Chapter 5
Biological Bases of Cardiac Function and the Pro-regenerative Potential of Stem Cells in the Treatment of Myocardial Disorder

Karolina Anna Bednarowicz and Maciej Kurpisz

5.1 Cardiac Function and Pathophysiology of Myocardial Infarction

The heart is one of the most important organs of the organism. It pumps and distributes the blood in a closed system of vessels throughout the whole organism, nourishing and supplying oxygen to even distant tissues.

The heart consists of four cavities: the right and left atria, and two ventricles—right and left (Fig. 5.1). The atria are connected to the ventricles, while the right and left halves of the heart are completely separated from each other. The right atrium is separated from the left by the atrial septum; the ventricles are separated by the interventricular septum. Atrioventricular canals, which are surrounded by fibrous rings, to which the valves are attached, are located between the atria and ventricles. The right atrioventricular valve (tricuspid valve) is located in the right atrioventricular canal; while in the left atrioventricular canal—left atrioventricular valve, also called the mitral valve (bicuspid valve). The valves do not allow the retraction of the blood into the atria during the contraction of ventricles.

Blood to the atria is supplied from the veins, while the beginning of the arteries takes from the ventricles. From the left ventricle exits the main artery (aorta). Its branches supply the tissues with oxygenated blood and nutrients. After passing through the capillaries, blood deoxidizes and collects carbon dioxide (from the body) and then flows into the right atrium (large circulation or systemic circulation). From the right atrium, the blood enters the right ventricle and thus through the pulmonary trunk and pulmonary arteries—to the lungs. In the lungs, the blood returns carbon dioxide, receives oxygen, and is delivered to the left atrium through the pulmonary veins (small circulation or pulmonary circulation). There is a clear

K. A. Bednarowicz · M. Kurpisz (✉)
Institute of Human Genetics Polish Academy of Science,
Strzeszynska 32, Poznan 60-479, Poland
e-mail: kurpimac@man.poznan.pl

Z. Brzozka and E. Jastrzebska (eds.), *Cardiac Cell Culture Technologies*,
https://doi.org/10.1007/978-3-319-70685-6_5

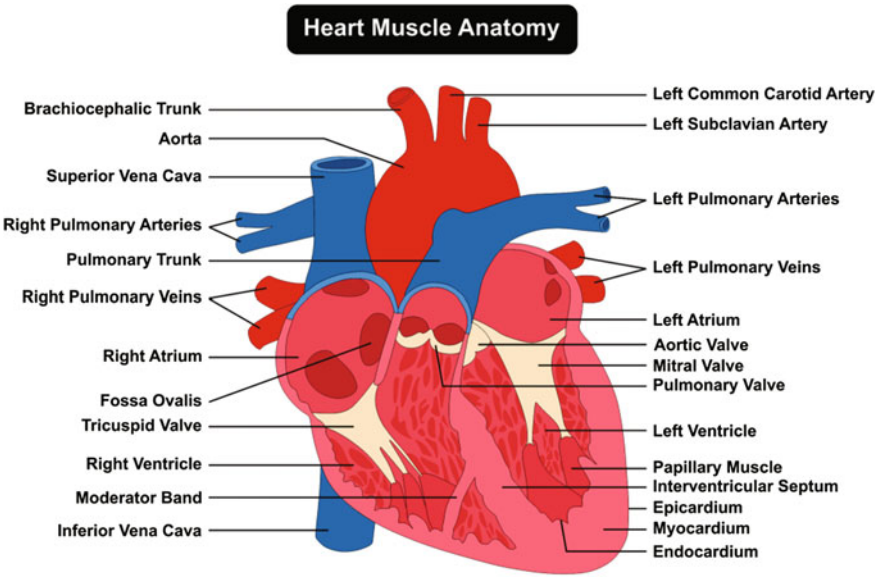


Fig. 5.1 Heart muscle anatomy. Reprinted with permission from https://www.shutterstock.com/image-illustration/human-heart-muscle-anatomy-infographic-chart-552509575

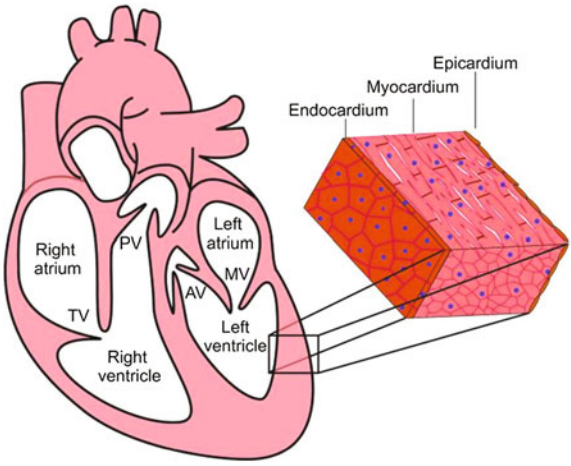


Fig. 5.2 Structure of a mammalian heart. The wall of each chamber consists of three tissue layers: endocardium, myocardium, and epicardium. *PV* pulmonary valve; *TV* tricuspid valve; *AV* atrial valve; *MV* mitral valve. Reprinted with permission from Lin et al. 2012. Copyright 2012 The Company of Biologists

division on the right half of the heart with deoxygenated blood and the left half with arterial (oxygenated) blood.

The heart wall is made up of three histological layers: the inner endocardium, middle myocardium, and outer epicardium (Fig. 5.2).

The epicardium is a thin serous membrane covering the outer surface of the myocardium along with the coronary vessels lying on its surface. At the starting point of the large vessels, the endocardium converts into outer pericardium (pericardial sac).

The endocardium is a thin, transparent membrane. It consists of a thin layer of connective tissue covered with a layer of flat epithelial cells. It covers the walls of the atria and ventricular chambers, passing directly into the membrane lining the internal surface of the vessels. The valves are made of fibrous connective tissue, covered on both sides by the endocardium.

