stem cells. J. Cell. Biol., 2003; 163: 303-314

[78] Pasha Z., Wang Y., Sheikh R., Zhang D., Zhao T., Ashraf M.: Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. Cardiovasc. Res., 2008; 77: 134-142

[79] Perin E.C., Sanchez P.L., Ruiz R.S., Perez-Cano R., Lasso J., Alonso-Farto J.C., Fernandez-Pina L., Serruys P.W., Duckers H.J., Kastrup J., Chameleau S., Zheng Y., Silva G.V., Milstein A.M., Martin M.T., et al.: First in man transendocardial injection of autologous adipose-derived stem cells in patients with non revascularizable ischemic myocardium (PRECISE). Circulation, 2010; 122: A17966

[80] Planat-Bénard V., Menard C., André M., Puceat M., Perez A., Garcia-Verdugo J.M., Pénicaud L., Casteilla L.: Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. Circ. Res., 2004; 94: 223-229

[81] Ravichandran R., Venugopal J.R., Mueller M., Sundarrajan S., Mukherjee S., Pliska D., Wintermantel E., Ramakrishna S.: Buckled

structures and 5-azacytidine enhance cardiogenic differentiation of adipose-derived stem cells. Nanomedicine, 2013; 8: 1985-1997

[82] Ravichandran R., Venugopal J.R., Sundarrajan S., Mukherjee S., Sridhar R., Ramakrishna S.: Minimally invasive injectable short nanofibers of poly(glycerol sebacate) for cardiac tissue engineering. Nanotechnology, 2012; 23: 385102

[83] Razban V., Lotfi A.S., Soleimani M., Ahmadi H., Massumi M., Khajeh S., Ghaedi M., Arjmand S., Najavand S., Khoshdel A.: HIF-1α overexpression induces angiogenesis in mesenchymal stem cells. Biores. Open Access, 2012; 1: 174-183

[84] Reing J.E., Zhang L., Myers-Irvin J., Cordero K.E., Freytes D.O., Heber-Katz E., Bedelbaeva K., McIntosh D., Dewilde A., Braunhut S.J., Badylak S.F.: Degradation products of extracellular matrix affect cell migration and proliferation. Tissue. Eng. Part. A., 2009; 15: 605-614

[85] Rizzi S.C., Ehrbar M., Halstenberg S., Raeber G.P., Schmoekel H.G., Hagenmüller H., Müller R., Weber F.E., Hubbell J.A.: Recombinant protein-co-PEG networks as cell-adhesive and proteolytically degradable hydrogel matrixes. Part II: biofunctional characteristics. Biomacromolecules, 2006; 7: 3019-3029

[86] Robinton D.A., Daley G.Q.: The promise of induced pluripotent stem cells in research and therapy. Nature, 2012; 481: 295-305

[87] Salomon C., Ryan J., Sobrevia L., Kobayashi M., Ashman K., Mitchell M., Rice G.E.: Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. PLoS One, 2013; 8: e68451

[88] Schuleri K.H., Feigenbaum G.S., Centola M., Weiss E.S., Zimmet J.M., Turney J., Kellner J., Zviman M.M., Hatzistergos K.E., Detrick B., Conte J.V., McNiece I., Steenbergen C., Lardo A.C., Hare J.M.: Autologous mesenchymal stem cells produce reverse remodeling in chronic ischaemic cardiomyopathy. Eur. Heart. J., 2009; 30: 2722-2732

[89] Sekiya S., Shimizu T., Yamato M., Kikuchi A., Okano T.: Bioengineered cardiac cell sheet grafts have intrinsic angiogenic potential. Biochem. Biophys. Res. Commun., 2006; 341: 573-582

[90] Shimizu T., Sekine H., Isoi Y., Yamato M., Kikuchi A., Okano T.: Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. Tissue. Eng., 2006; 12: 499-507

[91] Shimizu T., Yamato M., Kikuchi A., Okano T.: Cell sheet engineering for myocardial tissue reconstruction. Biomaterials, 2003; 24: 2309-2316

