J. Biomedical Science and Engineering, 2016, 9, 191-215
Published Online March 2016 in SciRes. http://www.scirp.org/journal/jbise
http://dx.doi.org/10.4236/jbise.2016.94015

Stem Cell Therapy of Ischemic Heart Disease

Mikhail Konoplyannikov, Vladimir Kalsin, Alexander Averyanov, Alexander Troitsky

Federal Research Clinical Center of the FMBA of Russia, Moscow, Russia
Email: mkonopl@mail.ru

Received 28 January 2016; accepted 26 March 2016; published 29 March 2016

Abstract

Ischemic heart disease (IHD) accelerates death of cardiomyocytes and leads to the onset of cardiac failure. Due to the application of stem cells, there exists a potential for the regeneration of a damaged myocardium. Here we present a brief review of the modern data on the application of various types of stem cells for the IHD therapy. We consider different types of stem cells, which are most preferable for the clinical application, including mesenchymal stem cells, cardiac stem cells, embryonic stem cells, iPS cells and others. In particular, we discuss their advantages and strategies which can be applied in order to boost their regenerative potential, as well as optimization of their delivery. Besides, our review refers to the contemporary achievements in the field of tissue engineering of heart, using both polymer scaffolds and scaffold-free constructs. We also discuss the most prominent known clinical trials on stem cell therapy of ischemic heart disease.

Keywords

IHD, Remodeling, Regeneration, Stem Cells, MSC, Pre-Conditioning, Paracrine Factors

1. Introduction

Ischemic heart disease (IHD), or coronary artery disease, is a condition in which the blood flow to the cardiac muscle is reduced, potentially leading to a heart attack. Atherosclerosis is the main cause of IHD, with an atherosclerotic plaque's rupture being the culprit for coronary artery narrowing or blockage resulting in a severe angina and a heart attack. As a result of a heart attack, the cardiac muscle remains permanently damaged by the cut-off of the blood supply.

According to the Mayo Clinic (www.mayoclinic.org), the most common symptom of IHD is a typically left-sided chest pressure or pain (angina pectoris). Angina may also be presented as a painful feeling in a shoulder, arms, neck, back, or jaw. Other common symptoms include a faster heartbeat, palpitations, shortness of breath, dizziness or weakness, sweating, nausea and vomiting.

IHD-induced myocardial infarction (MI) is the most common cause of mortality and disability in the Western

How to cite this paper: Konoplyannikov, M., Kalsin, V., Averyanov, A. and Troitsky, A. (2016) Stem Cell Therapy of Ischemic Heart Disease. *J. Biomedical Science and Engineering*, **9**, 191-215. http://dx.doi.org/10.4236/jbise.2016.94015

society [1]. In MI, necrosis and apoptosis of cardiomyocytes accelerate, resulting in an extremal heart loading and finally in appearance of heart failure. Progressing loss of cardiomyocytes occurs not only due to an ischemic condition, but also as an adverse effect of chronic hypertension [2]. On the contrary, suppressing apoptosis of cardiomyocytes leads to the heart function improvement in animal models [3]. Pharmaceutical drugs, medical interventions and heart transplants, being common methods of therapy for patients with cardiovascular diseases, have shown their limitedness. To overcome these limits, various new techniques for IHD treatment have been developed as a result of scientific research of the last decades. In particular, active studies have been performed on the possibility of applications of various types of stem cells for IHD therapy since 2000s [4]. It was believed earlier that cardiomyocytes could not proliferate into the adult human heart. However, as it has been found recently, adult cardiomyocytes are still capable of division and proliferation, although their ability to independently regenerate the heart, damaged by ischemic heart disease, is not sufficient [5]. A large number of experiments on animals have clearly demonstrated that the damaged heart function may be restored by administration of stem cells, which were preliminarily reproduced in a significant quantity *in vitro*. Some of the stem cells proliferated and differentiated into cardiomyocytes in the region of the heart damage [4]. However, in the majority of cases such a differentiation was limited and did not have an essential effect on the observed functional improvement of the heart, which determined a necessity of a versatile analysis of the heart regeneration mechanisms caused by stem cells. Since the heart is a post-mitotic organ, cardiomyocytes exist in the condition of the cell cycle arrest. Nevertheless, certain experiments on newts and Danio rerio fishes have shown that a damaged heart can regenerate due to resident stem cells with ability to self-renewal and differentiation into cardiomyocytes, leading to the damage repair.

Cardiomyocytes do not proliferate in the normal conditions, but in the case of a heart damage they undergo division, thus restarting the DNA synthesis and propagation via the cell cycle [6] [7]. In an adult human organism, a small ability of cardiomyocytes to renewal still exists; however, only about 1% or 0.4% of cardiomyocytes renew each year in humans of 20 and 75 years of age, respectively [8]. Opposite to the above, in patients with MI about 4% of cardiomyocytes in the peri-infarct zone are positive by the Ki-67 marker of cell proliferation [9], and they also show an elevated expression of specific genes, which facilitate the cardiomyocytes division and their release form the state of the cell cycle arrest [10].

Not only stem cells have an ability to self-renewal, they are also multipotent, and they can differentiate into various types of cells, including cardiomyocytes. Especially interesting are the following types of stem cells, as applied to the study of the heart regeneration in IHD: embryonic stem cells (ESC); induced pluripotent stem cells (iPSC); bone marrow stem cells (BMSC), including hematopoietic stem cells(HSC) and endothelial progenitor cells (EPC); mesenchymal stem cells (MSC); skeletal myoblasts (SkM); resident cardiac stem cells (CSC) (**Figure 1**).

1.1. Embryonic Stem Cells (ESC)

ESC are stem cells prepared from the inner cell mass of a blastocyst and having a totipotency. Theoretically, ESC are the most attractive type of cells for regeneration, since they can differentiate into any cell types [12]. ESC have been shown to differentiate into cardiomyocytes under specific culturing conditions, such as co-culturing with cells of murine visceral endoderma, with different factors facilitating ESC differentiation via the paracrine regulation [13]. Xue *et al.* showed that, during transplantation of human cardiomyocytes, prepared by differentiation from ESC, to experimental animals, the transplanted cells incorporated into the recipient's heart tissue and performed electric and functional interaction with resident cardiomyocytes of the host organism [14]. Besides, such a transplantation played an important role in the regeneration of the ischemic region in the process of the MI treatment, showing a positive effect on the condition of the left ventricle, according to the hemodynamic analysis of the heart contraction, analysis of the local disruption of the heart wall kinetics and the diastolic function of the left ventricle [15]. But in general, in spite of the obtained positive results, it has been shown that only a small fraction of cardiomyocytes prepared by ESC differentiation have an innate contractile ability [16]. Besides, the mechanism of ESC differentiation into cardiomyocytes has not been studied in detail [17]. Besides, the use of this type of stem cells is unfavorable from the ethical viewpoint, since ESC preparation involves destruction of a human embryo which has started to develop. Moreover, ESC transplantation may lead to a tumor (teratoma) growth or an immune rejection reaction. According to these reasons, no clinical studies have been conducted in which ESC would be used as a cell therapy agent for IHD patients.

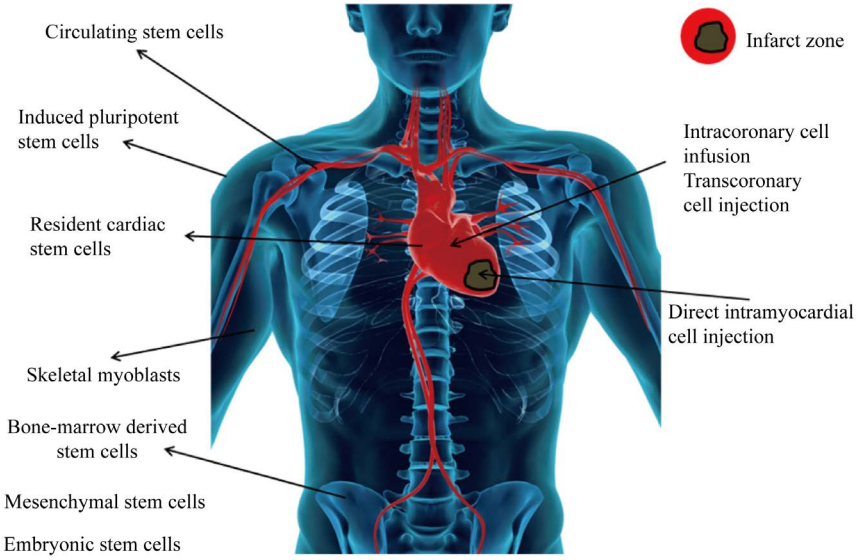

Figure 1. Stem and progenitor cells which are being studied for a potential cell therapy of IHD, and the ways of their delivery onto the heart [11].

1.2. Induced Pluripotent Stem Cells (iPSC)

In 2007, Yamanaka *et al*. obtained artificially created cells, similar to ESC by their plutipotency, but prepared from mature diploid somatic cells with the use of the following transcription factors—Oct3/4, Sox2, Klf4 and c-Myc, which were called "induced pluripotent stem cells" (iPSC) by the authors [18]. iPSC, similar to ESC, can differentiate into various types of cells, including cardiomyocytes [19], but they are not unfavorable from the ethical point of view. Nelson *et al*. have reported that cell therapy for the murine IHD model applying murine iPSCs differentiated towards cardiomyocytes, induced by human transcription factors, restores the impaired heart functions [20].

As in the ESC case, in spite of the positive results, the clinical use of iPSC is presumed disadvantageous, first, because of the low yield of iPSC prepared using the 4 gene types, second, because of the low efficiency of their differentiation into cardiomyocytes, third, because of the fact that the differentiated cells represent a heterogeneous mixture of different cell types, including those which are not cardiomyocytes [21]. Moreover, the therapeutic application of iPSC creates the same problems as that of ESC, namely, growth of a teratoma and a potential rejection reaction after the transplantation. The iPSC studies are currently at the pre-clinnical stage, the techniques of their preparation and application are gradually perfected.

1.3. Induced Cardiomyocytes and Direct Fibroblasts Reprogramming into Cardiomyocytes

Recently performed studies have shown that postnatal cardiac or dermal fibroblasts may be directly reprogrammed into cells similar to cardiomyocytes by using three cardiac growth transcription factors: Gata4, Mef2c and Tbx5 (GMT) [22]. Moreover, fibroblasts may be easily reprogrammed into cardiomyocytes if using exogenously expressed genes of pluripotency, such as Oct4, Sox2 and Klf4 [23]. Indeed, Qian *et al*. demonstrated that, after local application of the GMT-retrovirus in a murine model of MI, genetically labeled myocyte-like cells infiltrated into the peri-infarct zone; it was confirmed that these cells were the offspring of cardiac fibroblasts [24]. It was also shown that the GMT delivery *in vivo* reduced the size of the infarction region and somewhat relieved the heart dysfunction for up to 3 months after the coronary artery ligation. In spite of the impressive results, the gene manipulations were performed with the use of viral vectors which is undesirable in the clinical practice from the safety viewpoint.

1.4. Bone Marrow Stem Cells: Hematopoietic Stem Cells and Endothelial Progenitor Cells

Bone marrow stem cells (BMSC) are the most well-characterized stem cells, both by the characteristics of sur-

face antigens and by the growth properties *in vitro* and *in vivo*. Numerous studies have shown a positive effect of BMSC on the restoration of the heart function in the therapy of cardiovascular diseases [25]-[27]. A recently performed analysis of the results of 33 randomized clinical studies (1765 MI patients in total) has demonstrated that mortality and disability of the patients after the MBSC therapy only insignificantly differ from those of the patients treated with a standard therapy. At the same time, the BMSC therapy improved the indices of the left ventricular ejection fraction (LVEF) for a long period after the transplantation (12 - 61 months) [28]. It should be noted that BMSC are not a homogeneous cell population, they include cells of various origin, including hematopoietic stem cells (HSC) and endothelial progenitor cells (EPC). It has been shown, that c-kit$^+$/Lin$^-$ and c-kit$^+$/Sca-1$^+$/Lin$^-$ populations of HSC improve the heart function [19] [29]. Moreover, the results of the clinical studies (COMPARE-AMI) have demonstrated that CD133$^+$ HSC also improve the left ventricular function after transplantation [30]. In spite of the obtained results, the discussion on the potential differentiation of this stem cell type into cardiomyocytes is still going on [31] [32].

EPC are progenitor cells capable of differentiation into endothelial cells. In a number of studies, a positive effect of EPC transplantation on the damaged heart function has been found [33] [34], although this type of stem cells does not differentiate into cardiomyocytes after transplantation [35]. The observed positive effect is most likely explained by the EPC role in the stimulation of angiogenesis and sufficient recipient's cardiomyocytes supply with nutrients and oxygen, which are necessary for their survival and division, as well as for cardiomyocytes renewal from the endogenous cardiac stem cells and enhancement of the newly formed cardomyocytes' viability via the paracrine effect. It should be noted that the EPC definition remains not ultimately established, since a clear identification of markers for their separation is absent, hence, additional experiments are necessary for this problem solution.

1.5. Mesenchymal Stem Cells (MSC)

MSC are one of the most convenient for use types of stem cells of adult organism, since, on the one hand, they can be easily isolated from various tissues: bone marrow, adipose tissue, umbilical and peripheral blood and some other sources, and, on the other hand, they can be rather easily reproduced in a cell culture and, if necessary, differentiated into different cell types: adipocytes, osteoblasts, chondrocytes, cells of muscle (myoblasts and cardiomyocyte) and nervous tissues [36]-[38]. Moreover, MSC are immune-privileged cells [39], hence, allogeneic MSC may be successfully used along with autologous MSC.

In 1999, Makino *et al*. prepared cardiomyogenic cell line from the murine bone marrow MSC by the MSC treatment with 5-azacytidine *in vitro*. It was shown that in that case about 30% of cells differentiated into cardiomyocytes which expressed a number of specific genes and had a phenotype similar to that of fetal ventricular cardiomyocytes [40]. The same results were demonstrated by Tomita *et al*. (1999), who provoked a myocardial cryo-damage by administration of liquid nitrogen [41]. Other researchers (Davani *et al*., 2003) showed that MSC, along with formation of cardiomyocytes, also formed smooth muscle and endothelial cells which also promoted the heart function improvement [42]. Wang *et al*. launched a hypothesis that the myocardial microenvironment creates certain conditions and signals for cardiomyogenic MSC differentiation [43].

Thus, MSC carry a number of properties which make them favorable for transplantation to patients suffering from IHD: in local or systemic administration MSC are capable of homing in damaged fragments of the heart muscle, MSC are immune-privileged, the apoptosis of transplanted MSC is lower than that for other stem cell types; MSC can differentiate into cardiomyocytes; between the MSC-derived cardiomyocytes, intercalated discs are present with gap junctions allowing action potentials to spread from the host's cardiomyocytes to the transplanted cells; transplanted MSC have a strong paracrine effect.

Locally or systemically transplanted MSC into the infarcted heart provide a powerful cardiac repair action (**Figure 2**).

The therapeutic effect of MSC in the IHD treatment was initially associated with their ability to differentiate into cardiomyocytes, endothelial cells and smooth muscle cells [41]. According to [42], MSC, cultured for 15 days, expressed α-smooth muscle actin and β-actin filaments, characteristic of smooth muscle cells and non-muscle cells, correspondingly, but no CD31 expression was observed. After injection into infarcted heart, MSC demonstrated either a smooth muscle cell (αSM actin$^+$) or an endothelial cell (CD31$^+$) phenotype, as was shown by immunofluorescence studies.