The myocardium consists of two types of muscles. These are atrial and ventricular muscles, separated from each other by the fibrous rings surrounding the atrioventricular atrium. The myocardium is made of striated muscle tissue. It differs in its structure from skeletal muscles in that the individual cells connect the branches of neighboring cells, creating a specific muscle network with distinct histological pattern. The myocardium is made up of myogenic cells that have muscular fibrils and basic contractile elements, among others, cell filaments (Fig. 5.3). The thin filaments are formed from interconnected protein molecules—actin, and the thick filaments are built out of myosin. Thick and thin filaments are alternately arranged, with little overlap on each other. According to microscopic observations, this is visible in the form of alternating bright (actin) and dark (myosin) stripes. At half-length of the light stripe, you can see a thin, dark line (line Z). It is a membrane that divides muscle fibers into parts called sarcomers. Due to this membrane and the gap junctions, stimulating signals can be transferred from one cell to another and the cardiac muscle forms a functional syncytium. The muscle contractile phenomenon explains the "sliding" model, according to which a contraction is the result of the insertion of actin filaments between the myosin heads. This phenomenon can be observed at the level of each sarcomere, and the final effect of muscle fiber contraction is due to the sum of contractions within individual sarcomere.

Myocytes as well as the extracellular space can be distinguished in the myocardium (being the most active structural components). The space can be divided into extracellular fluid (interstitium), collagen, and fibronectin fibers. The other morphotic elements may belong to coronary vessels, i.e., endothelial cells, smooth muscle cells as well as fibroblasts underlying the myocyte layer. Myocytes represent only about 40% of the total cell population in myocardium but occupy about 75% of its volume. Extramyocyte part creates an environment, in which myocytes work. Therefore, the condition of textured extramyocytes space for cardiac function and its capacity is not less important than the myocytes themselves.

The role of the heart as a pump depends on the synchronized changes in the physical properties of the muscles. During relaxation time, the heart maintains plasticity so that blood flows into the individual cavities causing them to stretch. During stimulation this muscle generates mechanical stress and shortens, so that blood can enter the vessels, leaving the heart chambers.

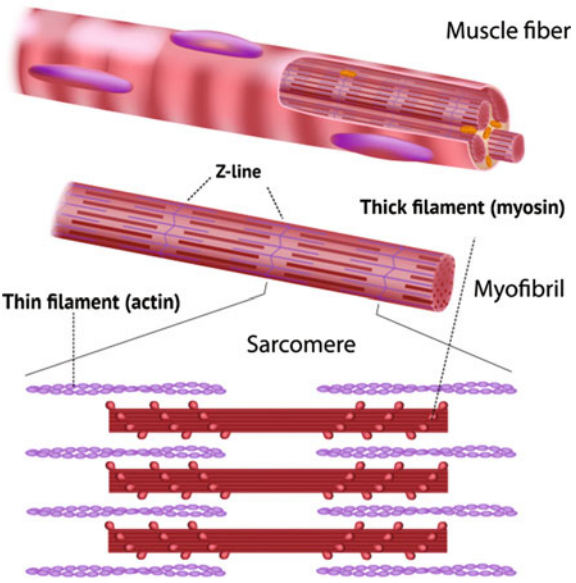


Fig. 5.3 Structure of a sarcomere. Reprinted with permission from https://www.shutterstock.com/pl/image-vector/structure-skeletal-muscle-fiber-116411524?src=5LX4lyg3Lx_7dGHE8BhYtw-1-57

The heart muscle controls its own contractions. Stimulus inducing contraction is produced in conductive heart muscle cells called Purkinje fibers (Fig. 5.4). The density as well as morphology and function of these cells is different from the other regions of the heart. They lack myofibrils and are more like smooth muscle. In these cells, there are no visible clear cross-striations. They contain more glycogen and the sarcoplasm. The cells of the conductive system stimulate themselves spontaneously and rhythmically, forming the so-called, autonomous system of the heart, in which they create contractions through depolarization. Cells of the conductive system are arranged in a very characteristic way. The sinoatrial node is the main place where synchronized stimulus arises (Keith-Flack). Stimulation propagates the first wave in the atria and then to the main chambers. In the event that the sinoatrial node is damaged, the atrioventricular one takes over as the trigger (Aschoff-Tavary). A bundle of atrioventricular (His) fibers runs from the trabecular and papillary muscles. Interruption of the connection between the atria and ventricular chambers causes on the atrioventricular block, which is manifested by the fact that the atria contract independently of the chambers.

Cardiovascular diseases (CVDs) are known to be the main cause of morbidity and mortality, particularly in highly developed countries, and are usually connected with myocardial infarction (MI). According to the World Health Organization, myocardial infarction and coronary artery disease are the reason for 29% of global mortality (Mendis et al. 2011). The predominance has been increasing constantly due to an ageing population and changes in a lifestyle.

Myocardial infarction can be a model for an acute heart ischemia. Ischemia is the result of inhibited coronary perfusion, typically because of narrowing or occlusion

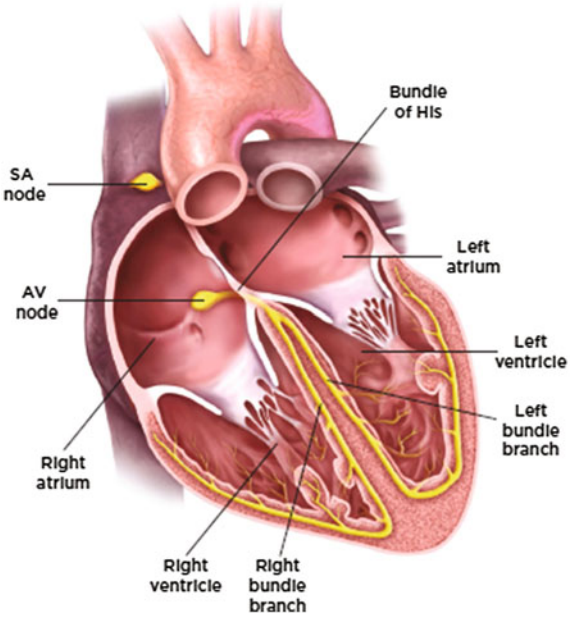


Fig. 5.4 Anatomy of the heart electrophysiological system. AV = atrioventricular; SA = sinoatrial. Reprinted with permission from Roquin 2006. Copyright 2015 Medmovie

of the coronary artery. However, human coronary collateral circulation is quite well developed. Therefore, total coronary occlusion often results in only a partial (and inhomogeneous) decrease in perfusion in the basin of the occluded artery. The accompanied decline in ATP production, proportional to the degree of perfusion limitation, increased lactic acid and cellular acidosis, decrease of myocardial contractility, and finally the death of cardiomyocytes (necrosis) may occur. The myocardial contractions may be weakened within the first few seconds of ischemia, and hypoxia is its earliest symptom. Hypoxia results in rapid acidification of heart cells, which is caused by the decomposition of ATP and the accumulation of lactic acid. Cellular acidosis is the most probable cause of early contractility disorders in ischemia, as it reduces affinity of troponin to Ca^{2+}, increases uptake, and inhibit Ca^{2+} release from the endoplasmic reticulum membrane, thus diminishing the activity of calcium channels. At a later stage of severe hypoxia (minutes, hours), loss of contractility correlates with the loss of cellular ATP and the progressive irreversible damage of cardiomyocytes. It is known that the extensive myocardial infarction reduces about 20–30% of the total heart mass (approximately 300 g), which is about 1.8–2.7 trillion cardiomyocytes.