[92] Siminiak T., Kalawski R., Fiszer D., Jerzykowska O., Rzeźniczak J., Rozwadowska N., Kurpisz M.: Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardiual injury: phase I clinical study with 12 months of follow- up. Am. Heart. J., 2004; 148: 531-537

[93] Stancovski I., Baltimore D.: NF-κB activation: the IκB kinase revealed? Cell, 1997; 91: 299-302

[94] Stevens K.R., Pabon L., Muskheli V., Murry C.E.: Scaffold-free human cardiac tissue patch created from embryonic stem cells. Tissue Eng. Part A, 2009; 15: 1211-1222

[95] Stile R.A., Chung E., Burghardt W.R., Healy K.E.: Poly(N-isopropylacrylamide)-based semi-interpenetrating polymer networks for tissue engineering applications. Effects of linear poly(acrylic acid) chains on rheology. J. Biomater. Sci. Polym. Ed., 2004; 15: 865-878

[96] Stroorvogel W.: Functional transfer of microRNA by exosomes. Blood, 2012; 119: 646-648

[97] Sun C.K., Zhen Y.Y., Leu S., Tsai T.H., Chang L.T., Sheu J.J., Chen Y.L., Chua.S, Chai H.T., Lu H.I., Chang H.W., Lee F.Y., Yip H.K.: Direct implantation versus platelet-rich fibrin-embedded adipose-derived mesenchymal stem cells in treating rat acute myocardial infarction. Int. J. Cardiol., 2014; 173: 410-423

[98] Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K., Yamanaka S.: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 2007; 131: 861-872

[99] Takahashi K., Yamanaka S.: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 2006; 126: 663-676

[100] Takehara N., Tsutsumi Y., Tateishi K., Ogata T., Tanaka H., Ueyama T., Takahashi T., Takamatsu T., Fukushima M., Komeda M., Yamagishi M., Yaku H., Tabata Y., Matsubara H., Oh H.: Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. J. Am. Coll. Cardiol., 2008; 52: 1858-1865

[101] Tang Y.L., Tang Y., Zhang Y.C., Qian K., Shen L., Phillips M.I.: Improved graft mesenchymal stem cell survival in ischemic heart with a hypoxia- regulated heme oxygenase-1 vector. J. Am. Coll. Cardiol., 2005; 46: 1339-1350

[102] Toma C., Pittenger M.F., Cahill K.S., Byrne B.J., Kessler P.D.: Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation, 2002; 105: 93-98

[103] Treskes P., Neef K., Perumal Srinivasan S., Halbach M., Stamm C., Cowan D., Scherner M., Madershahian N., Wittwer T., Hescheler J., Wahlers T., Choi Y.H.: Preconditioning of skeletal myoblast-based engineered tissue constructs enables functional coupling to myocardium in vivo. J. Thorac. Cardiovasc. Surg., 2015; 149: 348-356

[104] Tulloch N.L., Muskheli V., Razumova M.V., Korte F.S., Regnier M., Hauch K.D., Pabon L., Reinecke H., Murry C.E.: Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ. Res., 2011; 109: 47-59

[105] Uemura R., Xu M., Ahmad N., Ashraf M.: Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. Circ. Res., 2006; 98: 1414-1421

[106] Wang D., Shen W., Zhang F., Chen M., Chen H., Cao K.: Connexin43 promotes survival of mesenchymal stem cells in ischaemic heart. Cell. Biol. Int., 2010; 34: 415-423

[107] Wang K., Zhao X., Kuang C., Qian D., Wang H., Jiang H., Deng M., Huang L.: Overexpression of SDF-1α enhanced migration and engraftment of cardiac stem cells and reduced infarcted size via CXCR4/PI3K pathway. PLoS One, 2012; 7: e43922

[108] Wang L., Gu H., Turrentine M., Wang M.: Estradiol treatment promotes cardiac stem cell (CSC)-derived growth factors, thus improving CSC-mediated cardioprotection after acute ischemia/reperfusion. Surgery, 2014; 156: 243-252