An increased vascular density was also found in a dog model of chronic IHD [45], for which a colocalization

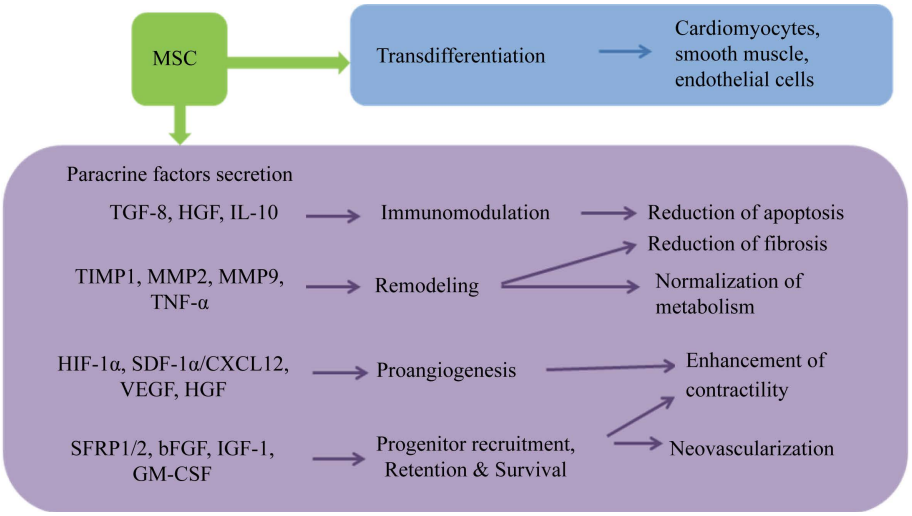

Figure 2. Mechanisms of MSC action [44].

of engrafted MSC with the markers of endothelial cells and smooth muscle cells was evidenced by immunohistological studies.

Although, in MSC transplantation the degree of their spontaneous differentiation into cardiomyocytes is low [46]. Recent studies have revealed that the number of cells created by MSC differentiation is insufficient for serious functional improvements, and the paracrine mechanisms of MSC action probably play the key role in the cardiac repair in IHD [47].

MSC provide a powerful paracrine effect, secreting numerous cytokines and growth factors (SDF-1/CXCL12, HGF, IGF-1, bFGF, HIF-1α, VEGF, Ang-1, MCP-1, IL-1, IL-6, PIGF, PLAT, TNF-α etc.). They affect the neighboring cells preventing their apoptosis and also have have immunomodulatory and antifibrotic effect and stimulate angiogenesis in the ischemic damage region.

Immunomodulation is at the top place among various paracrine mechanisms of MSC therapeutic effect in IHD. Since most injuries are associated with inflammation, the MSC ability to quench inflammatory processes at the site of injury appears highly likely to underlie their reparative effect in the damage of cardiac tissue. Monocyte and macrophage immunomodulation by MSC have recently been shown to play an important role. MSC were shown to inhibit production of macrophage pro-inflammatory cytokines (TNF-a, IL-6 and IFN-g), in the studies by Maggini *et al*. and other researchers [48]-[50].

At the same time, MSC were found to stimulate production of anti-inflammatory cytokines IL-10 and IL-12p40 [48] [49]. By doing so, MSC restrict the local inflammation and minimize the cardiac tissue damage, as well as facilitate clearing the site of injury by enhancing apoptotic cell phagocytosis. Monocyte-derived dendritic cell differentiation is also controlled by MSC [51]. PGE2 secretion, induced by MSC, appears to be responsible for these positive effects due to its direct action on macrophage activation.

Reduction of fibrosis and scar tissue formation [52] is another reason for the therapeutic efficiency of MSC. The studies performed on a rat model of myocardial infarction demonstrated that transplanted MSC regulated remodeling of left ventricle via decreasing mRNA expression and protein levels of TGF-β, collagens of type I and type III, as well as of tissue inhibitor of metalloproteinase (TIMP)-1 [53].

Spatial changes in matrix metalloproteinases (MMPs) and TIMPs altered the dynamics of collagen after MSC injections in sheep. MMPs-1, -2, -3, -7, -9, -13, MT1-MMP, and TIMPs-1, -2, -4 were shown to undergo changes in the remote, border zone, and infarcted zones after the injection [54].

VEGF is an important paracrine factor, as well, as far as MSC-related cardioprotection is considered. Some types of MSC also release insulin-like growth factor (IGF)-1, transforming growth factor (TGF)-β2, and EGF [55]-[57]. Adipose-derived MSC can secrete angiogenic, arteriogenic factors, as well as chemotactic, and anti-apoptotic growth factors thus enhancing angiogenesis [58]-[60].

Adipose-derived MSC were shown to increase both capillary and arteriole density in infarcted areas, as a result of paracrine signaling [61]. Other studies of adult stem cells in the treatment of myocardial infarction sup-

port this mechanism [59] [62] [63].

The enhanced angiogenesis and decreased infarct size, leading to improved cardiac function, have also been associated with hepatocyte growth factor (HGF) and angiopoietin (Ang)-1 released by MSC [61] [64] [65].

A correlation between the increase in capillary density and mRNA and protein levels of VEGF was found in [56] after a treatment with adipose-derived MSC.

There exists an interesting hypothesis on the paracrine mechanisms of MSC action which is based upon endogenous cardiac regeneration provided by activation of resident cardiac stem cells and stimulation of cardiomyocytes replication by MSC transplantation. In [66], MSC were injected into infarcted pig hearts, and the newly created cardiomyocytes were found to stain positive either for c-kit or for Ki67. Transplanted BMSC may also control the host cardiomyocytes proliferation; a direct evidence of BMSC-induced proliferation and endogenous CSC differentiation was recently obtained in [67].

To date, the MSC delivery in the therapy of acute or chronic MI has been achieved mainly by intracoronary administration, sometimes combined with EPC [68]-[71]. Recently, an IM MSC infusion using a catheter has been performed [72]. In both cases, inversion of the left cardiac ventricle remodeling was observed, along with reduction of the infarction region size and enhancement of the regional contractile function of the myocardium (the patients' observations were conducted for 1 year). A possibility of rapid preparation of cells in the sufficient amount for transplantation, and a possibility of application of both autologous and allogeneic MSC for elderly patients with comorbidities make MSC exceptionally attractive for the cell therapy. Creation of a donor bank of MSC from young healthy donors makes it possible to provide an appropriate system of the cells' quality and consistency of their preparation. An example is randomized, double blind, placebo-controlled study of 53 patients after acute MI, in which the patients were injected allogeneic MSC (the cell drug Prochymal, Osiris Therapeutics, Inc., Baltimore, Maryland) [73]. It should be noted that, due to the rate of MSC reproduction, it is possible to obtain up to 5000 ready-to-use doses of cells from one donor. MSC were infused intravenously for 10 days. Allogeneic product was well tolerated by the patients after systemic administration [74]. 6 months post-infusion, the LVEF increase and inversion of remodeling and, moreover, reduction of arrhythmia were observed. Similar results were obtained in Russia in the studies conducted at the Medical Radiological Research Center of the Russian Ministry of Health (MRRC, the city of Obninsk) [75]. In those studies, systemic (intravenous) transplantation of (150 - 200) $\times 10^6$ autologous bone marrow MSC was applied to patients with a severe chronic heart failure (including that after MI). It has been shown that such a type of cell therapy is minimally invasive, does not cause any complications (allergic reactions, life-threatening arrhythmias, embolism, severe hemodynamic disorders etc.) and does not lead to worsening of the condition after the therapy. In the first 3 - 6 months after the systemic transplantation of such cardiomyocyte progenitors, the patients experienced improvement in the myocardium contractile ability and perfusion, which manifested itself clinically as a reduction of the heart failure degree with the subsequent prolonged stable compensation (1-2 and more years of observation).

At the University Hospital, Touluse, a clinical trial "Mesenchymal Stem Cells and Myocardial Ischemia (MESAMI)" Clinical Trial Registration: NCT01076920 is conducted, which has been reported to produce rather impressive results [76]. The study objective is to confirm the safety and prospects of transendocardial injections of autologous bone marrow MSC guided by 3-dimensional NogaStar XP mapping, for treatment of chronic myocardial ischemia and left ventricular dysfunction. This bicentric phase 1 study recruited 10 patients with chronic myocardial ischemia, with the following inclusion criteria: NYHA Class II-IV and/or angina pectoris CCS Class III or IV, chronic ischemic cardiomyopathy with LVEF $\leq$ 35%. MSC were cultured for 17 days, from the iliac crest-aspirated autologous bone marrow. 14 - 16 MSC transendocardial injections of the 16.8 ± 2.0 ml volume, containing 61.5 $\times 10^6$ MSC, were performed into the intact muscle in the left ventricular scar border regions. According to the 1 month post-procedure follow-up, all patients tolerated the procedure well, no adverse events were registered. Thus, the primary end-point showed both safety and feasibility of the procedure. At 3 months post-procedure, the secondary endpoint showed that the summed stress score measured by SPECT essentially decreased from 34.1 ± 8 to 25.3 ± 9.5 ($p < 0.05$), although the effect did not last (32.1 ± 8.0, NS, at 12 months). The LVEF by echocardiography at 3 months was enhanced from 29.4% ± 6.5% to 35.9% ± 6.7% (6%, $p < 0.001$) and remained the same (35.7% ± 8.1%; $p < 0.001$) until the 12 months time point. The other assessed parameters were NYHA, 6-minute walk test, VO_2 peak, and Quality of life. All of them improved at 3 months and lasted until 12 months time point (except for the Quality of life). Thus, the MESAMI clinical trial has shown the safety and feasibility of transendocardial injections of autologous bone marrow MSC. MESAMI 2, a randomized, double blind, multicenter, placebo-controlled trial, Registration No. NCT01076920 is meant to assess the

procedure efficiency in a larger group of patients.

1.6. Skeletal Myoblasts (SkM)

Chrolologically, autologous skeletal myoblasts (SkM) were the first type of stem cells used in limited clinical studies for IHD therapy. While pluripotent stem cells, such as BMSC, can potentially differentiate into fibroblasts after transplantation into the post-infarction fibrosis region, SkM, being direct myocytes' precursors, can differentiate only into myocytes [77]-[79]. Their preparation and application pose no complications of ethical or immunological character.

SkM manifested a pronounced resistance under the ischemia conditions [78]. In the animal experiments, it was demonstrated that SkM transplantation led to improvement of the heart function, as judging by the LVEF enhancement [77] [79]. Unfortunately, in spite of the positive effect, it has been convincingly proven that the transplanted SkM are incapable of transdifferentiation into cardiomyocytes [80]. Moreover, the joint clinical studies taken place in several European clinics, MAGIC (Myoblast Autologous Grafting in Ischemic Cardiomyopathy) have shown that SkM, transplanted to IHD patients, caused serious adverse effects, namely, arrythmia [81].

1.7. Resident Cardiac Stem Cells (CSC)

Several research groups have identified a number of populations of resident cardiac stem cells (CSC), based on different markers characteristic of adult stem cells.

2. Sca-1$^+$ (Stem Cell Antigen-1) Cells

Sca-1 is an antigen used for HSC isolation. However, certain results were obtained showing that Sca-1$^+$ cells may be isolated from an adult heart and may undergo transdifferentiation into beating cardiomyocytes when cultured under specific conditions [82] [83]. Transplanted Sca-1$^+$ manifest homing into the peri-infarct zone, where they differentiate into cardiomyocytes expressing specific cardiac markers, such as Nkx2-5 и GATA4. According to the results of Wang and coauthors, transplantation of the Sca-1$^+$/CD31$^-$ population of CSC led to improvement of the left ventricular function in the murine MI model due to angiogenesis stimulation [84].

3. c-kit$^+$ Cells

c-kit$^+$ cells can differentiate into cardiomyocytes, endothelial cells and smooth muscle cells *in vitro*, and they contribute to the myocardium regeneration after transplantation *in vivo* [85]. The results of the SCIPIO clinical trial (Stem Cell Infusion in Patients with Ischaemic cardiOmyopathy) have shown that transplantation of c-kit$^+$ cells improved the left ventricular function [86]. Zaruba *et al*. analyzed the cardiomyogenic potential of the c-kit$^+$ CSC isolated from a normal neonatal, normal adult and infarct adult murine heart [87]. In the neonatal group, only the c-kit$^+$/CD45$^-$ subpopulation of cells demonstrated a notable cardiomyogenic activity, but not c-kit$^+$/CD45$^+$ or c-kit$^-$/CD45$^+$ cells subpopulations from bone marrow. It should be noted that c-kit$^+$ cells from a normal adult heart did not undergo cardiomyogenic differentiation either when co-cultured with fetal cardiomyocytes, or when transplantated into a normal or infarcted heart of an adult mouse. The obtained data testify that, in the process of an organism development, resident c-kit$^+$ cardiac cells lose their ability of acquiring the cardiomyogenic phenotype. These results differ from numerous earlier data on the c-kit$^+$ cells isolated from the rat or human heart. The co-culture of these CSC with cardiomyocytes or their injection into a damaged myocardium resulted in a more powerful cardiomyogenic differentiation [88] [89].

It is worth attention that the genesis of the c-kit$^+$ cells in the heart still remains not entirely known. In particular, experiments of Fazel and coauthors [90] on the bone marrow transplantation suggest that many of the c-kit$^+$ cells in the adult heart are of a bone marrow origin, indeed, the c-kit is expressed not only in CSC, but also in the bone marrow cell populations [30].

4. Isl-1$^+$ Cells

Isl-1 play an important role in the development of different organs, including the heart [91]. Laugwitz *et al*. first identified Isl-1$^+$ CSC in a mouse, rat and human postnatal myocardium [92]. The further investigations showed

that Isl-1 were absent in an adult heart [93]-[96]. It has been recently demonstrated that Isl-1^+ cells participate in the formation of all the basic types of a murine heart cells [91] [92] [97] [98]. Isolated Isl-1^+ cells proliferate and differentiate into cardiomyocytes, endothelial cells and smooth muscle cells after transplantation [98]. In contrast to Sca-1^+ CSC and c-kit^+ CSC, Isl-1^+ CSC do not express other markers of cell surface.

5. Side Population Cells (SP)

SP cells were isolated using a vital dye (Hoechst 33342 or Rhodamine 123), by sorting out the cells mostly "excluding" the dye and thus obtaining so-called "side population cells". SP cells were found in different organs, including bone marrow, skeletal muscle, adipose tissue, and the heart [99] [100]. SP cells express Sca-1 and can be transdifferentiate into cardiomyocytes with leads to improvement of the left ventricular function after transplantation [101]. These stem cells express Nkx2.5, GATA4 and Mef2c after differentiation into cardiomyocytes. SP cells can migrate to the damaged heart fragment after appearance of the damage. In general, SP cells are promising for cell therapy, but the use of a toxic dye for their preparation is currently limiting their application by only experimental animal models.

6. Cardiosphere-Derived Cardiac Cells (CDC)

CSC populations have a tendency to clonogenicity and can form spheroid aggregates in culture, so-called cardiospheres [102]. CDC cells—cells, isolated from cardiospheres—can differentiate into endothelial, smooth muscle cells, as well as into cardiomyocytes [103]. Various studies have shown that CDC cells improve the left ventricular function [89] [104] [105]. Unfortunately, in contrast to other CSC populations, CDC cells preparation requires a rather long time because of the slow growth of cardiospheres. Besides, CDC are frequently contaminated with other types of cells, such as with heart fibroblasts.

In Vitro Modification of Stem Cells

Stem cells modification *in vitro* is used to improve their biological functions playing a major role in their transplantation *in vivo*. Among those are: their capability of homing, *i.e*. migration into a target tissue, survival in the ischemia conditions, proliferation, synthesis of paracrine factors, ability to transdifferentiation. Here we consider the techniques for stem cells modification, by the example of MSC.