The term "postinfarction heart remodeling" defines the structural changes in the heart following a myocardial infarction. It marks the changes at the center of the infarction zone, replacing dead myocardial tissue with connective tissue (early phase of reconstruction) as well as changes in the remaining unaffected parts of the myocardium to adapt the structures to the pathological conditions (late phase of reconstruction). The early reconstruction phase can be divided into two stages:

infarct extension associated with extension of necrosis due to the loss of cardiomyocytes in the border zone (between ischemic and healthy areas) and infarct expansion associated with passive stretching by intraventricular blood pressure and tension in the wall of the ischemic chamber. Just a few hours after the infarct, this region gets thinner, the left ventricle enlarges (becomes dilated), and its elliptical shape becomes more spherical.

Myocardial infarction is related to the interstitial fibrosis and the left ventricular (LV) dilatation, which continue to weaken cardiac efficiency and can be independent causes of morbidity and mortality after MI. LV remodeling is the consequence of overexpression of multiple factors, including angiotensin II, norepinephrine, and proinflammatory cytokines, which exert pathological effects on cardiac myocytes, non-myocyte cells, and the extracellular matrix. Current therapeutic approaches to MI (pharmacological treatment and interventional strategies) are focused on alleviation of symptoms and life extension, and they do not concentrate on the fundamental issue, which is the physical decrease (death) of cardiac viable tissue. Therefore, there is a dramatic need for new therapies that will contribute to the repair and improvement of the heart function. We shall further understand how the changes in the mechanical properties of myocardial tissue would affect the phenotype and function of cells after MI.

The most efficient therapy is the heart transplantation, but it may cause numerous problems, among others: insufficient number of donors, immunological rejection, age limitations (both of donor and recipient), complications before and after surgical intervention, and substantial medical costs. Therefore, current studies taking place around the world have been focused on stem cell-based therapies, which aim to rebuild the wasted myocardium with new functional cardiac cells. However, the results of functional stem cell therapies are limited due to multiple obstacles such as post-transplantation low cell survival, insufficient capacity of cell engraftment and their retention.

The clinical trials and experimental research attending to overpass these limitations focus on finding optimal stem cell candidates, perfect systems of cell delivery, adequate cell doses, and timing of their administration. There are two main strategies used to improve current stem cell therapy system. The first one is short term and has been focused on the pretreatment of the cells to stimulate their directed migration, retention, differentiation, and survival. The second strategy aims to ensure the optimal environment for the cells, their long-term engraftment, differentiation, and function in pathological conditions. In next section, we will summarize the latest advances on stem cell therapy and methods used to increase their efficacy for clinical application.

5.2 Stem Cells in Cardiac Regenerative Therapy; Candidates

Various types of stem cells have been included in many preclinical and clinical trials to determine their pro-regenerative ability that could be used for either direct or indirect (paracrine) effects in undertaken cell therapy. The optimal cell type should meet the postulated features: safety (no induction of immunity or tumorigenesis), pro-regenerating ability, and differentiation toward target tissues and/or organs, ease of being to be obtained with no danger of rejection and/or ethical controversy involved.

5.2.1 Embryonic Stem Cells (ESCs)

Promising candidates for cardiac pro-regenerative therapy were embryonic stem cells because of their strong ability to proliferate and differentiate into the cells from all the three germ layers: ectoderm, endoderm, and mesoderm. Nevertheless, clinical trials using these cells in humans have rarely been conducted due to bioethical issues associated with their source of origin. Other ESCs-related obstacles were possibilities for teratoma formation or immune rejection. Recently, Menasche group at Georges Pompidou European Hospital in Paris started a clinical trial (ESCORT—transplantation of human Embryonic Stem Cells-derived prOgenitors in severe heaRT failure) using pericardial flaps seeded with cardiomyocytes obtained from hESCs (Baas 2014).

5.2.2 Adult Stem Cells (ASCs)

Different populations of cells for cardiac pro-regenerative therapy have been represented by the family of adult stem cells (ASCs) including: skeletal myoblasts (SKMs), bone marrow cells (BMCs), adipose-derived stem cells (ADSCs), and endogenous cardiac stem cells (CSCs). Despite their various limitations in amount, low or variable differentiation and proliferation abilities when compared to ESCs, the efficiency and simplicity of their acquisition from the patient were the reasons that ASCs are considered to be optimal candidates for cell therapy of the heart. Moreover, the transplantation of autologous patient-derived cells eliminates ethical controversies and danger of immune rejection.

5.2.2.1 Skeletal Myoblasts (SKMs)

Mature skeletal muscle contains a reservoir of inactive and undifferentiated satellite cells, which may be transformed in a mixture of myoblasts. Myoblasts exhibit proliferative potential and can differentiate into muscle fibers regenerating damaged skeletal muscle parts. But transplanted skeletal myoblasts are mechanically and electrically detached from the host myocardium, which has been a serious obstacle for their application in cardiac pro-regenerative therapy. SKMs are one of the first cells brought into the clinical trials for heart regeneration. Small non-randomized phase I trials revealed that SKMs have a functional advantage (over the other candidates) in increasing left ventricular ejection fraction (LVEF) and improving myocardial viability. However, ventricular arrhythmias and a high loss rate of skeletal myoblasts in situ have been observed in the treated patients (Hagège et al. 2006; Siminiak et al. 2004). The first prospective randomized placebo-controlled phase II SKM trial, called MAGIC, using autologous skeletal myoblasts, showed limited or no benefits to regeneration of the postinfarction scar region (Menaschè et al. 2008). However, successive research conducted to test skeletal myoblasts as possible candidates for cardiac pro-regenerative therapy has been rather optimistic. The outcome of recent experiments demonstrated that mechanical preconditioning of transplanted skeletal myoblasts improved their interaction with recipient organ's cardiomyocytes in vivo.