[109] Wang L., Pasha Z., Wang S., Li N., Feng Y., Lu G., Millard R.W., Ashraf M.: Protein kinase G1α overexpression increases stem cell survival and cardiac function after myocardial infarction. PLoS One, 2013; 8: e60087

[110] Xi J., Khalil M., Shishechian N., Hannes T., Pfannkuche K., Liang H., Fatima A., Haustein M., Suhr F., Bloch W., Reppel M., Sarić T., Wernig M., Jänisch R., Brockmeier K., et al.: Comparison of contractile behavior of native murine ventricular tissue and cariomyocytes derived from embryonic or induced pluripotent stem cells. FASEB J., 2010; 24: 2739-2751

[111] Xiong Q., Ye L., Zhang P., Lepley M., Tian J., Li J., Zhang L., Swingen C., Vaughan J.T., Kaufman D.S., Zhang J.: Functional consequences of human induced pluripotent stem cell therapy: myocardial ATP turnover rate in the in vivo swine heart with postinfarction remodeling. Circulation, 2013; 127: 997-1008

[112] Xu R., Chen J., Cong X., Hu S., Chen X.: Lovastatin protects mesenchymal stem cells against hypoxia- and serum deprivation-induced apoptosis by activation of PI3K/Akt and ERK1/2. J. Cell. Biochem., 2008; 103: 256-269

[113] Xu Y., Patnaik S., Guo X., Li Z., Lo W., Butler R., Claude A., Liu Z., Zhang G., Liao J., Anderson P.M., Guan J.: Cardiac differentiation

of cardiosphere-derived cells in scaffolds mimicking morphology of the cardiac extracellular matrix. Acta Biomater., 2014; 10: 3449-3462

[114] Yin Q., Jin P., Liu X., Wei H., Lin X., Chi C., Liu Y., Sun C., Wei Y.: SDF-1α inhibits hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells through PI3K/Akt and ERK1/2 signaling pathways. Mol. Biol. Rep., 2011; 38: 9-16

[115] Yin Q., Pei Z., Wang H., Zhao Y.: Cyclosporine A-nanoparticles enhance the therapeutic benefit of adipose tissue-derived stem cell transplantation in a swine myocardial infarction model. Int. J. Nanomedicine., 2014; 9: 17-26

[116] Yu B., Gong M., Wang Y., Millard R.W., Pasha Z., Yang Y., Ashraf M., Xu M.: Cardiomyocyte protection by GATA-4 gene engineered mesenchymal stem cells is partially mediated by translocation of miR-221 in microvesicles. PLoS One, 2013; 8: e73304

[117] Zeng H., Li L., Chen J.X.: Overexpression of angiopoietin-1 increases CD133+/c-kit+ cells and reduces myocardial apoptosis in db/db mouse infarcted hearts. PLoS One, 2012; 7: e35905

[118] Zhang Y., Li W., Ou.L, Wang W., Delyagina E., Lux C., Sorg H., Riehemann K., Steinhoff G., Ma N.: Targeted delivery of human VEGF gene via complexes of magnetic nanoparticle-adenoviral vectors enhanced cardiac regeneration. PLoS One, 2012; 7: e39490

[119] Zhang Z., Li S., Cui M., Gao X., Sun D., Qin X., Narsinh K., Li C., Jia H., Li C., Han Y., Wang H., Cao F.: Rosuvastin enhances the therapeutic efficacy of adipose-derived mesenchymal stem cells for myocardial infarction via PI3K/Akt and MEK/ERK pathways. Basic Res. Cardiol., 2013; 108: 333

[120] Zimmermann W.H., Schneiderbanger K., Schubert P., Didié M., Münzel F., Heubach J.F., Kostin S., Neuhuber W.L., Eschenhagen T.: Tissue engineering of a differentiated cardiac muscle construct. Circ. Res., 2002; 90: 223-230

The authors have no potential conflicts of interest to declare.