7. MSC Preconditioning

In the course of MI, the blood flow to a part of myocardium is interrupted, hence, cardiomyocytes are undersupplied with oxygen, the level of which drops to 0.2% [106]. Soon after that, the combination of residual tissue hypoxia, oxidative stress during "ischemia-reperfusion", death of cardiomyocytes and inflow of inflammatory cells create an unfriendly medium for stem cells potentially transplanted into the damaged myocardium. Since MSC naturally exist in the bone marrow in the conditions of low oxygenation (~2%) [107], they are presumably rather tolerant to the ischemic conditions in infarcted myocardium. However, there exist possibilities for even better enhancement of the inherent MSC stability, if they are undergone pre-conditioning (prior to transplantation and incubation) with different factors. Indeed, it has been shown in *in vitro* and *in vivo* stidues that MSC subjected to hypoxia produce an elevated amount of transcription and growth factors, enhancing the cells' survival, including VEGF, HIF-1α, survivin and Bcl-2, as compared to MSC incubated in normal conditions [108]. The enhanced survival and functional advantages of MSC, preconditioned in the hypoxia conditions, have been demonstrated in their transplantation in MI models [106] and in diabetic cardiomyopathy models [109].

In particular, hypoxic pre-conditioning may provide advantages for enhancing the cells' survival via the induction and stabilization of intracellular HIF-1α, which undergoes nuclear translocation to bind several important promoters. The subsequent target it can affect is glucose-6-phosphate transporter serving to increase the glucose concentration in cells via enhancement of gluconeogenesis. Besides, MSC can be pre-conditioned with agents causing formation of nitrogen oxide [110], with hydrogen peroxide or diazoxide [109] to boost their survival and paracrine effect.

Diazoxide is known as a stimulator for opening of mitochondrial ATP-sensitive potassium channels (MitoKATP), which is believed to provide a cell's protection in the ischemic stress. A brief incubation of MSC with diazoxide has been shown to strengthen the cell resistance due to increased expression of factors facilitating the

survival, as well as of signal factors (VEGF, HGF, NF-κB, Akt factors of signal pathway, microRNA-146a etc.) [111] [112]. It has been known for several decades that heat shock proteins (HSP) play a key role in a cell's protection from a number of ecological stress factors. HSP transcription may be induced in cultured cells under hyperthermia (e.g., incubation at 42˚C for 1 hour). This approach was applied to skeletal myoblasts pre-conditioning to increase their survival inside myocardium *in vivo* [113], while MSC were genetically modified for an elevated expression of various HSP before transplantation [114] [115]. Although the specific mechanisms of the HSP effect on MSC remain unclear, the experimental data testify that these proteins inhibit regulators of necrosis and apoptosis pathways and finally activate Akt. Moreover, they also may enhance the trophic properties of MSC via increasing the secretion of various soluble factors, such as VEGF, FGF-2 and IGF-1 [114]. A wide spectrum of pharmaceutical drugs have been investigated either in combination with MSC, or as an additional therapy during cell transplantation (e.g., statins [116] [117], sildenafil [118]), or as drugs for cells pre-conditioning. The last examples include application of phosphodiesterase inhibitors [119], modulators of angiotensin signal pathways [120] and neuropeptide Y [121]. These agents are believed to have different mechanisms of action. It has been recently suggested that a block of angiotensin II may enhance human MSC both in experimental and clinical conditions. However, the MSC nature is rather complicated, especially regarding their translocation from a controlled microenvironment in a culture into a dynamic and unpredictable microenvironment of a damaged heart. One of the primary tasks in the field of transdifferentiation towards cardiomyocytes [120]. A great attraction of preconditionining is related to its simplicity cell therapy optimization is deciphering the most important signal pathways involved in the processes of cell survival and accomplishment of the cell's repair function after transplantation, thus the strategy of preconditioning should be directed towards the highest benefit in this context.

8. Enhancing the Effect of Paracrine Factors

As described above, a significant part of the MSC reparative potential in IHD is related to production of a wide spectrum of soluble paracrine factors. Essential efforts have been directed towards the support of this paracrine potential with the use of either transfer of appropriate genes, or pre-incubation with agents which cause an elevated expression of cytokines or growth factors.

VEGF (vascular endothelial growth factor) is an important regulator of MSC-induced vasculogenesis and angiogenesis a [122] and can be excessively expressed in MSC via the induction of HIF-1α. MSC pre-incubation with TGF-β [123], SDF-1α [124] and lipopolysaccharides [125] has also been shown to increase the VEGF cell production accompanied by advantages for cell survival after transplantation and enhancement of angiogenesis and myocardium regeneration in rodent MI models. Adenoviral rat MSC transfection with the human VEGF165 gene was also a successful "hybrid" strategy for transplantation of cells and genetic therapy to improve the therapeutic angiogenesis after MI [126].

IGF-1 (insulin-like growth factor-1) possesses a pleiotropic activity, as well, which affects the growth, proliferation and survival of cells, predominantly via the activation of signal pathways of Akt and MAP-kinase. MSC preconditioning with IGF-1 enhanced homing and survival of cells in the myocardium after a systemic administration and led to functional advantages, as compared to common MSC [127].

An important component of the MSC paracrine effect is their ability to attract the corresponding cells, including hematopoietic, endothelial or cardiac progenitor cells, into the region of myocardium damage. SDF-1α and its G-protein transmembrane receptor (CXCR4) plays a key role in the cell attraction from the bone marrow into the region of infarcted myocardium [128]. Immediately after MI, the SDF-1α level in the blood serum and in the myocardium rapidly grows and reaches a peak in 48 and 72 hours, respectively, then it returns to the basic level. This facilitates homing of progenitor cells in the region of myocardium damage, including circulating, pro-angiogenic $CD34^+$ cells which promote neovascularization. Besides, SDF-1α may also have a direct pro-angiogenic effect, inducing expression of HIF-1α and VEGF in cells [129]. In models of cardiovascular diseases, mechanisms of SDF-1α/CXCR4 were manipulated in different ways to enhance the myocardium function and perfusion, as well as the reparative properties of transplanted MSC. In one of the studies on a rodent model of MI, SDF-1α was administered directly into myocardium as an addition to an intravenous MSC injection which resulted in a better homing and survival of those exogenous cells in the recipient's heart, the effect being neutralized if a functional blocking antibody was added [130]. MSC pre-incubation with SDF-1α also demonstrated pro-mitogenic and anti-apoptotic effects in the course of incubation *in vitro* with hydrogen peroxide and after

transplantation into an infarcted myocardium [124]. In the latter case, it was accompanied by a decrease in the size of the infarction and fibrosis region, as well as by improvement in the heart function, as compared to the common MSC. A similar improvement of the MSC properties was observed in the cells transduction for a stably elevated SDF-1α expression [131]. After transplantation into the heart, transduced MSC were more resistant to apoptosis and better survived than unmodified MSC which led to a decrease in the collagen deposition and to expression of matrix proteinases. In other preclinical studies, MSC were used with hyperexpression of HGF [132] and other factors (e.g. CCR1 [133]) for boosting their therapeutic potential. Additional advantages were obtained when performing a double transfection of MSC with VEGF and SDF-1α genes [134].

9. Activation of Cytoprotector Pathways

Cytokines, described above, accomplish sophisticated interactions and functions with different cell and tissue substrates. A significant part of their ability to protect cells from death is regulated via the effect on mechanisms of the Akt signaling pathway (**Figure 3**).

The Akt gene codes serine-threonine protein kinase which stimulates further signal pathways (e.g., PI3K/mTOR) under activation and finally, as a result, inhibits BAD, a protein of the Bcl-2 family and, vice versa, activates NFκB [135]. This leads to inhibition of apoptosis and transcription of genes activating the cells' survival, respectively [136]. The activation of the Akt gene expression is also known to stimulate angiogenesis and overcoming the cell cycle arrest [137]. Correspondingly, manipulations with mechanisms of the Akt signal pathway in stem cells are considered positively as a way of survival optimization for both transplanted cells and cardiac and vascular cells of the host organism. In a series of highly cited articles on fundamental and preclinical studies, Dzau *et al*. have demonstrated that not only the reparative MSC properties are mostly provided by their paracrine effect, but also these properties can be notably improved via the genetic modification of MSC for Akt [138] [139]. The advantages of Akt-transfected MSC vs. intact cells have been demonstrated *in vivo* not later than 72 hours after transplantation into an infarcted rat myocardium. In particular, the cardiac cells apoptosis was essentially reduced, the MSC survival was enhanced, and formation of VEGF, HGF and IGF-1 in the myocardium was stimulated. Impressing results were obtained even when using only the conditioning medium from cultures of Akt-transfected MSC which emphasized the paracrine basis of their cytoprotector and reparative properties. It has also been demonstrated very recently that the cell therapy of MI with the use of Akt-transfected MSC is also accompanied by restoration of the normal metabolic function of the heart, with a limited use of highly energetic phosphates and normalization of the myocardium pH and glucose metabolism [140]. The genomic analysis has

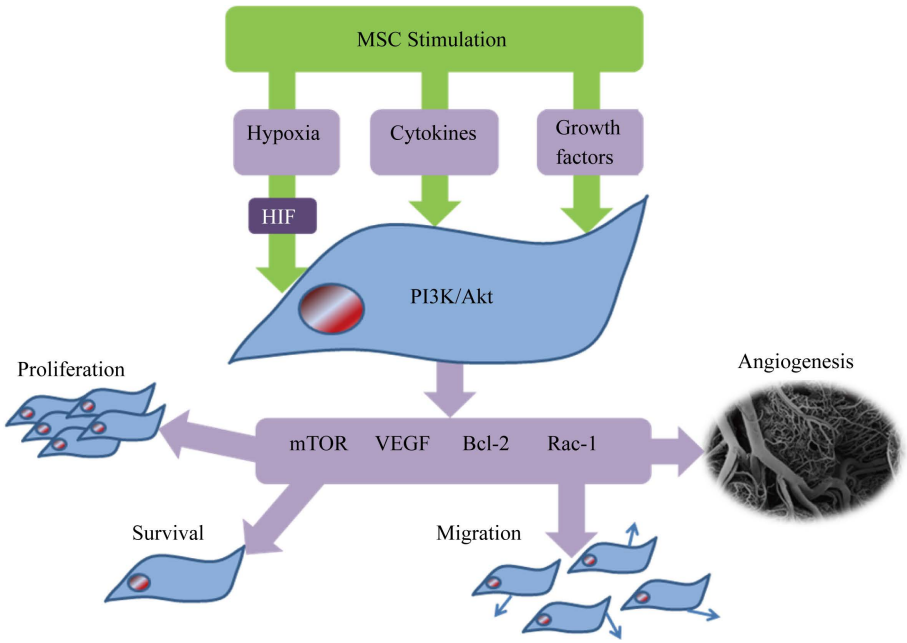

Figure 3. Activation of PI3K/Akt signaling pathway in MSC for cytoprotection [135].

identified that Akt-transfected MSC have an elevated content of the Sfrp2 protein, a key mediator of the cytoprotector paracrine properties of these cells [141].

Another strategy used for enhancement of the MSC cytoprotector properties is their modification for hyperexpression of integrin-linked kinase (ILK) [142] and heme cyclooxygenase-1 (HO-1) [143]. Heme cyclooxygenase-1 enzymatically destroys heme to bilirubin, carbon monoxide and free iron and has an anti-inflammatory, antioxidant, anti-apoptotic and pro-angiogenic effect. Elevated HO-1 expression in MSC enhances the survival of transplanted cells and improves the left ventricular function of a porcine heart in the "ischemia-reperfusion" model [143]. Thus, the studies described above demonstrate good opportunities for boosting the trophic potential of MSC, their resistance to stress and apoptosis, as well as their ability to the heart repair. Besides, they help to disclose many of the critically important molecules and signal pathways, regulating the adult stem cells functions (survival, proliferation and migration).

To date, a development of technologies for the clinical use of the above strategic methods is in need. Although the genetic modification of MSC and other cell types creates a number of formal questions concerning the safety of their application, some results have already been obtained. Currently, the ENACT-AMI clinical trial is going on (clinicaltrials.gov NCT00936819), in which the patients with severe MI undergo administration of circulating mononuclear cells (MNC), transfected with the gene of human endothelial NO-synthase (eNOS) [144].

10. Manipulation of the MSC Cardiomyogenic Potential

Efforts are also applied to increase the MSC potential towards their cardiomyogenic differentiation. As was mentioned before, the initial attempts to verify and then to manipulate the cardiomyogenic potential of MSC were focused on the use of 5-azacytidine, a DNA-methyl transferase inhibitor [40]. Although this non-specific agent may promote a moderate MSC transdifferentiation into cardiomyocytes, its action was less reproduclible in human MSC, than that in murine MSC [40]. Besides, pre-conditioning with 5-azacytidine may have a genotoxic effect on cells with consequences for their safe transplantation *in vivo*. Preconditioned MSC also appear in the state of the cell cycle arrest, thus losing their replication potential required for cardiomyocytes repopulation.

Another strategy consists in a directed MSC cardiopoiesis. Cardiopoietic programming was developed as a way of ESC modification, in which the cells are subjected to exposure to a combination of specific recombinant factors usually present in embryonic medium, in order to boost their cardiomyogenic regenerative potential [145]. Important mechanisms of differentiation into cardiomyocytes have been discovered, where the members of TNF-α, TGF-β and FGF families act as essential regulators of stem cells cardiopoiesis, which, in combination with each other, can reprogram ESC into cardiomyocytes without the carcinogenesis risk [146].

Recently, the principle of directed cardiopoiesis has been spread to MSC from the human bone marrow [147]. As a result of different IHD patients screening, a rare patients' subpopulation was identified, whose MSC demonstrated a spontaneous ability to improve the myocardium contractile function, along with a high expression of *de novo* early and late cardiac transcription factors (e.g., Nkx2.5, TBX5, MESP1, MEFC2). These cells underwent the action of a combination of recombinant factors consisting of TGF-β1, BMP4, activin A, retinoic acid, IGF-1, FGF-2, alpha-thrombin и interleukin-6 which consolidated the cardiospecific MSC potential. In a murine MI model, transferred into the chronic phase of the heart muscle damage, the delivery of cardiopoietic MSC allowed stable functional and structural benefits without adverse consequences, as compared to intact MSC. It was related to a higher degree of the MSC capture within the myocardium, their more efficient transformation into cardiomyocytes *in vivo* and in a higher paracrine stimulation of endogenous c-kit$^+$ cardiac stem cells (CSC).

This work was continued in the form of clinical studies in Europe, in which cardiopoietic MSC were used for the therapy of patients with chronic ischemic cardiomyopathy [148]. Cardiopoietic MSC ($0.6 - 1.2 \times 10^9$) were transendocardially transplanted to 21 patients into a viable dysfunctional myocardium of the left ventricle. At the average, in slightly less than a year, the left ventricular ejection fraction increased more notably in MSC recipients than it did in the control group (absolute increase by 5.2% vs. 1% in the control group), besides, an improvement of the functional state was observed (by the results of the 6-min walking test) and reduction of the arrhythmia episodes.

10.1. MicroRNA and Cardiomyogenic Differentiation of MSC

As was found in the last years, microRNAs play an active role in the processes of MSC renewal and differentia-

tion [149] [150]. MicroRNAs (miRNAs) are endogenous, non-coding RNA molecules, of 20 - 23 nucleotides of length, negatively regulating gene expression in different biological and pathological processes, including cell differentiation, proliferation, apoptosis in cardiac diseases, neurological diseases and cancer in humans [151]-[155]. MicroRNAs participate in MSC regulation at different stages of this cell type development. In general, the mechanisms of microRNA expression differ for intact MSC and for completely differentiated cells, e.g. osteoblasts, adipocytes and chondrocytes, which suggests that microRNAs are important at the stage of MSC differentiation decision. Indeed, a low, or, on the contrary, a high level of a certain microRNA expression may be a preliminary determinant condition for MSC commitment and differentiation into specific cell lines, as was demonstrated in the studies by Guo and coauthors [156]. For instance, it was demonstrated that the reduced level of miR-138 expression was associated with osteogenic [157] and adipogenic [158] differentiation of human MSC. Other microRNAs, miR-204/211 and miR-637 control the balance between MSC differentiation into adipocytes and osteoblasts [159] [160]. It is extremely interesting that such microRNAs as miR-1, -206, -24 and -181 may induce MSC differentiation into cardiomyocytes *in vitro*. In the presence of 5-azacytidine, miR-143 and miR-181 are expressed, while indirect co-culture of MSC with neonatal rat myocytes leads to an elevated expression of miR-143, -206, -208 and -181 [161]. AT the same time, miR-133 may block this differentiation [161]. Moreover, Liu *et al*. (2012) have recemtly shown that miR-16 is involved into myogenic differentiation of human MSC in the cardiac microenvironment (a niche of resident cardiac stem cells) [162].