5.2.2.2 Bone Marrow Cells (BMCs)

So far, autologous bone marrow cells (BMCs) have been the most commonly used for clinical therapy. The BMCs are a composition of endothelial progenitor cells (EPCs) and hematopoietic stem cells (HSCs) constituting 2–4% of BMCs, mesenchymal stem cells (MSCs) < 0.1% of BMCs, and some amounts of side cell population. The induction of two major subpopulations, HSC and MSCs, were tried to provide structural elements to myocardium. The administration of MSCs into the murine myocardium demonstrated potential to differentiate into cardiomyocytes. Also, MSCs do not possess the major histocompatibility complex (MHC) antigens of class II (HLA-DR), which allow them to be applied in allogeneic transplantations. The effect of MSCs in heart regeneration is not only based on their capacity to differentiate into cardiomyocytes but is mostly related to their paracrine activities. Mesenchymal stem cells actively secrete chemokines, growth factors, and cytoprotective cytokines, which promote proliferation, differentiation, vascularization of own cardiac progenitors cells (CPC), inhibit cardiomiocyte apoptosis and myocardial fibrosis (sometimes preventing scar formation). The mechanism of MSCs is characterized by their ability to decrease the activation of NF-κB, inhibiting the expression of TNF-α and IL-6, and increasing secretion of anti-inflammatory cytokine, IL-10.

Transplantation of autologous MSCs demonstrated an increase of left ventricular ejection fraction (LVEF), decrease of the infarct size, and reversed heart remodeling

developed due to the infarction. However, based on data from meta-analysis of 49 trials performed by Nowbar and colleagues in 2014, it was only found slight myocardial recovery after BMCs transplantation (Nowbar et al. 2014). Presently, the use of BMCs in cardiac pro-regenerative therapy has shown average to modest benefits in left ventricle (LV) function. The latest research discovered that the cooperation of human MSCs with CPC's produces a better effect on the reduction of infarction size and improvement of cardiac functions than MSCs alone (Williams et al. 2013). Finally, myocardial regeneration with the appearance of new blood vessels and new cardiomyocyte populations after the BMCs transplantation has not been reported.

5.2.2.3 Adipose-Derived Stem Cells (ADSCs)

The next population of stem cells representing therapeutic potential in cardiac pro-regenerative therapy have been adipose-derived stem cells (ADSCs). The adipose tissues mostly consist of endothelial progenitor cells and adult mesenchymal stem cells, which have been shown to differentiate into a variety of cell lineages including cardiomyocytes. The application of ADSCs can effectively improve left ventricular function in preclinical animal models of myocardial infarction. For example, transplantation of ADSCs in a postinfarction rat model increased LVEF, improved angiogenesis, and reduced myocardial fibrosis (Mazo et al. 2008). Recent research has also demonstrated that human ADSCs revealed perivascular characteristics through enhanced migration in response to platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), thus leading to increased microvascular density (neoangiogenesis) (Eng et al. 2016). Additionally, ADSCs using their paracrine mechanisms can trigger native cardiac resident stem or progenitor cells (CPC) to improve heart function. Several attempts ensured that ADSCs containing a population of adult multipotent mesenchymal stem cells can improve left ventricular function but especially by the paracrine action of growth factor-mediated effects. Because the presence of cardiomyocytes within the MSC transplant seems to be rare, it is believed that MSCs control the angiogenesis mostly through paracrine pathways.

The preliminary results of clinical trials assumed that ADSCs may also improve maximal oxygen consumption and ensure stabilization of infarct size. The positive impact of ADSCs with increased cardiovascular cytoprotection and angiogenesis is believed to be associated with their multipotency and ability to secrete growth factors. Because of the potential large amount of adipose tissue in the body and the fact that ADSCs can be simply and safely obtained, this population of adult stem cells become another accessible great cell source for future cardiovascular therapy.

5.2.2.4 Cardiac Stem/Progenitor Cells (CS/PCs)

Since 2003, it has been known that the adult heart contains a population of stem/progenitor cells that can be engaged in its own regeneration process. These cells are characterized by multipotent, self-renewed, and clonogenic ability, and develop to mature cardiomyocytes, endothelial cells, and smooth muscle cells. Cardiac stem/progenitor cells were initially obtained from the adult rat heart and described as expressing the tyrosine kinase receptor c-kit and lacking any markers of hematopoietic lineage (Beltrami et al. 2003). CS/PCs have been identified in humans as well (Bearzi et al. 2007), which initiated a new direction in clinical trials. Various populations of CS/PCs have been identified and phenotyped including c-kit +, sca-1+, Is11+, cardiospheres, side cell population, epicardial, and SSEA-1+ progenitor cells. But human c-kit-positive cardiac cells are the best known and the most intensively studied CS/PC population. Some research documented the potential of CS/PCs to enhance regeneration and improvement in both LV function and structure, and heart remodeling inhibition in different animal models of post-MI cardiomyopathy (Li et al. 2011a; Linke et al. 2005; Tang et al. 2010). The results of the first clinical trial (SCIPIO) are similar to that ones obtained with the preclinical studies and assumed that intracoronary infusion of autologous CSCs provided a decrease of infarct size and an improvement of LV systolic function (Bolli et al. 2011).

Cardiosphere cardiac stem cells have also been described as a self-renewed, multipotent, and clonogenic cell subpopulation with the pro-regenerative potential to the myocardium in vivo. In the first clinical trial performed with autologous cardiosphere-derived cells (CADUCEUS), beneficial effects have been obtained (Makkar et al. 2012). The latest studies, based on this trial, but with the use of cardiosphere-derived cells from an allogenic source (ALLSTAR), resulted also in promising effects after phase I clinical trials. This led to the start of the randomized, double-blind, placebo-controlled phase 2 clinical trials to further estimate safety and efficacy of allogenic cardiosphere-derived cells in decreasing scar size in a postinfarction heart (Chakravarty et al. 2017).

The obstacle associated with CS/PCs is mainly caused by their poor retention and cell engraftment efficacy. Unfortunately, less than 1% of engrafted cells can be indentified at 4 weeks after transplantation, whereas most of the initially retained cells die due to inflammatory reaction, apoptosis, or hypoxia.

Several studies concentrated on comparing the ability of different myocardial adult stem cells types to repair damaged region. The highest rated was CSCs because of their ability to extensively produce paracrine factors, the greatest functional benefits, and the lowest dose to obtain therapeutic effects. But still, as already mentioned, the combined therapy with both CSCs and MSCs showed better results than any of these cell populations alone.

Summarizing, clinical trials focused on the postinfarction heart and based on BMSCs, CSCs, and ADSCs showed heart function improvement, also increasing the left ventricular ejection fraction and finally extending the life span of individual. However, this was mainly due to paracrine effects instead of direct differentiation

into cardiomyocytes. The new strategies, however, demonstrated another line of research when using cardiopoietic driven cell populations out of bone marrow-derived stem cells. Such manipulation could modify traditional "paracrine procedures" into more structurally oriented cells of cardiac origin (CHART-1 design) (Bartunek et al. 2016). However, conclusive documentation of such cell modification is lacking. First, genotypic and phenotypic cell characterizations were made on the grounds of MEFc nuclear/cytosol ratio, and long-term cell structural conversion has not been yet confirmed. Clinical endpoints were established at a six-month follow-up time frame, typical for "paracrine procedures" heart improvement. Further documentation is immediately needed including transplanted cell imaging to document the proof of concept.

5.2.3 Induced Pluripotent Stem Cells (IPSCs)

A pluripotent stem cell population (iPSCs) was created in 2006 by Takahashi and Yamanaka overexpressing four specific transcription factors: OCT3/4, Sox2, c-Myc, and Klf4 (so-called Yamanaka factors) in mouse fibroblasts. In this way, cells obtained revealed overexpression similar to ESC marker genes and morphology, and growth properties similar to embryonic stem cells, thus creating an opportunity to use them for regenerative medicine. Also, it is known that iPSCs differ from ESCs in respect to intensity in gene expression and DNA methylation scheme. Appeared also a concept of somatic cell reprogramming making autologous pluripotent stem cells that can be simply obtained in the laboratory, individually tailored and differentiated to specific precursors in order to eliminate rejection from the immune system of individual after aimed cellular therapy.

iPSCs may differentiate into three types of cardiomyocytes (atrial, nodal, and ventricular) with similar characteristics to "native" cardiomyocytes. No significant differences have been noticed between cardiomyocytes derived from either ESCs or iPSCs.

Most iPSCs have been obtained inducing overexpression of specific transcription factors using retroviral and lentiviral vectors (Takahashi et al. 2007), associated with the insertion of transgenes into the host cell genome. Some transcription factors, however, which generate iPSCs (c-Myc and Klf4), have been defined as oncogenes. To solve the other problems associated with induction of pluripotent stem cells, which among others is the low efficiency of iPSC output (0.001–2%), low kinetics, and no absolute safety in reprogramming, a lot of methodological corrections have been invented. For example, the reprogramming factors using Sendai viral system (SeV), established on a negative-strand RNA virus with no possibility of being stably inserted into the host cell genome, decreased the danger of random integration. Also, different methods have been expanded to fulfill the clinical application requirements through, e.g., virus-free iPSCs reprogramming (Kaji et al. 2009), piggyBac transposons (Woltjen et al. 2009), recombinant proteins (Kim et al. 2009), episomes (Yu et al. 2009), synthetic mRNAs (Warren et al. 2010),

and microRNAs (Miyoshi et al. 2011) or minicircle systems (Jia et al. 2010). Reprogramming fibroblasts to iPSCs with modified mRNA exhibited approximately 1.1% efficacy, while lentiviral transduction with miR302/367 showed 10%. Furthermore, chemical modifications have been considered as the method of choice to improve the iPSC generation (CiPSCs). Numerous small chemical molecules have been declared to replace traditional reprogramming factors in order to improve safe iPSC generation. Thus, the disadvantages of current methods could be avoided, such as the risk of tumorigenesis from random genomic integration or overexpression of harmful oncogenes.

It has been mentioned that iPSCs epigenetic characteristics, among others, histone modification, and CG methylation, may influence the pattern of the iPSC-derived cells. Since the iPSCs reprogramming from somatic cell involves global epigenetic remodeling, the efficient reprogramming process relies on chromatin modifying enzymes, for instance: inhibitors of DNA methyltransferase (e.g., 5-azacytidine) and histone deacetylase (HDAC) inhibitors (Mikkelsen et al. 2008; Huangfu et al. 2008a). For example, Valproic acid (VPA), an HDAC inhibitor, increases reprogramming efficacy by more than 100-fold. Also, VPA allows the induction of pluripotent stem cells with only Oct4 and Sox2, without using oncogene, c-Myc (Huangfu et al. 2008b).

New approaches to genetically modify human iPS cells at "safe harbor" places in the genome have been expanded, to reduce the perturbation resulting from neighboring gene expression. Safety standards made to estimate potential safe harbors are as following: a distance of at least 50 kb from the 5' end of any gene, and at least 300 kb from any cancer-related gene and microRNA, and a position outside of transcriptional units and ultraconserved regions (Papapetrou et al. 2011). Cerbini and colleagues reported observations of their latest experiments, in which they created transcription activator-like effector nucleases (TALENs) targeting the safe-harbor like gene CLYBL. It has been revealed that a target for TALEN–enhanced integrative gene-transfer, situated in intron 2 of the Citrate Lyase Beta-Like (CLYBL) gene, assured up to tenfold higher transgene expression compared to commonly used AAVS1 (Cerbini et al. 2015).

Zhang and coworkers developed a novel method of isolating a cell population with great proliferation ability and cardiovascular differentiation capacity after successful myogenic differentiation from mouse fibroblasts. They have been called, ieCPCs—induced expandable cardiovascular progenitor cells, which can differentiate into functional cardiomyocytes, endothelial cells, and smooth muscle cells. Moreover, after injection of ieCPCs into MI mouse heart the cells started to differentiate into all three subpopulations of the heart improving cardiac functions. This suggests, that ieCPCs could constitute a novel approach in pro-regenerative strategy to the heart (Zhang et al. 2016). Summary of cardiac regenerative medicine products is shown in Fig. 5.5.