Hyperexpression of miR-16 significantly increased the delay of human MSC in the G1-phase of the cell cycle and enhanced expression of the cardiac tissue marker genes: GATA4, Nkx2-5, MEF2C and TNNI3. Finally, it induced MSC differentiation into cardiomyocytes in the cardiac microenvironment. The use of miR-16 may represent a promising approach to MSC pre-modification before their transplantation to IHD patients.

10.2. Optimization of MSC Delivery

An imperative requirement for efficient myocardium repair is the sufficient number of viable MSC to reach the corresponding target sites soon after transplantation and remain there for a long time, with appropriate survival, proliferation and functioning.

The cells can be administered to the heart: 1) via a systemic intravenous injection, 2) regionally, via an infusion into a coronary artery or vein, 3) locally, via a direct transepicardial, transendocardial or intrapericardial implantation. Unfortunately, in spite of some differences in the efficiency of these various ways of cell delivery, the MSC preservation and their survival in the myocardium remains imperfect in all the cases [163].

Fluoroscopically guided intracoronary infusions is one of the most wide-spread techniques applied in the clinical cell therapy. This technique is used to distribute the cells in the affected ischemic artery region [164]-[166]. Its advantages include a low cost, minimal invasiveness, relatively short duration and a possibility of immediate accomplishment via the primary transcutaneous coronary intervention in the acute MI therapy. But the use of this technique is associated with a potential risk of adhesive MSC aggregation inside the coronary microvessels [167]-[169]. MSC administration via an IM injection (open transepicardial or transendocardial, transcutaneous using a catheter) delivers MSC into the corresponding myocardial regions more specifically. Such a direct injection probably has advantages related to cardiac cells preservation [170], capture of non-resident cells [168] and the general therapeutic effect [171], as compared to systemic or intracoronary delivery. There is some potential risk of creation of foci with electrophysiological heterogeneity and arrhythmia. However, it is not characteristic of MSC and mainly specific for skeletal myoblasts [172]. Although the cells' preservation and their distribution is similar for transendocardial and transepicardial injections [173], transcutaneous delivery via a catheter is less invasive and thus presents wider possibilities for the clinical use [174].

10.3. Cardiac Tissue Engineering

Cardiac tissue engineering is a complex of new technologies based on application of stem cells, biocompatible natural or synthetic matrices, and also, in certain cases, growth factors, differentiation and pro-angiogenic factors, inducing the processes of the cardiac tissue regeneration. A perfect artificial cardiac tissue will reproduce structural, mechanical and electrophysiological properties of the native cardiac tissue, which provide the viability of transplanted stem cells, as well as stimulate vasculogenesis in the implanted tissue.

Stem cell culturing in temperature-sensitive polymer plaques facilitates separation of cell monolayers without the need of using enzymes [175]. Such a construct adheres to the ischemic zone providing intra-cardiac implan-

tation of the monolayer cells. Besides, formation of new blood vessels and a general functional improvement are observed as a result of combined implantation of various MSC layers, derived from adipose tissue, in a murine model of chronic MI [62]. Moreover, the use of neonatal cardiomyocyte monolayers generated the processes of intercellular communication activating the myocardium contractile function and signal transfer inside the construct [176]. Besides, the intercalation of endothelial cell monolayers promotes formation of new blood vessels in the ischemia region.

Another approach is based on creation of natural hydrogels, such as Matrigel™ (laminin, type IV collagen and heparin sulphate) [177], collagen [178] or fibrin, in which stem cells are embedded for the subsequent intra-myocardial injection. Although the hydrogel effect was positive for the cells fixation [177]-[179], the injection pressure necessary for its delivery is too high and causes a notable cell death which finally decreases the therapeutic effect. Besides, it is difficult to sterilize and purify natural hydrogels [180]. As an alternative, synthetic hydrogels have been developed (polyethylene glycol, polylactic acid, polylactic acid-co-glycolic acid, polycaprolactone, polyacrilamide and polyurethane), in which such disadvantages are minimized [181], the US Food and Drug Administration having approved clinical application of polyethylene glycol, polylactic acid and polylactic acid-co-glycolic acid.

There exists an alternative to IM injections of hydrogel based on preparation of a new tissue *ex vivo* from stem cells incorporated into the hydrogel. Two recent studies report the contractile ability of prepared *in vitro* constructs involving embryonic cardiomyocytes [182] or neonatal rat cardiomyocytes [183]. The 3D environment favors the intercellular communication and prevents the cell death [184], and finally the prepared constructs form the extracellular matrix on their own [185]. In the clinical trial MAGNUM (Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium) a type I collagen matrix is used, with a size sufficient to completely cover the myocardium scar. In this trial, a common cardiomyoplastics was compared with combined cardiomyoplastics and tissue engineering, and the conclusion was made that the new alternative suggests better results from the viewpoint of functional restoration and ventricular remodeling [186].

For the development of cardiac tissue engineered constructs based on innovative materials (an elastomeric skeleton and hydrogel-PuraMatrix™), a European consortium has been recently opened (RECATABI, REgeneration of CArdiac Tissue Assisted by Bioactive Implants) [187]. The preliminary results have shown a cardiomyogenic differentiation of implanted cells and formation of vascular connections between the construct and native myocardium.

A promising material for tissue engineering is extracellular matrix. Extracellular matrix consists of functional and structural proteins, such as collagen, elastin, laminin, fibronectin, proteoglycans and many others [188] [189]. Since the matrix participates in many cellular processes, including proliferation, differentiation and migration [190], it is potentially attractive for cardiac tissue engineering, both due to favoring implantation of cells which repair the damaged myocardium, and due to a possibility of the damaged tissue replacement with the matrix itself. Extracellular matrix lamina was successfully isolated from different tissues, including native cardiac valves [191]-[193], and blood vessels [194] [195]. A possibility of application of animal matrix, in particular, porcine matrix, is also very promising in the human tissue regeneration [196].

11. Conclusion

The use of stem cell therapy is an exciting and dynamic field of research, with a great potential for improvement in the health of patients with cardiovascular diseases, being the main cause of death in developed countries. While the advantages of this therapy for the cardiac function improvement after MI and in heart failure have been extensively demonstrated in animal studies, the results of clinical studies have sometimes been disappointing. However, the results of the clinical trials, conducted in the last decade, give hope for a rather rapid creation and wide use of a safe and efficient medical technology based on transplantation of stem cells to cardiology patients, *i.e*. for realization of methods and approaches of regenerative medicine. The accumulated data allow expecting good prospectives for therapeutic use of stem cells, especially, autologous and allogeneic MSC, as most studied and safe cells among all the stem cell types. It is evident that the MSC application needs additional perfection of techniques for MSC isolation and identification, study of molecular mechanisms for their paracrine effect, as well as of possibility of survival, proliferation and differentiation of cells in the myocardium, use of accompanying pharmaceutical drugs for stem cells transplantation and other means of enhancing the therapeutic effect of stem cell therapy. Cardiac tissue engineering provides an excellent prospective in this field, since ar-

tificial cardiac tissue will be able to maintain structural, mechanical and contractile function of the native cardiac tissue, as well as viability of transplanted stem cells and vasculogenesis in the implanted tissue.

References

[1] Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T. and Murray, C.J. (2006) Global and Regional Burden of Disease and Risk Factors, 2001: Systematic Analysis of Population Health Data. *Lancet*, **367**, 1747-1757. http://dx.doi.org/10.1016/S0140-6736(06)68770-9

[2] Diwan, A. and Dorn II, G.W. (2007) Decompensation of Cardiac Hypertrophy: Cellular Mechanisms and Novel Therapeutic Targets. *Physiology*, **22**, 56-64. http://dx.doi.org/10.1152/physiol.00033.2006

[3] Diwan, A., Krenz, M., Syed, F.M., Wansapura, J., Ren, X., Koesters, A.G., *et al*. (2007) Inhibition of Ischemic Cardiomyocyte Apoptosis through Targeted Ablation of Bnip3 Restrains Postinfarction Remodeling in Mice. *Journal of Clinical Investigation*, **117**, 2825-2833. http://dx.doi.org/10.1172/JCI32490

[4] Abdel-Latif, A., Bolli, R., Tleyjeh, I.M., Montori, V.M., Perin, E.C., Hornung, C.A., *et al*. (2007) Adult Bone Marrow-Derived Cells for Cardiac Repair: A Systematic Review and Meta-Analysis. *Archives of Internal Medicine*, **167**, 989-997. http://dx.doi.org/10.1001/archinte.167.10.989

[5] Leri, A., Kajstura, J. and Anversa, P. (2005) Cardiac Stem Cells and Mechanisms of Myocardial Regeneration. *Physiological Reviews*, **85**, 1373-1416. http://dx.doi.org/10.1152/physrev.00013.2005

[6] Borchardt, T. and Braun, T. (2007) Cardiovascular Regeneration in Non-Mammalian Model Systems: What Are the Differences between Newts and Man? *Thrombosis and Haemostasis*, **98**, 311-318. http://dx.doi.org/10.1160/th07-02-0153

[7] Poss, K.D. (2007) Getting to the Heart of Regeneration in Zebrafish. *Seminars in Cell & Developmental Biology*, **18**, 36-45. http://dx.doi.org/10.1016/j.semcdb.2006.11.009

[8] Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabe-Heider, F., Walsh, S., *et al*. (2009) Evidence for Cardiomyocyte Renewal in Humans. *Science*, **324**, 98-102. http://dx.doi.org/10.1126/science.1164680

[9] Beltrami, A.P., Urbanek, K., Kajstura, J., Yan, S.M., Finato, N., Bussani, R., *et al*. (2001) Evidence That Human Cardiac Myocytes Divide after MI. *The New England Journal of Medicine*, **344**, 1750-1757. http://dx.doi.org/10.1056/NEJM200106073442303

[10] Ahuja, P., Sdek, P. and MacLellan, W.R. (2007) Cardiac Myocyte Cell Cycle Control in Development, Disease, and Regeneration. *Physiological Reviews*, **87**, 521-544. http://dx.doi.org/10.1152/physrev.00032.2006

[11] Tongers, J., Losordo, D.W. and Landmesser, U. (2011) Stem and Progenitor Cell-Based Therapy in Ischaemic Heart Disease: Promise, Uncertainties, and Challenges. *European Heart Journal*, **32**, 1197-1206. http://dx.doi.org/10.1093/eurheartj/ehr018

[12] Keller, G. (2005) Embryonic Stem Cell Differentiation: Emergence of a New Era in Biology and Medicine. *Genes & Development*, **19**, 1129-1155. http://dx.doi.org/10.1101/gad.1303605

[13] Mummery, C., Ward-van Oostwaard, D., Doevendans, P., Spijker, R., van den Brink, S., Hassink, R., *et al*. (2003) Differentiation of Human Embryonic Stem Cells to Cardiomyocytes: Role of Coculture with Visceral Endoderm-Like Cells. *Circulation*, **107**, 2733-2740. http://dx.doi.org/10.1161/01.CIR.0000068356.38592.68

[14] Xue, T., Cho, H.C., Akar, F.G., Tsang, S.Y., Jones, S.P., Marban, E., *et al*. (2005) Functional Integration of Electrically Active Cardiac Derivatives from Genetically Engineered Human Embryonic Stem Cells with Quiescent Recipient Ventricular Cardiomyocytes: Insights into the Development of Cell-Based Pacemakers. *Circulation*, **111**, 11-20. http://dx.doi.org/10.1161/01.CIR.0000151313.18547.A2

[15] Caspi, O., Huber, I., Kehat, I., Habib, M., Arbel, G., Gepstein, A., *et al*. (2007) Transplantation of Human Embryonic Stem Cell-Derived Cardiomyocytes Improves Myocardial Performance in Infarcted Rat Hearts. *Journal of the American College of Cardiology*, **50**, 1884-1893. http://dx.doi.org/10.1016/j.jacc.2007.07.054

[16] Mignone, J.L., Kreutziger, K.L., Paige, S.L. and Murry, C.E. (2010) Cardiogenesis from Human Embryonic Stem Cells. *Circulation Journal*, **74**, 2517-2526. http://dx.doi.org/10.1253/circj.CJ-10-0958

[17] Condorelli, G. and Catalucci, D. (2007) Human Stem Cells for Heart Failure Treatment Ready for Prime Time? *Journal of the American College of Cardiology*, **50**, 1894-1895. http://dx.doi.org/10.1016/j.jacc.2007.07.057

[18] Takahashi, K. and Yamanaka, S. (2006) Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*, **126**, 663-676. http://dx.doi.org/10.1016/j.cell.2006.07.024

[19] Zhang, J., Wilson, G.F., Soerens, A.G., Koonce, C.H., Yu, J., Palecek, S.P., *et al*. (2009) Functional Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells. *Circulation Research*, **104**, e30-e41. http://dx.doi.org/10.1161/circresaha.108.192237

[20] Nelson, T.J., Martinez-Fernandez, A., Yamada, S., Perez-Terzic, C., Ikeda, Y. and Terzic, A. (2009) Repair of Acute MI by Human Stemness Factors Induced Pluripotent Stem Cells. *Circulation*, **120**, 408-416. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.865154

[21] Yoshida, Y. and Yamanaka, S. (2011) IPS Cells: A Source of Cardiac Regeneration. *Journal of Molecular and Cellular Cardiology*, **50**, 327-332. http://dx.doi.org/10.1016/j.yjmcc.2010.10.026

[22] Ieda, M., Fu, J.D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., *et al*. (2010) Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. *Cell*, **142**, 375-386. http://dx.doi.org/10.1016/j.cell.2010.07.002

[23] Efe, J.A., Hilcove, S., Kim, J., Zhou, H., Ouyang, K., Wang, G., *et al*. (2011) Conversion of Mouse Fibroblasts into Cardiomyocytes Using a Direct Reprogramming Strategy. *Nature Cell Biology*, **13**, 215-222. http://dx.doi.org/10.1038/ncb2164

[24] Qian, L., Huang, Y., Spencer, C.I., Foley, A., Vedantham, V., Liu, L., *et al*. (2012) *In Vivo* Reprogramming of Murine Cardiac Fibroblasts into Induced Cardiomyocytes. *Nature*, **485**, 593-598. http://dx.doi.org/10.1038/nature11044

[25] Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., *et al*. (2001) Mobilized Bone Marrow Cells Repair the Infarcted Heart, Improving Function and Survival. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 10344-10349. http://dx.doi.org/10.1073/pnas.181177898

[26] Yoon, Y.S., Wecker, A., Heyd, L., Park, J.S., Tkebuchava, T., Kusano, K., *et al*. (2005) Clonally Expanded Novel Multipotent Stem Cells from Human Bone Marrow Regenerate Myocardium after MI. *Journal of Clinical Investigation*, **115**, 326-338. http://dx.doi.org/10.1172/JCI200522326

[27] Kajstura, J., Rota, M., Whang, B., Cascapera, S., Hosoda, T., Bearzi, C., *et al*. (2005) Bone Marrow Cells Differentiate in Cardiac Cell Lineages after Infarction Independently of Cell Fusion. *Circulation Research*, **96**, 127-137. http://dx.doi.org/10.1161/01.RES.0000151843.79801.60

[28] Clifford, D.M., Fisher, S.A., Brunskill, S.J., Doree, C., Mathur, A., Watt, S., *et al*. (2012) Stem Cell Treatment for Acute MI. *Cochrane Database of Systematic Reviews*, **2**, CD006536.