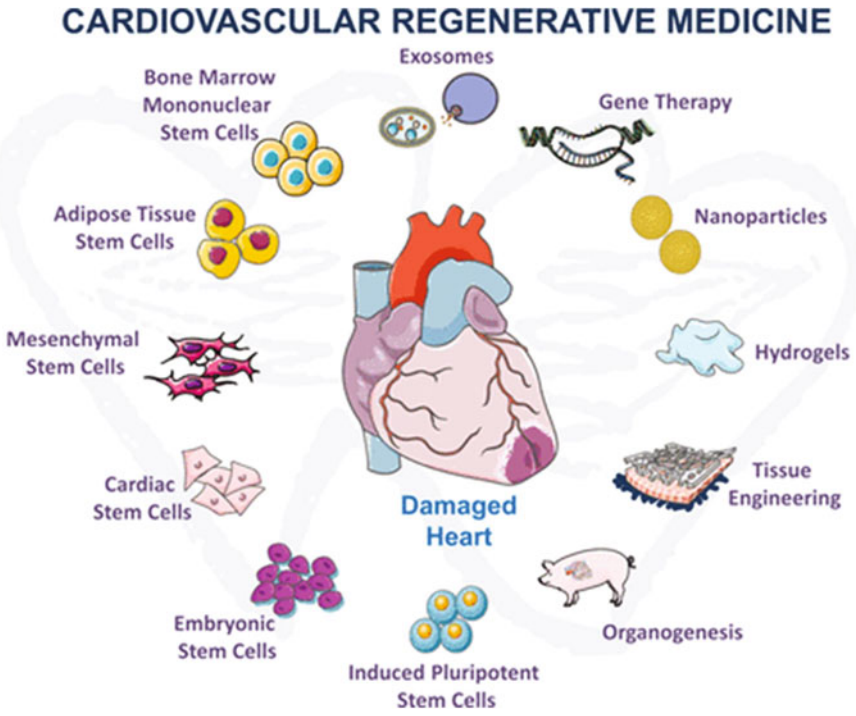


Fig. 5.5 Summary of cardiac regenerative medicine products. Reprinted with permission from Climent et al. 2016. Available from: http://circres.ahajournals.org/content/119/3/409

5.3 Novel Strategies in Cardiac Cell Therapy with Use of the Adult Stem Cells

The major issue in the transplantation of stem cells is an insufficient number of cells which may be retained in desired regions of intervention and overall low survival rate in the recipient organ. It has been proved that more than 90% of the stem cells disappear within 24 h after transplantation; this is associated with hypoxia, local ischemia, and pro-apoptotic inflammatory conditions. Furthermore, it has been documented that two hours after injection just 1.3–2.6% stem cells were retained in the myocardium and after 20 h about only 1.49% of the initial cell population was reported.

Researchers revealed that heart failure therapy based on stem cells operates within general mechanisms connected with immune surveillance, apoptosis, and angiogenesis. To address these issues, new approaches to improve the present strategies have been expanded, e.g., different methods of cells preconditioning before transplantation into infarcted myocardium.

5.3.1 Cell Preconditioning

It is known that mesenchymal stem cells cultured in hypoxic conditions exhibited increased expression of pro-angiogenic and pro-survival factors, e.g., angiopoietin-1, vascular endothelial growth factor, erythropoietin, hypoxia-inducible factor 1, Flk-1, Bcl-2, and Bcl-xL (Hu et al. 2008).

Since the time that paracrine factors were discovered to inhibit apoptosis, promote angiogenesis, and myocyte proliferation, they have been widely used to improve the therapeutic effect of stem cells transplanted into damaged myocardium. Some benefits were gained after MSCs preconditioning with cytokines and growth factors (e.g., SDF-1α), which inhibited cells apoptosis, increased their survival, engraftment, vascular density, and cooperated with SDF/CXCR4 signaling pathway improving myocardial function (Pasha et al. 2008). Recent studies reported that the preconditioning of BMSCs using the hypoxia—inducible factor 1α (HIF-1α) prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG), increased their life span and paracrine function (Liu et al. 2014), while pretreatment of adipose-derived stem cells (ADSCs) using 5-azacytidine enhanced cardiogenic differentiation (Ravichandran et al. 2013).

Similarly, preconditioning using physical factors, among others: magnetic fields, mechanical stress, and low O_2 pre-culture, has been involved in cardiac stem cell preparation. Mechanical stress inhibits the proliferation of CSCs but supports production of angiogenic factors and inflammatory cytokines (Kurazumi et al. 2011). A magnetic field was used to lead cardiac—specific differentiation into adult cardiac progenitor cells (Gaetani et al. 2009), while low O_2 conditions increased cells propagation and quality (viability) (Li et al. 2011a).

5.3.2 Genetic Stem Cell Modifications

Another strategy to improve the therapeutic ability of stem cells and enhance their chance of survival, engraftment, homing, and pro-regenerative efficiency may be a combination of the cell and gene therapies. Genetically modified stem cells have been designed to reduce the pro-apoptotic pressure after transplantation. The survival rate of MCSs has been extended after the cell modification with the anti-apoptotic gene *Bcl-2*. The results have shown the reduction of the infarct size and progress in cardiac functioning (Li et al. 2007). Improved cell survival has also been documented with MSCs transduced with heme oxygenase (*Hmox-1*), connexin43 (*Cx43*), and *Akt*, known as protein kinase B (*PKB*). Similarly, the transplantation of autologous ADSCs linked with *Hmox-1* transduction indicated improved heart function and inhibition of postinfarcted myocardium remodeling (Li and Verma 2002). Methods of genetic engineering have also been adapted to increase CPCs cell survival and proliferation. For example, overexpression of *Pim-1* kinase caused a renewal of phenotypic and functional stem cell characteristics

probably by increasing telomerase activity and extending telomere lengths (Mohsin et al. 2013). Additionally, genes responsible for the promotion of angiogenesis have also been modified, e.g., angiopoietin-1 (*Ang-1*), the vascular endothelial growth factor (VEGF), granulocyte chemotactic protein (*GCP-2*). The results obtained after MSCs transplantations in a mouse/rat model revealed significant progress in angiogenesis, cell survival index, cardiac function, and reduction of infarct size (Hua et al. 2014; Liu et al. 2012; Kim et al. 2012). Also, increased pro-angiogenic effects have been demonstrated in studies with CPCs after VEGF modification (Zhang et al. 2012) or those with high expressed Ang-1 (Zeng et al. 2012). Furthermore, human ADSCs transduced with the VEGF gene (Rehman et al. 2004) and hepatocyte growth factor (*HGF*) (Zhu et al. 2009) brought an improvement in angiogenesis and cardiac function.