[29] Templin, C., Kotlarz, D., Faulhaber, J., Schnabel, S., Grote, K., Salguero, G., *et al*. (2008) *Ex Vivo* Expanded Hematopoietic Progenitor Cells Improve Cardiac Function after MI: Role of Beta-Catenin Transduction and Cell Dose. *Journal of Molecular and Cellular Cardiology*, **45**, 394-403. http://dx.doi.org/10.1016/j.yjmcc.2008.06.010

[30] Mansour, S., Roy, D.C., Bouchard, V., Nguyen, B.K., Stevens, L.M., Gobeil, F., *et al*. (2010) COMPARE-AMI Trial: Comparison of Intracoronary Injection of $CD133^+$ Bone Marrow Stem Cells to Placebo in Patients after Acute MI and Left Ventricular Dysfunction: Study Rationale and Design. *Journal of Cardiovascular Translational Research*, **3**, 153-159. http://dx.doi.org/10.1007/s12265-009-9145-2

[31] Balsam, L.B., Wagers, A.J., Christensen, J.L., Kofidis, T., Weissman, I.L. and Robbins, R.C. (2004) Haematopoietic Stem Cells Adopt Mature Haematopoietic Fates in Ischaemic Myocardium. *Nature*, **428**, 668-673. http://dx.doi.org/10.1038/nature02460

[32] Murry, C.E., Soonpaa, M.H., Reinecke, H., Nakajima, H., Nakajima, H.O., Rubart, M., *et al*. (2004) Haematopoietic Stem Cells Do Not Transdifferentiate into Cardiac Myocytes in Myocardial Infarcts. *Nature*, **428**, 664-668. http://dx.doi.org/10.1038/nature02446

[33] Jujo, K., Ii, M. and Losordo, D.W. (2008) Endothelial Progenitor Cells in Neovascularization of Infarcted Myocardium. *Journal of Molecular and Cellular Cardiology*, **45**, 530-544. http://dx.doi.org/10.1016/j.yjmcc.2008.08.003

[34] Leone, A.M., Rutella, S., Giannico, M.B., Perfetti, M., Zaccone, V., Brugaletta, S., *et al*. (2008) Effect of Intensive vs Standard Statin Therapy on Endothelial Progenitor Cells and Left Ventricular Function in Patients with Acute MI: Statins for Regeneration after Acute MI and PCI (STRAP) Trial. *International Journal of Cardiology*, **130**, 457-462. http://dx.doi.org/10.1016/j.ijcard.2008.05.036

[35] Gruh, I., Beilner, J., Blomer, U., Schmiedl, A., Schmidt-Richter, I., Kruse, M.L., *et al*. (2006) No Evidence of Transdifferentiation of Human Endothelial Progenitor Cells into Cardiomyocytes after Coculture with Neonatal Rat Cardiomyocytes. *Circulation*, **113**, 1326-1334. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.559005

[36] Barry, F.P. and Murphy, J.M. (2004) Mesenchymal Stem Cells: Clinical Applications and Biological Characterization. *The International Journal of Biochemistry & Cell Biology*, **36**, 568-584. http://dx.doi.org/10.1016/j.biocel.2003.11.001

[37] Xu, X., Xu, Z., Xu, Y. and Cui, G. (2005) Selective Down-Regulation of Extracellular Matrix Gene Expression by Bone Marrow Derived Stem Cell Transplantation into Infarcted Myocardium. *Circulation Journal*, **69**, 1275-1283. http://dx.doi.org/10.1253/circj.69.1275

[38] Xu, H., Yang, Y.J., Qian, H.Y., Tang, Y.D., Wang, H. and Zhang, Q. (2011) Rosuvastatin Treatment Activates JAK-STAT Pathway and Increases Efficacy of Allogeneic Mesenchymal Stem Cell Transplantation in Infracted Hearts. *Circulation Journal*, **75**, 1476-1485. http://dx.doi.org/10.1253/circj.CJ-10-1275

[39] Aggarwal, S. and Pittenger, M.F. (2005) Human Mesenchymal Stem Cells Modulate Allogeneic Immune Cell Res-

ponses. *Blood*, **105**, 1815-1822. http://dx.doi.org/10.1182/blood-2004-04-1559

[40] Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A. and Ogawa, S. (1999) Cardiomyocytes Can Be Generated from Marrow Stromal Cells *in Vitro*. *Journal of Clinical Investigation*, **103**, 697-705. http://dx.doi.org/10.1172/JCI5298

[41] Tomita, S., Li, R.K., Weisel, R.D., Mickle, D.A., Kim, E.J., Sakai, T. and Jia, Z.Q. (1999) Autologous Transplantation of Bone Marrow Cells Improves Damaged Heart Function. *Circulation*, **100**, II247-II256. http://dx.doi.org/10.1161/01.cir.100.suppl_2.ii-247

[42] Davani, S., Marandin, A., Mersin, N., Royer, B., Kantelip, B., Hervé, P., Etievent, J.P. and Kantelip, J.P. (2003) Mesenchymal Progenitor Cells Differentiate into an Endothelial Phenotype, Enhance Vascular Density, and Improve Heart Function in a Rat Cellular Cardiomyoplasty Model. *Circulation*, **108**, II253-II258. http://dx.doi.org/10.1161/01.cir.0000089186.09692.fa

[43] Wang, J.-S., Shum-Tim, D. and Chedrawy, E. (2000) Marrow Stromal Cells for Cellular Cardiomyoplasty: The Importance of Microenvironment for Milieu Dependent Differentiation. *Circulation*, **102**, II-683.

[44] Richardson, J.D., Nelson, A.J., Zannettino, A.C., Gronthos, S., Worthley, S.G. and Psaltis, P.J. (2013) Optimization of the Cardiovascular Therapeutic Properties of Mesenchymal Stromal/Stem Cells-Taking the Next Step. *Stem Cell Reviews and Reports*, **9**, 281-302. http://dx.doi.org/10.1007/s12015-012-9366-7

[45] Silva, G.V., Litovsky, S., Assad, J.A., Sousa, A.L., Martin, B.J., Vela, D., Coulter, S.C., Lin, J., Ober, J., Vaughn, W.K., Branco, R.V., Oliveira, E.M., He, R., Geng, Y.J., Willerson, J.T. and Perin, E.C. (2005) Mesenchymal Stem Cells Differentiate into an Endothelial Phenotype, Enhance Vascular Density, and Improve Heart Function in a Canine Chronic Ischemia Model. *Circulation*, **111**, 150-156. http://dx.doi.org/10.1161/01.CIR.0000151812.86142.45

[46] Amado, L.C., Saliaris, A.P., Schuleri, K.H., St John, M., Xie, J.S., Cattaneo, S., *et al*. (2005) Cardiac Repair with IM Injection of Allogeneic Mesenchymal Stem Cells after MI. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11474-11479. http://dx.doi.org/10.1073/pnas.0504388102

[47] Gnecchi, M., Zhang, Z., Ni, A. and Dzau, V.J. (2008) Paracrine Mechanisms in Adult Stem Cell Signaling and Therapy. *Circulation Research*, **103**, 1204-1219. http://dx.doi.org/10.1161/CIRCRESAHA.108.176826

[48] Maggini, J., Mirkin, G., Bognanni, I., Holmberg, J., Piazzon, I.M., Nepomnaschy, I., *et al*. (2010) Mouse Bone Marrow-Derived Mesenchymal Stromal Cells Turn Activated Macrophages into a Regulatory-Like Profile. *PLoS ONE*, **5**, e9252. http://dx.doi.org/10.1371/journal.pone.0009252

[49] Nemeth, K., Leelahavanichkul, A., Yuen, P.S., Mayer, B., Parmelee, A., Doi, K., *et al*. (2009) Bone Marrow Stromal Cells Attenuate Sepsis via Prostaglandin E_2-Dependent Reprogramming of Host Macrophages to Increase Their Interleukin-10 Production. *Nature Medicine*, **15**, 42-49. http://dx.doi.org/10.1038/nm.1905

[50] Zhang, Q.Z., Su, W.R., Shi, S.H., Wilder-Smith, P., Xiang, A.P., Wong, A., *et al*. (2010) Human Gingival Derived Mesenchymal Stem Cells Elicit Polarization of m2 Macrophages and Enhance Cutaneous Wound Healing. *Stem Cells*, **28**, 1856-1868. http://dx.doi.org/10.1002/stem.503

[51] Kronsteiner, B., Peterbauer-Scherb, A., Grillari-Voglauer, R., Redl, H., Gabriel, C., van Griensven, M., *et al*. (2011) Human Mesenchymal Stem Cells and Renal Tubular Epithelial Cells Differentially Influence Monocyte-Derived Dendritic Cell Differentiation and Maturation. *Cellular Immunology*, **267**, 30-38. http://dx.doi.org/10.1016/j.cellimm.2010.11.001

[52] Li, Q., Turdi, S., Thomas, D.P., Zhou, T. and Ren, J. (2010) Intra-Myocardial Delivery of Mesenchymal Stem Cells Ameliorates Left Ventricular and Cardiomyocyte Contractile Dysfunction Following Myocardial Infarction. *Toxicology Letters*, **195**, 119-126. http://dx.doi.org/10.1016/j.toxlet.2010.03.009

[53] Xu, X., Xu, Z., Xu, Y. and Cui, G. (2005) Effects of Mesenchymal Stem Cell Transplantation on Extracellular Matrix after Myocardial Infarction in Rats. *Coronary Artery Disease*, **16**, 245-255. http://dx.doi.org/10.1097/00019501-200506000-00006

[54] Dixon, J.A., Gorman, R.C., Stroud, R.E., Bouges, S., Hirotsugu, H., Gorman, J.H., Martens, T.P., Itescu, S., Schuster, M.D., Plappert, T., *et al*. (2009) Mesenchymal Cell Transplantation and Myocardial Remodeling after Myocardial Infarction. *Circulation*, **120**, S220-S229. http://dx.doi.org/10.1161/CIRCULATIONAHA.108.842302

[55] Jiang, Z., Hu, X., Yu, H., Xu, Y., Wang, L., Chen, H., *et al*. (2013) Human Endometrial Stem Cells Confer Enhanced Myocardial Salvage and Regeneration by Paracrine Mechanisms. *Journal of Cellular and Molecular Medicine*, **17**, 1247-1260. http://dx.doi.org/10.1111/jcmm.12100

[56] Li, B., Zeng, Q., Wang, H., Shao, S., Mao, X., Zhang, F., *et al*. (2007) Adipose Tissue Stromal Cells Transplantation in Rats of Acute Myocardial Infarction. *Coronary Artery Disease*, **18**, 221-227. http://dx.doi.org/10.1097/MCA.0b013e32801235da

[57] Sadat, S., Gehmert, S., Song, Y.-H., Yen, Y., Bai, X., Gaiser, S., *et al*. (2007) The Cardioprotective Effect of Mesenchymal Stem Cells Is Mediated by IGF-I and VEGF. *Biochemical and Biophysical Research Communications*, **363**,

674-679. http://dx.doi.org/10.1016/j.bbrc.2007.09.058

[58] Madonna, R., Geng, Y.-J. and Caterina, R.D. (2009) Adipose Tissue-Derived Stem Cells Characterization and Potential for Cardiovascular Repair. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **29**, 1723-1729. http://dx.doi.org/10.1161/ATVBAHA.109.187179

[59] Nagaya, N., Fujii, T., Iwase, T., Ohgushi, H., Itoh, T., Uematsu, M., *et al*. (2004) Intravenous Administration of Mesenchymal Stem Cells Improves Cardiac Function in Rats with Acute Myocardial Infarction through Angiogenesis and Myogenesis. *American Journal of Physiology*: *Heart and Circulatory Physiology*, **287**, H2670-H2676. http://dx.doi.org/10.1152/ajpheart.01071.2003

[60] Rehman, J., Traktuev, D., Li, J., Merfeld-Clauss, S., Temm-Grove, C.J., Bovenkerk, J.E., *et al*. (2004) Secretion of Angiogenic and Antiapoptotic Factors by Human Adipose Stromal Cells. *Circulation*, **109**, 1292-1298. http://dx.doi.org/10.1161/01.CIR.0000121425.42966.F1

[61] Schenke-Layland, K., Strem, B.M., Jordan, M.C., DeEmedio, M.T., Hedrick, M.H., Roos, K.P., *et al*. (2009) Adipose Tissue-Derived Cells Improve Cardiac Function Following Myocardial Infarction. *Journal of Surgical Research*, **153**, 217-223. http://dx.doi.org/10.1016/j.jss.2008.03.019

[62] Miyahara, Y., Nagaya, N., Kataoka, M., Yanagawa, B., Tanaka, K., Hao, H., *et al*. (2006) Monolayered Mesenchymal Stem Cells Repair Scarred Myocardium after Myocardial Infarction. *Nature Medicine*, **12**, 459-465. http://dx.doi.org/10.1038/nm1391

[63] Uemura, R., Xu, M., Ahmad, N. and Ashraf, M. (2006) Bone Marrow Stem Cells Prevent Left Ventricular Remodeling of Ischemic Heart through Paracrine Signaling. *Circulation Research*, **98**, 1414-1421. http://dx.doi.org/10.1161/01.RES.0000225952.61196.39

[64] Bai, X. and Alt, E. (2010) Myocardial Regeneration Potential of Adipose Tissue-Derived Stem Cells. *Biochemical and Biophysical Research Communications*, **401**, 321-326. http://dx.doi.org/10.1016/j.bbrc.2010.09.012

[65] Bai, X., Yan, Y., Song, Y.-H., Seidensticker, M., Rabinovich, B., Metzele, R., *et al*. (2010) Both Cultured and Freshly Isolated Adipose Tissue-Derived Stem Cells Enhance Cardiac Function after Acute Myocardial Infarction. *European Heart Journal*, **31**, 489-501. http://dx.doi.org/10.1093/eurheartj/ehp568

[66] Schuleri, K.H., Amado, L.C., Boyle, A.J., Centola, M., Saliaris, A.P., Gutman, M.R., Hatzistergos, K.E., Oskouei, B.N., Zimmet, J.M., Young, R.G., Heldman, A.W., Lardo, A.C. and Hare, J.M. (2008) Early Improvement in Cardiac Tissue Perfusion Due to Mesenchymal Stem Cells. *American Journal of Physiology—Heart and Circulatory Physiology*, **294**, H2002-H2011. http://ajpheart.physiology.org/content/294/5/H2002.long

[67] Hatzistergos, K.E., Quevedo, H., Oskouei, B.N., Hu, Q., Feigenbaum, G.S., Margitich, I.S., Mazhari, R., Boyle, A.J., Zambrano, J.P., Rodriguez, J.E., Dulce, R., Pattany, P.M., Valdes, D., Revilla, C., Heldman, A.W., McNiece, I. and Hare, J.M. (2010) Bone Marrow Mesenchymal Stem Cells Stimulate Cardiac Stem Cell Proliferation and Differentiation. *Circulation Research*, **107**, 913-922. http://dx.doi.org/10.1161/CIRCRESAHA.110.222703

[68] Katritsis, D.G., Sotiropoulou, P.A., Karvouni, E., Karabinos, I., Korovesis, S., Perez, S.A., *et al*. (2005) Transcoronary Transplantation of Autologous mesenchymal Stem Cells and Endothelial Progenitors into Infarcted Human Myocardium. *Catheterization and Cardiovascular Interventions*, **65**, 321-329. http://dx.doi.org/10.1002/ccd.20406

[69] Yang, Z., Zhang, F., Ma, W., Chen, B., Zhou, F., Xu, Z., *et al*. (2010) A Novel Approach to Transplanting Bone Marrow Stem Cells to Repair Human MI: Delivery via a Noninfarct-Relative Artery. *Cardiovascular Therapeutics*, **28**, 380-385. http://dx.doi.org/10.1111/j.1755-5922.2009.00116.x

[70] Chen, S., Liu, Z., Tian, N., Zhang, J., Yei, F., Duan, B., Zhu, Z., Lin, S. and Kwan, T.W. (2006) Intracoronary Transplantation of Autologous Bone Marrow Mesenchymal Stem Cells for Ischemic Cardiomyopathy Due to Isolated Chronic Occluded Left Anterior Descending Artery. *The Journal of Invasive Cardiology*, **18**, 552-556.

[71] Katritsis, D.G., Sotiropoulou, P., Giazitzoglou, E., Karvouni, E. and Papamichail, M. (2007) Electrophysiological Effects of Intracoronary Transplantation of Autologous Mesenchymal and Endothelial Progenitor Cells. *Europace*, **9**, 167-171. http://dx.doi.org/10.1093/europace/eul184

[72] Williams, A.R., Trachtenberg, B., Velazquez, D.L., McNiece, I., Altman, P., Rouy, D., *et al*. (2011) IM Stem Cell Injection in Patients with Ischemic Cardiomyopathy: Functional Recovery and Reverse Remodeling. *Circulation Research*, **108**, 792-796. http://dx.doi.org/10.1161/CIRCRESAHA.111.242610

[73] Hare, J.M., Traverse, J.H., Henry, T.D., Dib, N., Strumpf, R.K., Schulman, S.P., *et al*. (2009) A randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of Intravenous Adult Human Mesenchymal Stem Cells (Prochymal) after Acute MI. *Journal of the American College of Cardiology*, **54**, 2277-2286. http://dx.doi.org/10.1016/j.jacc.2009.06.055

[74] Fischer, U.M., Harting, M.T., Jimenez, F., Monzon-Posadas, W.O., Xue, H., Savitz, S.I., Laine, G.A. and Cox Jr., C.S. (2009) Pulmonary Passage Is a Major Obstacle for Intravenous Stem Cell Delivery: The Pulmonary First-Pass Effect. *Stem Cells and Development*, **18**, 683-692. http://dx.doi.org/10.1089/scd.2008.0253

[75] Tsyb, A.F., Konoplyannikov, A.G., Kaplan, M.A., *et al*. (2009) Systemic Transplantation of Autologous Mesenchymal Stem Cells for Coplex Therapy of Patients with Chronic Heart Failure. *Cell Transplantology and Tissue Engineering*, **4**, 78-84.

[76] Guijarro, D., Lebrin, M., Lairez, O., Bourin, P., Piriou, N., Pozzo, J., *et al*. (2015) 0148: IM Transplantation of Mesenchymal Stromal Cells for Chronic Myocardial Ischemia and Decreased Left Ventricular Function: 1-Year Results of the MESAMI Phase I Clinical Trial. *Archives of Cardiovascular Diseases Supplements*, **7**, 20. http://dx.doi.org/10.1016/S1878-6480(15)71543-0

[77] Menasche, P. (2007) Skeletal Myoblasts as a Therapeutic Agent. *Progress in Cardiovascular Diseases*, **50**, 7-17. http://dx.doi.org/10.1016/j.pcad.2007.02.002

[78] Pagani, F.D., DerSimonian, H., Zawadzka, A., Wetzel, K., Edge, A.S., Jacoby, D.B., *et al*. (2003) Autologous Skeletal Myoblasts Transplanted to Ischemia Damaged Myocardium in Humans: Histological Analysis of Cell Survival and Differentiation. *Journal of the American College of Cardiology*, **41**, 879-888. http://dx.doi.org/10.1016/S0735-1097(03)00081-0

[79] Ghostine, S., Carrion, C., Souza, L.C., Richard, P., Bruneval, P., Vilquin, J.T., *et al*. (2002) Long-Term Efficacy of Myoblast Transplantation on Regional Structure and Function after MI. *Circulation*, **106**, I131-I136.

[80] Reinecke, H., Poppa, V. and Murry, C.E. (2002) Skeletal Muscle Stem Cells Do Not Transdifferentiate into Cardiomyocytes after Cardiac Grafting. *Journal of Molecular and Cellular Cardiology*, **34**, 241-249. http://dx.doi.org/10.1006/jmcc.2001.1507

[81] Eisen, H.J. (2008) Skeletal Myoblast Transplantation: No Magic Bullet for Ischemic Cardiomyopathy. *Nature Clinical Practice Cardiovascular Medicine*, **5**, 520-521. http://dx.doi.org/10.1038/ncpcardio1299

[82] Oh, H., Bradfute, S.B., Gallardo, T.D., Nakamura, T., Gaussin, V., Mishina, Y., *et al*. (2003) Cardiac Progenitor Cells from Adult Myocardium: Homing, Differentiation, and Fusion after Infarction. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 12313-12318. http://dx.doi.org/10.1073/pnas.2132126100

[83] Matsuura, K., Nagai, T., Nishigaki, N., Oyama, T., Nishi, J., Wada, H., *et al*. (2004) Adult Cardiac Sca-1-Positive Cells Differentiate into Beating Cardiomyocytes. *The Journal of Biological Chemistry*, **279**, 11384-11391. http://dx.doi.org/10.1074/jbc.M310822200

[84] Wang, X., Hu, Q., Nakamura, Y., Lee, J., Zhang, G., From, A.H., *et al*. (2006) The Role of the Sca-1^{+}/CD31^{-} Cardiac Progenitor Cell Population in Postinfarction Left Ventricular Remodeling. *Stem Cells*, **24**, 1779-1788. http://dx.doi.org/10.1634/stemcells.2005-0386

[85] Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., *et al*. (2003) Adult Cardiac Stem Cells Are Multipotent and Support Myocardial Regeneration. *Cell*, **114**, 763-776. http://dx.doi.org/10.1016/S0092-8674(03)00687-1

[86] Bolli, R., Chugh, A.R., D'Amario, D., Loughran, J.H., Stoddard, M.F., Ikram, S., *et al*. (2011) Cardiac Stem Cells in Patients with Ischaemic Cardiomyopathy (SCIPIO): Initial Results of a Randomised Phase 1 Trial. *Lancet*, **378**, 1847-1857. http://dx.doi.org/10.1016/S0140-6736(11)61590-0

[87] Zaruba, M.M., Soonpaa, M., Reuter, S. and Field, L.J. (2010) Cardiomyogenic Potential of c-kit^{+}-Expressing Cells Derived from Neonatal and Adult Mouse Hearts. *Circulation*, **121**, 1992-2000. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.909093

[88] 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. and Anversa, P. (2003) Adult Cardiac Stem Cells Are Multipotent and Support Myocardial Regeneration. *Cell*, **114**, 763-776. http://dx.doi.org/10.1016/S0092-8674(03)00687-1

[89] Dawn, B., Stein, A.B., Urbanek, K., Rota, M., Whang, B., Rastaldo, R., Torella, D., Tang, X.-L., Rezazadeh, A., Kajstura, J., Leri, A., Hunt, G., Varma, J., Prabhu, S.D., Anversa, P. and Bolli, R. (2005) Cardiac Stem Cells Delivered Intravascularly Traverse the Vessel Barrier, Regenerate Infarcted Myocardium, and Improve Cardiac Function. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 3766-3771. http://dx.doi.org/10.1073/pnas.0405957102

[90] Fazel, S., Cimini, M., Chen, L., Li, S., Angoulvant, D., Fedak, P., *et al*. (2006) Cardioprotective c-kit^{+} Cells Are from the Bone Marrow and Regulate the Myocardial Balance of Angiogenic Cytokines. *Journal of Clinical Investigation*, **116**, 1865-1877. http://dx.doi.org/10.1172/JCI27019

[91] Cai, C.L., Liang, X., Shi, Y., Chu, P.H., Pfaff, S.L., Chen, J., *et al*. (2003) Isl1 Identifies a Cardiac Progenitor Population That Proliferates Prior to Differentiation and Contributes a Majority of Cells to the Heart. *Developmental Cell*, **5**, 877-889. http://dx.doi.org/10.1016/S1534-5807(03)00363-0

[92] Laugwitz, K.L., Moretti, A., Lam, J., Gruber, P., Chen, Y., Woodard, S., *et al*. (2005) Postnatal Isl1^{+} Cardioblasts Enter Fully Differentiated Cardiomyocyte Lineages. *Nature*, **433**, 647-653. http://dx.doi.org/10.1038/nature03215

[93] Chien, K.R., Domian, I.J. and Parker, K.K. (2008) Cardiogenesis and the Complex Biology of Regenerative Cardi-

ovascular Medicine. *Science*, **322**, 1494-1497. http://dx.doi.org/10.1126/science.1163267

[94] Laugwitz, K.L., Moretti, A., Caron, L., Nakano, A. and Chien, K.R. (2008) Islet1 Cardiovascular Progenitors: A Single Source for Heart Lineages? *Development*, **135**, 193-205. http://dx.doi.org/10.1242/dev.001883

[95] Martin-Puig, S., Wang, Z. and Chien, K.R. (2008) Lives of a Heart Cell: Tracing the Origins of Cardiac Progenitors. *Cell Stem Cell*, **2**, 320-331. http://dx.doi.org/10.1016/j.stem.2008.03.010

[96] Wu, S.M., Chien, K.R. and Mummery, C. (2008) Origins and Fates of Cardiovascular Progenitor Cells. *Cell*, **132**, 537-543. http://dx.doi.org/10.1016/j.cell.2008.02.002

[97] Kattman, S.J., Huber, T.L. and Keller, G.M. (2006) Multipotent Flk-1^+ Cardiovascular Progenitor Cells Give Rise to the Cardiomyocyte, Endothelial, and Vascular Smooth Muscle Lineages. *Developmental Cell*, **11**, 723-732. http://dx.doi.org/10.1016/j.devcel.2006.10.002

[98] Moretti, A., Caron, L., Nakano, A., Lam, J.T., Bernshausen, A., Chen, Y., *et al*. (2006) Multipotent Embryonic Isl1^+ Progenitor Cells Lead to Cardiac, Smooth Muscle, and Endothelial Cell Diversification. *Cell*, **127**, 1151-1165. http://dx.doi.org/10.1016/j.cell.2006.10.029

[99] Challen, G.A. and Little, M.H. (2006) A Side Order of Stem Cells: The SP Phenotype. *Stem Cells*, **24**, 3-12. http://dx.doi.org/10.1634/stemcells.2005-0116

[100] Martin, C.M., Meeson, A.P., Robertson, S.M., Hawke, T.J., Richardson, J.A., Bates, S., *et al*. (2004) Persistent Expression of the ATP-Binding Cassette Transporter, abcg2, Identifies Cardiac SP Cells in the Developing and Adult Heart. *Developmental Biology*, **265**, 262-275. http://dx.doi.org/10.1016/j.ydbio.2003.09.028

[101] Oyama, T., Nagai, T., Wada, H., Naito, A.T., Matsuura, K., Iwanaga, K., *et al*. (2007) Cardiac Side Population Cells Have a Potential to Migrate and Differentiate into Cardiomyocytes *in Vitro* and *in Vivo*. *The Journal of Cell Biology*, **176**, 329-341. http://dx.doi.org/10.1083/jcb.200603014

[102] Messina, E., De Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., *et al*. (2004) Isolation and Expansion of Adult Cardiac Stem Cells from Human and Murine Heart. *Circulation Research*, **95**, 911-921. http://dx.doi.org/10.1161/01.RES.0000147315.71699.51

[103] Takehara, N., Tsutsumi, Y., Tateishi, K., Ogata, T., Tanaka, H., Ueyama, T., *et al*. (2008) Controlled Delivery of Basic Fibroblast Growth Factor Promotes Human Cardiosphere-Derived Cell Engraftment to Enhance Cardiac Repair for Chronic MI. *Journal of the American College of Cardiology*, **52**, 1858-1865. http://dx.doi.org/10.1016/j.jacc.2008.06.052

[104] Tang, X.L., Rokosh, G., Sanganalmath, S.K., Tokita, Y., Keith, M.C., Shirk, G., Stowers, H., Hunt, G.N., Wu, W., Dawn, B. and Bolli, R. (2015) Effects of Intracoronary Infusion of Escalating Doses of Cardiac Stem Cells in Rats with Acute Myocardial Infarction. *Circ Heart Fail*, **8**, 757-65. http://circheartfailure.ahajournals.org/content/8/4/757.long

[105] Smith, R.R., Barile, L., Cho, H.C., Leppo, M.K., Hare, J.M., Messina, E., *et al*. (2007) Regenerative Potential of Cardiosphere-Derived Cells Expanded from Percutaneous Endomyocardial Biopsy Specimens. *Circulation*, **115**, 896-908. http://dx.doi.org/10.1161/CIRCULATIONAHA.106.655209

[106] Khan, M., Kwiatkowski, P., Rivera, B.K. and Kuppusamy, P. (2010) Oxygen and Oxygenation in Stem-Cell Therapy for MI. *Life Sciences*, **87**, 269-274. http://dx.doi.org/10.1016/j.lfs.2010.06.013

[107] Kofoed, H., Sjontoft, E., Siemssen, S.O. and Olesen, H.P. (1985) Bone Marrow Circulation after Osteotomy. Blood Flow, pO_2, pCO_2, and Pressure Studied in Dogs. *Acta Orthopaedica Scandinavica*, **56**, 400-403. http://dx.doi.org/10.3109/17453678508994357

[108] Hu, X., Yu, S.P., Fraser, J.L., Lu, Z., Ogle, M.E., Wang, J.-A. and Wei, L. (2008) Transplantation of Hypoxia-Preconditioned Mesenchymal Stem Cells Improves Infarcted Heart Function via Enhanced Survival of Implanted Cells and Angiogenesis. *The Journal of Thoracic and Cardiovascular Surgery*, **135**, 799-808. http://dx.doi.org/10.1016/j.jtcvs.2007.07.071

[109] Li, J.H., Zhang, N. and Wang, J.A. (2008) Improved Antiapoptotic and Anti-Remodeling Potency of Bone Marrow Mesenchymal Stem Cells by Anoxic Pre-Conditioning in Diabetic Cardiomyopathy. *Journal of Endocrinological Investigation*, **31**, 103-110. http://dx.doi.org/10.1007/BF03345575

[110] Rebelatto, C.K., Aguiar, A.M., Senegaglia, A.C., Aita, C.M., Hansen, P., Barchiki, F., *et al*. (2009) Expression of Cardiac Function Genes in Adult Stem Cells Is Increased by Treatment with Nitric Oxide Agents. *Biochemical and Biophysical Research Communications*, **378**, 456-461. http://dx.doi.org/10.1016/j.bbrc.2008.11.061

[111] Afzal, M.R., Haider, H., Idris, N.M., Jiang, S., Ahmed, R.P. and Ashraf, M. (2010) Pre-Conditioning Promotes Survival and Angiomyogenic Potential of Mesenchymal Stem Cells in the Infarcted Heart via NF-kappaB Signaling. *Antioxidants & Redox Signaling*, **12**, 693-702. http://dx.doi.org/10.1089/ars.2009.2755

[112] Suzuki, Y., Kim, H.W., Ashraf, M. and Haider, H. (2010) Diazoxide Potentiates Mesenchymal Stem Cell Survival via NFkappaB-Dependent miR-146a Expression by Targeting Fas. *American Journal of Physiology*: *Heart and Circulatory Physiology*, **299**, H1077-H1082. http://dx.doi.org/10.1152/ajpheart.00212.2010