Also, it has been reported that the overexpression of protein kinase G1 α (*PKG1α*) extended MSCs survival and their angiomyogenic ability in damaged myocardium. The heart function and cell survival rate were increased, but simultaneously the levels of paracrine factors and anti-apoptotic proteins (Akt, GSK3β, and Bcl-2) were raised (Wang et al. 2013).

It has been documented that genetically modified stem cells, present some problems associated with migration, hypoxic conditions, and immune response from the recipient organ. However, genetically modified MSCs by hypoxia-inducible factor-1α (*HIF-1α*) overexpression revealed an improved angiogenesis under hypoxic conditions and developed better autocrine and paracrine activities (Razban et al. 2012). Besides, intramyocardial transfection of *HIF-1α* and transplantation of genetically modified MSCs in an experimental model of rat MI showed improved angiogenesis, engraftment, cell survival, and inhibited apoptosis (Huang et al. 2014). Trying to reduce the obstacles associated with migration of the transplanted MSCs from the postinfarcted region, attempts at delivery of stromal derived factor-1α (SDF-1α), a pro-angiogenic and cardiomyocyte protective protein were initiated (Askari et al. 2003). Moreover, SDF-1α stopped hypoxia/SD-induced MSCs apoptosis through ERK1/and 2 PI3 K/Akt signaling pathways (Yin et al. 2011). It has also been shown that the transfection of mesenchymal stem cells with tumor necrosis factor receptor (*TNFR*) gene resulted in the anti-apoptotic and anti-inflammatory effects after transplantation into MI region (Bao et al. 2008). Besides, it has been documented that the enhancement of CSCs recruitment, engraftment, and reduction of infarct size was regulated through the CXCR4/PI3 K signaling pathway (Wang et al. 2012). Some research also revealed that CSCs differentiation and engraftment potential were increased simply after fibroblast growth factor (bFGF) introduction (Takehara et al. 2008).

Recently, some studies have demonstrated a new method of reprogramming mouse fibroblasts into cardiac cells using a small molecule cocktail (containing ALK4/5/6 inhibitor, GSK3 inhibitor, parnate, and forskolin) and leaving only one transcription factor—Oct4, without entering into the pluripotent state. These conditions have been very efficient and led to the creation of spontaneously beating cardiomyocytes from fibroblasts presenting heart ventricle phenotype. The introduction of small molecules reduced the dependence on genetically manipulated

transcription factors when transforming into a total pharmacological induction of cardiomyocyte development (Wang 2014).

Stem cell's ability to contract, respond, and integrate with their surrounding electrophysiological environment is critical to their reprogramming into cardiomyocytes. Vunjak-Novakovic and colleagues assumed that biomimetic electrical signals may control the internal beating properties of cardiomyocytes. They performed an electrical preconditioning of human stem cells-derived cardiomyocytes in three-dimensional (3D) culture and observed an enhancement of cardiomyocyte maturation, modification of their automaticity and increasing connexin expression, and sarcomeric pattern. Cardiomyocytes adjusted their autonomous beating rate to the frequency at which they were stimulated. This rate-adaptive action was long lasting and transferable to the adjacent cardiomyocytes. Such an electrical preconditioning brings an optimistic prospect of the implication for cell-based decrease of arrhythmia during heart regeneration (Eng et al. 2016).

5.3.3 MicroRNAs/Exosomes

It is assumed that both normal and dysfunctional cardiac mechanisms, e.g., apoptosis, myocardial fibrosis, myocyte hypertrophy, and ventricular dilation are regulated by microRNAs. The latest discovery has suggested that miR-133 and miR-1 are the main regulators of cardiac hypertrophy (Carè et al. 2007), whereas miR-499 supports differentiation of CSCs into mechanically integrated cardiomyocytes (Hosoda et al. 2011), implicating their potential therapeutic use in cardiology. Novel studies described let-7 family as the most highly upregulated family of microRNAs in cardiac maturation. Overexpression of let-7 in human embryonic stem cells-derived cardiomyocytes increased cell size, maturation of the cells, sarcomere length and contractility (Kuppusamy et al. 2015).

Another mechanism adapting the therapeutic potential of MSCs concerns the function of exosomes. They are used as vectors for miRNAs communication between cells. MicroRNA transferred via exosomes can silence compatible mRNA in target cells (Stroorvogel 2012). MSCs-derived exosomes play an important role in therapy by many anti-apoptotic miRNAs (e.g., miR-221), which initiate cell survival signaling pathway. Latest studies have simply reported that exosomal miR-221 inhibit the expression of p53 upregulated modulator of apoptosis (PUMA). Since PUMA showed an interaction with BCL-xL and p53, and activation of pro-apoptotic proteins, its inhibition by miR-221 improved CMCs survival (Yu et al. 2013). Further, last findings indicated the possibility to use the exosomes from MSCs in a few pro-regenerative attempts such as: neovascularization, anti-cardiac remodeling, and anti-inflammatory effects (Arslan et al. 2013; Salomon et al. 2013).