[113] Suzuki, K., Smolenski, R.T., Jayakumar, J., Murtuza, B., Brand, N.J. and Yacoub, M.H. (2000) Heat Shock Treatment Enhances Graft Cell Survival in Skeletal Myoblast Transplantation to the Heart. *Circulation*, **102**, III216-III221. http://dx.doi.org/10.1161/01.cir.102.suppl_3.iii-216

[114] Wang, X., Zhao, T., Huang, W., Wang, T., Qian, J., Xu, M., *et al*. (2009) Hsp20-Engineered Mesenchymal Stem Cells Are Resistant to Oxidative Stress via Enhanced Activation of Akt and Increased Secretion of Growth Factors. *Stem Cells*, **27**, 3021-3031. http://dx.doi.org/10.1002/stem.230

[115] Chang, W., Song, B.-W., Lim, S., Song, H., Shim, C.Y., Cha, M.-J., *et al*. (2008) Mesenchymal Stem Cells Pretreated with Delivered Hph-1-Hsp70 Protein Are Protected from Hypoxia-Mediated Cell Death and Rescue Heart Functions from Myocardial Injury. *Stem Cells*, **27**, 2283-2292. http://dx.doi.org/10.1002/stem.153

[116] Yang, Y.J., Qian, H.Y., Huang, J., Li, J.-J., Gao, R.-L., Dou, K.-F., *et al*. (2009) Combined Therapy with Simvastatin and Bone Marrow-Derived Mesenchymal Stem Cells Increases Benefits in Infarcted Swine Hearts. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **29**, 2076-2082. http://dx.doi.org/10.1161/ATVBAHA.109.189662

[117] Yang, Y., Mou, Y., Hu, S.J. and Fu, M. (2009) Beneficial Effect of Rosuvastatin on Cardiac Dysfunction Is Associated with Alterations in Calcium-Regulatory Proteins. *European Journal of Heart Failure*, **11**, 6-13. http://dx.doi.org/10.1093/eurjhf/hfn002

[118] Lin, Y.C., Leu, S., Sun, C.K., Yen, C.-H., Kao, Y.-H., Chang, L.-T., *et al*. (2010) Early Combined Treatment with Sildenafil and Adipose-Derived Mesenchymal Stem Cells Preserves Heart Function in Rat Dilated Cardiomyopathy. *Journal of Translational Medicine*, **8**, 88. http://dx.doi.org/10.1186/1479-5876-8-88

[119] Haider, H., Lee, Y.J., Jiang, S., Ahmed, R.P., Ryon, M. and Ashraf, M. (2010) Phosphodiesterase Inhibition with Tadalafil Provides Longer and Sustained Protection of Stem Cells. *American Journal of Physiology*: *Heart and Circulatory Physiology*, **299**, H1395-H1404. http://dx.doi.org/10.1152/ajpheart.00437.2010

[120] Numasawa, Y., Kimura, T., Miyoshi, S., Nishiyama, N., Hida, N., Tsuji, H., Tsuruta, H., Segawa, K., Ogawa, S. and Umezawa, A. (2011) Treatment of Human Mesenchymal Stem Cells with Angiotensin Receptor Blocker Improved Efficiency of Cardiomyogenic Transdifferentiation and Improved Cardiac Function via Angiogenesis. *Stem Cells*, **29**, 1405-1414. http://dx.doi.org/10.1002/stem.691

[121] Wang, Y., Zhang, D., Ashraf, M., Zhao, T., Huang, W., Ashraf, A. and Balasubramaniam, A. (2010) Combining Neuropeptide Y and Mesenchymal Stem Cells Reverses Remodeling after MI. *American Journal of Physiology*: *Heart and Circulatory Physiology*, **298**, H275-H286. http://dx.doi.org/10.1152/ajpheart.00765.2009

[122] Kinnaird, T., Stabile, E., Burnett, M.S., Shou, M., Lee, C.W., Barr, S., Fuchs, S. and Epstein, S.E. (2004) Local Delivery of Marrow-Derived Stromal Cells Augments Collateral Perfusion through Paracrine Mechanisms. *Circulation*, **109**, 1543-1549. http://dx.doi.org/10.1161/01.CIR.0000124062.31102.57

[123] Herrmann, J.L., Wang, Y., Abarbanell, A.M., Weil, B.R., Tan, J. and Meldrum, D.R. (2010) Pre-Conditioning Mesenchymal Stem Cells with Transforming Growth Factor-Alpha Improves Mesenchymal Stem Cell-Mediated Cardioprotection. *Shock*, **33**, 24-30. http://dx.doi.org/10.1097/SHK.0b013e3181b7d137

[124] Pasha, Z., Wang, Y., Sheikh, R., Zhang, D., Zhao, T. and Ashraf, M. (2008) Pre-Conditioning Enhances Cell Survival and Differentiation of Stem Cells during Transplantation in Infarcted Myocardium. *Cardiovascular Research*, **77**, 134-142. http://dx.doi.org/10.1093/cvr/cvm025

[125] Yao, Y., Zhang, F., Wang, L., Zhang, G., Wang, Z., Chen, J. and Gao, X. (2009) Lipopolysaccharide Pre-Conditioning Enhances the Efficacy of Mesenchymal Stem Cells Transplantation in a Rat Model of Acute MI. *Journal of Biomedical Science*, **16**, 74. http://dx.doi.org/10.1186/1423-0127-16-74

[126] Matsumoto, R., Omura, T., Yoshiyama, M., Hayashi, T., Inamoto, S., Koh, K.-R., *et al*. (2005) Vascular Endothelial Growth Factor-Expressing Mesenchymal Stem Cell Transplantation for the Treatment of Acute MI. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **25**, 1168-1173. http://dx.doi.org/10.1161/01.ATV.0000165696.25680.ce

[127] Guo, J., Lin, G., Bao, C., Hu, Z., Chu, H. and Hu, M. (2008) Insulin-Like Growth Factor 1 Improves the Efficacy of Mesenchymal Stem Cells Transplantation in a Rat Model of MI. *Journal of Biomedical Science*, **15**, 89-97. http://dx.doi.org/10.1007/s11373-007-9207-x

[128] Abbott, J.D., Huang, Y., Liu, D., Hickey, R., Krause, D.S. and Giordano, F.J. (2004) Stromal Cell-Derived Factor-1α Plays a Critical Role in Stem Cell Recruitment to the Heart after MI but Is Not Sufficient to Induce Homing in the Absence of Injury. *Circulation*, **110**, 3300-3305. http://dx.doi.org/10.1161/01.CIR.0000147780.30124.CF

[129] Kijowski, J., Baj-Krzyworzeka, M., Majka, M., Reca, R., Marquez, L.A., Christofidou-Solomidou, M., *et al*. (2001) The SDF-1-CXCR4 Axis Stimulates VEGF Secretion and Activates Integrins but Does Not Affect Proliferation and Survival in Lymphohematopoietic Cells. *Stem Cells*, **19**, 453-466. http://dx.doi.org/10.1634/stemcells.19-5-453

[130] Zhuang, Y., Chen, X., Xu, M., Zhang, L.Y. and Xiang, F. (2009) Chemokine Stromal Cell-Derived Factor 1/CXCL12 Increases Homing of Mesenchymal Stem Cells to Injured Myocardium and Neovascularization Following MI. *Chinese Medical Journal*, **122**, 183-187.

[131] Tang, J., Wang, J., Guo, L., Kong, X., Yang, J., Zheng, F., Zhang, L. and Huang, Y. (2010) Mesenchymal Stem Cells Modified with Stromal Cell-Derived Factor 1 Alpha Improve Cardiac Remodeling via Paracrine Activation of Hepatocyte Growth Factor in a Rat Model of MI. *Molecules and Cells*, **29**, 9-19. http://dx.doi.org/10.1007/s10059-010-0001-7

[132] Guo, Y.H., He, J.G., Wu, J.L., Yang, L., Zhang, D.-S., Tan, X.-Y. and Qi, R.-D. (2008) Hepatocyte Growth Factor and Granulocyte Colony-Stimulating Factor Form a Combined Neovasculogenic Therapy for Ischemic Cardiomyopathy. *Cytotherapy*, **10**, 857-867. http://dx.doi.org/10.1080/14653240802419278

[133] Huang, J., Zhang, Z., Guo, J., Ni, A., Deb, A., Zhang, L., Mirotsou, M., Pratt, R.E. and Dzau, V.J. (2010) Genetic Modification of Mesenchymal Stem Cells Overexpressing CCR1 Increases Cell Viability, Migration, Engraftment, and Capillary Density in the Injured Myocardium. *Circulation Research*, **106**, 1753-1762. http://dx.doi.org/10.1161/CIRCRESAHA.109.196030

[134] Tang, J., Wang, J., Zheng, F., Kong, X., Guo, L., Yang, J., Zhang, L. and Huang, Y. (2010) Combination of Chemokine and Angiogenic Factor Genes and Mesenchymal Stem Cells Could Enhance Angiogenesis and Improve Cardiac Function after Acute MI in Rats. *Molecular and Cellular Biochemistry*, **339**, 107-118. http://dx.doi.org/10.1007/s11010-009-0374-0

[135] Chen, J., Crawford, R., Chen, C. and Xiao, Y. (2013) The Key Regulatory Roles of the PI3K/Akt Signaling Pathway in the Functionalities of Mesenchymal Stem Cells and Applications in Tissue Regeneration. *Tissue Engineering Part B—Reviews*, **19**, 516-528. http://online.liebertpub.com/doi/abs/10.1089/ten.TEB.2012.0672

[136] Datta, S.R., Brunet, A. and Greenberg, M.E. (1999) Cellular Survival: A Play in Three Akts. *Genes & Development*, **13**, 2905-2927. http://dx.doi.org/10.1101/gad.13.22.2905

[137] Somanath, P.R., Razorenova, O.V., Chen, J. and Byzova, T.V. (2006) Akt1 in Endothelial Cell and Angiogenesis. *Cell Cycle*, **5**, 512-518. http://dx.doi.org/10.4161/cc.5.5.2538

[138] Gnecchi, M., He, H., Liang, O.D., Melo, L.G., Morello, F., Mu, H., *et al*. (2005) Paracrine Action Accounts for Marked Protection of Ischemic Heart by Akt Modified Mesenchymal Stem Cells. *Nature Medicine*, **11**, 367-368. http://dx.doi.org/10.1038/nm0405-367

[139] Gnecchi, M., He, H., Noiseux, N., Liang, O.D., Zhang, L., Morello, F., *et al*. (2006) Evidence Supporting Paracrine Hypothesis for Akt-Modified Mesenchymal Stem Cell-Mediated Cardiac Protection and Functional Improvement. *The FASEB Journal*, **20**, 661-669. http://dx.doi.org/10.1096/fj.05-5211com

[140] Gnecchi, M., He, H., Melo, L.G., Noiseaux, N., Morello, F., de Boer, R.A., *et al*. (2009) Early Beneficial Effects of Bone Marrow-Derived Mesenchymal Stem Cells Overexpressing Akt on Cardiac Metabolism after MI. *Stem Cells*, **27**, 971-979. http://dx.doi.org/10.1002/stem.12

[141] Mirotsou, M., Zhang, Z., Deb, A., Zhang, L., Gnecchi, M., Noiseux, N., Mu, H., Pachori, A. and Dzau, V. (2007) Secreted Frizzled Related Protein 2 (Sfrp2) Is the Key Akt-Mesenchymal Stem Cellreleased Paracrine Factor Mediating Myocardial Survival and Repair. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 1643-1648. http://dx.doi.org/10.1073/pnas.0610024104

[142] Song, S.W., Chang, W., Song, B.W., Song, H., Lim, S., Kim, H.-J., *et al*. (2009) Integrin-Linked Kinase Is Required in Hypoxic Mesenchymal Stem Cells for Strengthening Cell Adhesion to Ischemic Myocardium. *Stem Cells*, **27**, 1358-1365. http://dx.doi.org/10.1002/stem.47

[143] Jiang, Y., Chen, L., Tang, Y., Ma, G., Shen, C., Qi, C., *et al*. (2010) HO-1 Gene Overexpression Enhances the Beneficial Effects of Superparamagnetic Iron Oxide Labeled Bone Marrow Stromal Cells Transplantation in Swine Hearts Underwent Ischemia/Reperfusion: An MRI Study. *Basic Research in Cardiology*, **105**, 431-442. http://dx.doi.org/10.1007/s00395-009-0079-2

[144] Taljaard, M., Ward, M.R., Kutryk, M.J., Courtman, D.W., Camack, N.J., Goodman, S.G., *et al*. (2010) Rationale and Design of Enhanced Angiogenic Cell Therapy in Acute MI (ENACT-AMI): The First Randomized Placebo-Controlled Trial of Enhanced Progenitor Cell Therapy for Acute MI. *American Heart Journal*, **159**, 354-360. http://dx.doi.org/10.1016/j.ahj.2009.12.021

[145] Behfar, A., Zingman, L.V., Hodgson, D.M., Rauzier, J.-M., Kane, G.C., Terzic, A. and Pucéat, M. (2002) Stem Cell Differentiation Requires a Paracrine Pathway in the Heart. *The FASEB Journal*, **16**, 1558-1566. http://dx.doi.org/10.1096/fj.02-0072com

[146] Behfar, A., Perez-Terzic, C., Faustino, R.S., Arrell, D.K., Hodgson, D.M., Yamada, S., *et al*. (2007) Cardiopoietic Programming of Embryonic Stem Cells for Tumor Free Heart Repair. *The Journal of Experimental Medicine*, **204**, 405-420. http://dx.doi.org/10.1084/jem.20061916

[147] Behfar, A., Yamada, S., Crespo-Diaz, R., Nesbitt, J.J., Rowe, L.A., Perez-Terzic, C., *et al*. (2010) Guided Cardiopoiesis Enhances Therapeutic Benefit of Bone Marrow Human Mesenchymal Stem Cells in Chronic MI. *Journal of the American College of Cardiology*, **56**, 721-734. http://dx.doi.org/10.1016/j.jacc.2010.03.066

[148] Bartunek, J., Wijns, W., Dolatabadi, D., Vanderheyden, M., Dens, J., Ostojic, M., *et al*. (2011) C-Cure Multicenter Trial: Lineage Specific Bone Marrow Derived Cardiopoietic Mesenchymal Stem Cells for the Treatment of Ischaemic Cardiomyopathy. *Journal of the American College of Cardiology*, **57**, E200. http://dx.doi.org/10.1016/s0735-1097(11)60200-3

[149] Lakshmipathy, U. and Hart, R.P. (2008) Concise Review: MicroRNA Expression in Multipotent Mesenchymal Stromal Cells. *Stem Cells*, **26**, 356-363. http://dx.doi.org/10.1634/stemcells.2007-0625

[150] Valtieri, M. and Sorrentino, A. (2008) The Mesenchymal Stromal Cell Contribution to Homeostasis. *Journal of Cellular Physiology*, **217**, 296-300. http://dx.doi.org/10.1002/jcp.21521

[151] Karp, X. and Ambros, V. (2005) Developmental Biology: Encountering microRNAs in Cell Fate Signaling. *Science*, **310**, 1288-1289. http://dx.doi.org/10.1126/science.1121566

[152] Kloosterman, W.P. and Plasterk, R.H. (2006) The Diverse Functions of microRNAs in Animal Development and Disease. *Developmental Cell*, **11**, 441-450. http://dx.doi.org/10.1016/j.devcel.2006.09.009

[153] van Rooij, E., Sutherland, L.B., Liu, N., Williams, A.H., McAnally, J., Gerard, R.D., *et al*. (2006) A Signature Pattern of Stress-Responsive microRNAs That Can Evoke Cardiac Hypertrophy and Heart Failure. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 18255-18260. http://dx.doi.org/10.1073/pnas.0608791103

[154] Kim, J., Inoue, K., Ishii, J., Vanti, W.B., Voronov, S.V., Murchison, E., *et al*. (2007) A microRNA Feedback Circuit in Midbrain Dopamine Neurons. *Science*, **317**, 1220-1224. http://dx.doi.org/10.1126/science.1140481

[155] Calin, G.A. and Croce, C.M. (2006) MicroRNA Signatures in Human Cancers. *Nature Reviews Cancer*, **6**, 857-866. http://dx.doi.org/10.1038/nrc1997

[156] Guo, L., Zhao, R.C. and Wu, Y. (2011) The Role of miRNAs in Self-Renewal and Differentiation of Mesenchymal Stem Cells. *Experimental Hematology*, **39**, 608-616. http://dx.doi.org/10.1016/j.exphem.2011.01.011

[157] Eskildsen, T., Taipaleenmäki, H., Stenvang, J., Abdallah, B.M., Ditzel, N., Nossent, A.Y., Bak, M., Kauppinen, S. and Kassem, M. (2011) MicroRNA-138 Regulates Osteogenic Differentiation of Human Stromal (Mesenchymal) Stem Cells *in Vivo*. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 6139-6144. http://dx.doi.org/10.1073/pnas.1016758108

[158] Yang, Z., Bian, C., Zhou, H., Huang, S., Wang, S., Liao, L. and Zhao, R.C. (2011) MicroRNA Hsa-miR-138 Inhibits Adipogenic Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells through Adenovirus EID-1. *Stem Cells and Development*, **20**, 259-267. http://dx.doi.org/10.1089/scd.2010.0072

[159] Huang, J., Zhao, L., Xing, L. and Chen, D. (2010) MicroRNA-204 Regulates Runx2 Protein Expression and Mesenchymal Progenitor Cell Differentiation. *Stem Cells*, **28**, 357-364.

[160] Zhang, J.F., Fu, W.M., He, M.L., Wang, H., Wang, W.M., Yu, S.C., *et al*. (2011) MiR-637 Maintains the Balance between Adipocytes and Osteoblasts by Directly Targeting Osterix. *Molecular Biology of the Cell*, **22**, 3955-3961. http://dx.doi.org/10.1091/mbc.E11-04-0356

[161] Shan, Z.X., Lin, Q.X., Yu, X.Y., Deng, C.Y., Li, X.H., Zhang, X.C., Liu, X.Y. and Fu, Y.H. (2007) MicroRNAs Can Be Expressed in Cardiomyocyte-Like Cells Differentiated from Human Mesenchymal Stem Cells. *Journal of Southern Medical University*, **27**, 1813-1816.

[162] Liu, J.L., Jiang, L., Lin, Q.X., Deng, C.Y., Mai, L.P., Zhu, J.N., Li, X.H., Yu, X.Y., Lin, S.G. and Shan, Z.X. (2012) MicroRNA 16 Enhances Differentiation of Human Bone Marrow Mesenchymal Stem Cells in a Cardiac Niche toward Myogenic Phenotypes *in Vitro*. *Life Sciences*, **90**, 1020-1026. http://dx.doi.org/10.1016/j.lfs.2012.05.011

[163] Psaltis, P.J., Simari, R.D. and Rodriguez-Porcel, M. (2012) Emerging Roles for Integrated Imaging Modalities in Cardiovascular Cell-Based Therapeutics: A Clinical Perspective. *European Journal of Nuclear Medicine and Molecular Imaging*, **39**, 165-181. http://dx.doi.org/10.1007/s00259-011-1925-7

[164] Lunde, K., Solheim, S., Aakhus, S., Arnesen, H., Abdelnoor, M., Egeland, T., *et al*. (2006) Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute MI. *The New England Journal of Medicine*, **355**, 1199-1209. http://dx.doi.org/10.1056/NEJMoa055706

[165] Schachinger, V., Erbs, S., Elsasser, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., *et al*. (2006) Intracoronary Bone Marrow-Derived Progenitor Cells in Acute MI. *The New England Journal of Medicine*, **355**, 1210-1221. http://dx.doi.org/10.1056/NEJMoa060186

[166] Traverse, J.H., Henry, T.D., Ellis, S.G., Pepine, C.J., Willerson, J.T., Zhao, D.X.M., *et al*. (2011) Effect of Intracoronary Delivery of Autologous Bone Marrow Mononuclear Cells 2 to 3 Weeks Following Acute MI on Left Ventricular Function: The LateTIME Randomized Trial. *Journal of the American Medical Association*, **306**, 2110-2119. http://dx.doi.org/10.1001/jama.2011.1670

[167] Vulliet, P.R., Greeley, M., Halloran, S.M., MacDonald, K.A. and Kittleson, M.D. (2004) Intra-Coronary Arterial Injection of Mesenchymal Stromal Cells and Microinfarction in Dogs. *Lancet*, **363**, 783-784.

http://dx.doi.org/10.1016/S0140-6736(04)15695-X

[168] Freyman, T., Polin, G., Osman, H., Crary, J., Lu, M.M., Cheng, L., *et al*. (2006) A Quantitative, Randomized Study Evaluating Three Methods of Mesenchymal Stem Cell Delivery Following MI. *European Heart Journal*, **27**, 1114-1122. http://dx.doi.org/10.1093/eurheartj/ehi818

[169] Ly, H.Q., Hoshino, K., Pomerantseva, I., Kawase, Y., Yoneyama, R., Takewa, Y., *et al*. (2009) *In Vivo* Myocardial Distribution of Multipotent Progenitor Cells Following Intracoronary Delivery in a Swine Model of MI. *European Heart Journal*, **30**, 2861-2868. http://dx.doi.org/10.1093/eurheartj/ehp322

[170] Hou, D., Youssef, E.A., Brinton, T.J., Zhang, P., Rogers, P., Price, E.T., *et al*. (2005) Radiolabeled Cell Distribution after Intramyocardial, Intracoronary, and Interstitial Retrograde Coronary Venous Delivery: Implications for Current Clinical Trials. *Circulation*, **112**, I150-I156.

[171] Perin, E.C., Silva, G.V., Assad, J.A., Vela, D., Buja, L.M., Sousa, A.L.S., *et al*. (2008) Comparison of Intracoronary and Transendocardial Delivery of Allogeneic Mesenchymal Cells in a Canine Model of Acute MI. *Journal of Molecular and Cellular Cardiology*, **44**, 486-495. http://dx.doi.org/10.1016/j.yjmcc.2007.09.012

[172] Menasche, P., Hagege, A.A., Vilquin, J.T., Desnos, M., Abergel, E., Pouzet, B., *et al*. (2003) Autologous Skeletal Myoblast Transplantation for Severe Postinfarction Left Ventricular Dysfunction. *Journal of the American College of Cardiology*, **41**, 1078-1083. http://dx.doi.org/10.1016/S0735-1097(03)00092-5

[173] Mitchell, A.J., Sabondjian, E., Sykes, J., Deans, L., Zhu, W., Lu, X., *et al*. (2010) Comparison of Initial Cell Retention and Clearance Kinetics after Subendocardial or Subepicardial Injections of Endothelial Progenitor Cells in a Canine MI Model. *Journal of Nuclear Medicine*, **51**, 413-417. http://dx.doi.org/10.2967/jnumed.109.069732

[174] Psaltis, P., Zannettino, A., Gronthos, S. and Worthley, S. (2010) IM Navigation and Mapping for Stem Cell Delivery. *Journal of Cardiovascular Translational Research*, **3**, 135-146. http://dx.doi.org/10.1007/s12265-009-9138-1

[175] Okano, T., Yamada, N., Okuhara, M., Sakai, H. and Sakurai, Y. (1995) Mechanism of Cell Detachment from Temperature-Modulated, Hydrophilic-Hydrophobic Polymer Surfaces. *Biomaterials*, **16**, 297-303. http://dx.doi.org/10.1016/0142-9612(95)93257-E

[176] Shimizu, T., Yamato, M., Isoi, Y., Akutsu, T., Setomaru, T., Abe, K., *et al*. (2002) Fabrication of Pulsatile Cardiac Tissue Grafts Using a Novel 3-Dimensional Cell Sheet Manipulation Technique and Temperature-Responsive Cell Culture Surfaces. *Circulation Research*, **90**, e40-e48. http://dx.doi.org/10.1161/hh0302.105722

[177] Kofidis, T., Lebl, D.R., Martinez, E.C., Hoyt, G., Tanaka, M. and Robbins, R.C. (2005) Novel Injectable Bioartificial Tissue Facilitates Targeted, Less Invasive, Large-Scale Tissue Restoration on the Beating Heart after Myocardial Injury. *Circulation*, **112**, I173-I177.

[178] Zhang, Y., Thorn, S., DaSilva, J.N., Lamoureux, M., DeKemp, R.A., Beanlands, R.S., *et al*. (2008) Collagen-Based Matrices Improve the Delivery of Transplanted Circulating Progenitor Cells: Development and Demonstration by *ex Vivo* Radionuclide Cell Labeling and *in Vivo* Tracking with Positron-Emission Tomography. *Circulation*: *Cardiovascular Imaging*, **1**, 197-204. http://dx.doi.org/10.1161/circimaging.108.781120

[179] Roura, S., Bagor, J.R., Soler-Botija, C., Pujal, J.M., Garlvez-Montorn, C., Prat-Vidal, C., *et al*. (2012) Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Promote Vascular Growth *in Vivo*. *PLoS ONE*, **7**, e49447. http://dx.doi.org/10.1161/circimaging.108.781120

[180] Dawson, E., Mapili, G., Erickson, K., Taqvi, S. and Roy, K. (2008) Biomaterials for Stem Cell Differentiation. *Advanced Drug Delivery Reviews*, **60**, 215-228. http://dx.doi.org/10.1016/j.addr.2007.08.037

[181] Li, Z. and Guan, J. (2011) Hydrogels for Cardiac Tissue Engineering. *Polymers*, **3**, 740-761. http://dx.doi.org/10.3390/polym3020740

[182] Eschenhagen, T., Fink, C., Remmers, U., Scholz, H., Wattchow, J., Weil, J., *et al*. (1997) Threedimensional Reconstitution of Embryonic Cardiomyocites in a Collagen Matrix: A New Heart Muscle Model System. *The FASEB Journal*, **11**, 683-694.

[183] Morritt, A.N., Bortolotto, S.K., Dilley, R.J., Han, X., Kompa, A.R., McCombe, D., *et al*. (2007) Cardiac Tissue Engineering in an *in Vivo* Vascularized Chamber. *Circulation*, **115**, 353-360. http://dx.doi.org/10.1161/CIRCULATIONAHA.106.657379

[184] Munoz, J., Zhou, Y. and Jarrett, H.W. (2010) LG4-5 Domains of Laminin-211 Binds α-Dystroglycan to Allow Myotube Attachment and Prevent Anoikis. *Journal of Cellular Physiology*, **222**, 111-119. http://dx.doi.org/10.1002/jcp.21927

[185] Eschenhagen, T., Eder, A., Vollert, I. and Hansen, A. (2012) Physiological Aspects of Cardiac Tissue Engineering. *American Journal of Physiology*: *Heart and Circulatory Physiology*, **303**, H133-H143. http://dx.doi.org/10.1152/ajpheart.00007.2012

[186] Chachques, J.C., Trainini, J.C., Lago, N., Cortes-Morichetti, M., Schussler, O. and Carpentier, A. (2008) Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM trial): Clinical Feasibility Study. *The*

Annals of Thoracic Surgery, **85**, 901-908. http://dx.doi.org/10.1016/j.athoracsur.2007.10.052

[187] RECATABI. Regeneration of Cardiac Tissue Assisted by Bioactive Implants. http://cordis.europa.eu/project/rcn/93530_en.html

[188] Laurie, G.W., Horikoshi, S., Killen, P.D., Segui-Real, B. and Yamada, Y. (1989) *In Situ* Hybridization Reveals Temporal and Spatial Changes in Cellular Expression of mRNA for a Laminin Receptor, Laminin, and Basement Membrane (Type IV) Collagen in the Developing Kidney. *The Journal of Cell Biology*, **109**, 1351-1362. http://dx.doi.org/10.1083/jcb.109.3.1351

[189] Baldwin, H.S. (1996) Early Embryonic Vascular Development. *Cardiovascular Research*, **31**, E34-E45. http://dx.doi.org/10.1016/S0008-6363(95)00215-4

[190] Midwood, K.S., Williams, L.V. and Schwarzbauer, J.E. (2004) Tissue Repair and the Dynamics of the Extracellular Matrix. *The International Journal of Biochemistry & Cell Biology*, **36**, 1031-1037. http://dx.doi.org/10.1016/j.biocel.2003.12.003

[191] Bader, A., Schilling, T., Teebken, O.E., Brandes, G., Herden, T., Steinhoff, G., *et al*. (1998) Tissue Engineering of Heart Valves—Human Endothelial Cell Seeding of Detergent Acellularized Porcine Valves. *European Journal Cardio-Thoracic Surgery*, **14**, 279-284. http://dx.doi.org/10.1016/S1010-7940(98)00171-7

[192] Booth, C., Korossis, S.A., Wilcox, H.E., Watterson, K.G., Kearney, J.N., Fisher, J., *et al*. (2002) Tissue Engineering of Cardiac Valve Prostheses I: Development and Histological Characterization of an Acellular Porcine Scaffold. *The Journal of Heart Valve Disease*, **11**, 457-462.

[193] Korossis, S.A., Booth, C., Wilcox, H.E., Watterson, K.G., Kearney, J.N., Fisher, J., *et al*. (2002) Tissue Engineering of Cardiac Valve Prostheses II: Biomechanical Characterization of Decellularized Porcine Aortic Heart Valves. *The Journal of Heart Valve Disease*, **11**, 463-471.

[194] Dahl, S.L., Koh, J., Prabhakar, V. and Niklason, L.E. (2003) Decellularized Native and Engineered Arterial Scaffolds for Transplantation. *Cell Transplant*, **12**, 659-666. http://dx.doi.org/10.3727/000000003108747136

[195] Schmidt, C.E. and Baier, J.M. (2000) Acellular Vascular Tissues: Natural Biomaterials for Tissue Repair and Tissue Engineering. *Biomaterials*, **21**, 2215-2231. http://dx.doi.org/10.1016/S0142-9612(00)00148-4

[196] Ekser, B. and Cooper, D.K. (2010) Overcoming the Barriers to Xenotransplantation: Prospects for the Future. *Expert Review of Clinical Immunology*, **6**, 219-230. http://dx.doi.org/10.1586/eci.09.81

Abbreviations

IHD: Ischemic heart disease
MI: Myocardial infarction
ESC: Embryonic stem cells
iPSC: Induced pluripotent stem cells
BMSC: Bone marrow stem cells
HSC: Hematopoietic stem cells
EPC: Endothelial progenitor cells
MSC: Mesenchymal stem cells
SkM: Skeletal myoblasts
CSC: Cardiac stem cells
SDF-1/CXCL12: Stromal cell-derived factor-1/ C-X-C motif ligand 12
HGF: Hepatocyte growth factor
IGF-1: Insulin-like growth factor 1
bFGF: Basic fibroblast growth factor
HIF-1α: Hypoxia-inducible factor-1 alpha
VEGF: Vascular endothelial growth factor
Ang-1: Angiopoietin-1
IL-1: Interleukin-1
IL-6: Interleukin-6
PIGF: Phosphatidylinositol-glycan biosynthesis class F protein
PLAT: Tissue plasminogen activator
TNF-α: Tumor necrosis factor alpha
LVEF: Left ventricular ejection fraction
NYHA: The New York Heart Association
CCS: Canadian Cardiovascular Society
SPECT: Single-photon emission computed tomography
VO_2: Volume of oxygen
Sca-1: Stem cell antigen-1
TGF-β: Transforming growth factor beta
IM: Intramyocardial