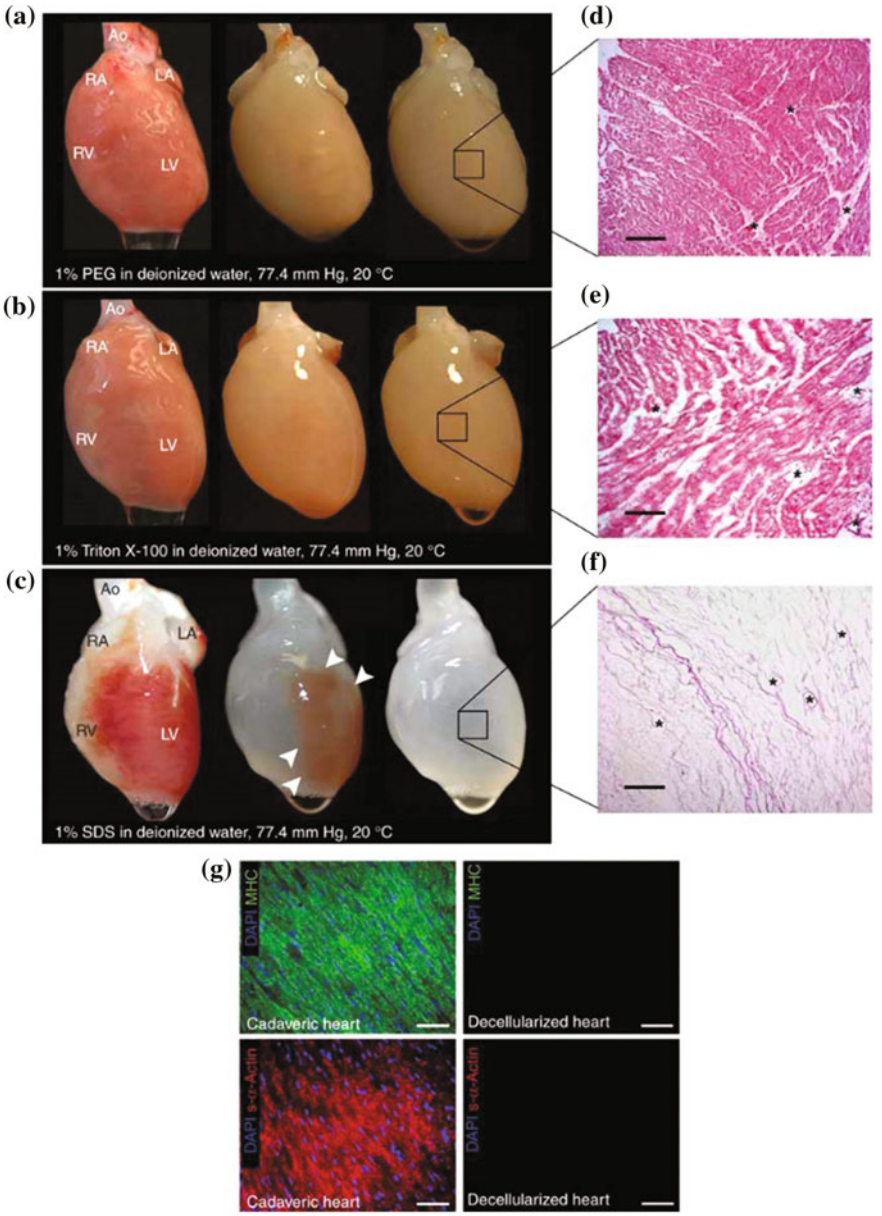
5.3.4 Drug Administration

Application of drug therapy after stem cell implantation has been tested to improve implant viability. The combined therapy with both MSCs and drugs resulted in the improvement of therapeutic effects after cell transplantation toward damaged myocardium. Statins, among others, lovastatin and rosuvastatin, are considered to have cytoprotective properties against hypoxia and serum deprivation-stimulated apoptosis via PI3 K/Akt and MEK/ERK1/2 pathways (Xu et al. 2008; Zhang et al. 2013). Also, rosuvastatin inhibited the pro-apoptotic murine proteins Bim and Bax and activated the anti-apoptotic proteins Bcl-xL, Bcl-2, and paracrine effects of MSCs (Zhang et al. 2013). Additionally, trimetazidine (TMZ) has also been described as having a protective influence on MSCs H/SD-induced apoptosis via Akt pathway as well as increasing the paracrine effect of MSCs (Gong et al. 2014). The latest research has also shown that the impact of the ASCs therapy for myocardial infarction is strengthened when the treatment is combined with cyclosporine A nanoparticles (CsA-NP) (Yin et al. 2014). Similarly, usage of 17β-estradiol (E2) increased CSCs therapeutic potential (Wang et al. 2014).

NF-κB is a transcription factor, which plays a role in the pathogenesis of heart failure activating fibrotic and inflammatory reactions (Stancovski and Baltimore 1997; Li et al. 2011b; Frangogiannis et al. 2002). The results have shown that the blockade of NF-κB action with its inhibitor decreased cell mortality and inhibited the LV remodeling. Furthermore, adapting the phosphorylation inhibitor of the IκB (the inhibitor of the NF-κB), IMD-0354 (IMD), the number of accumulated inflammatory cells in the infarcted heart regions decreased and the expression of chemokines and proinflammatory cytokines was reduced, with concomitant suppression of myocardial fibrosis (Onai et al. 2007).

5.4 Nanotechnology and Biodegradable 3D Scaffolds

A different issue in cardiac cell therapy is the stem cells injected directly into the myocardium and their migration to other distant organs. It has been reported that most of cells injected by intracoronary infusion cumulate in the border zone, not in the infarction region, whereas retention of cells within myocardium is critical for therapeutic effect. The eventual goal of tissue engineering is to replace or support infarcted regions by the transplantation of a mixture containing cells with biodegradable biomaterial scaffolds. The selection of the proper biomaterial for a scaffold that matches the conditions of the “native” microenvironment of cardiac tissue is essential. It should ensure adequate cell–material interactions and above all proper cell adhesion but not affecting the cell proliferation, differentiation, and maturation. Great progress in biomaterials production, which shows both structural


(a)
Ao
RA
LA
RV
LV
1% PEG in deionized water, 77.4 mm Hg, 20 °C
(b)
Ao
RA
LA
RV
LV
1% Triton X-100 in deionized water, 77.4 mm Hg, 20 °C
(c)
Ao
RA
LA
RV
LV
1% SDS in deionized water, 77.4 mm Hg, 20 °C
(d)
(e)
(f)
(g)
DAPI MHC
Cadaveric heart
DAPI MHC
Decellularized heart
DAPI α-cr-Actin
Cadaveric heart
DAPI α-cr-Actin
Decellularized heart

◂**Fig. 5.6** Perfusion decellularization of whole rat hearts. **a–c** Photographs of cadaveric rat hearts mounted on a Langendorff apparatus. Ao, aorta; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Retrograde perfusion of cadaveric rat heart using PEG (**a**), Triton-X-100 (**b**) or SDS (**c**) over 12 h. The heart becomes more translucent as cellular material is washed out from the right ventricle, then the atria and finally the left ventricle. **d, e** Corresponding H&E staining of thin sections from LV of rat hearts perfused with PEG (**d**) or Triton-X-100 (**e**), showing incomplete decellularization. Hearts treated with PEG or Triton-X-100 retained nuclei and myofibers. Scale bars, 200 mum. **f** H&E staining of thin section of SDS-treated heart showing no intact cells or nuclei. Scale bar, 200 mum. All three protocols maintain large vasculature conduits (black asterisks). **g** Immunofluorescent staining of cadaveric and SDS-decellularized rat heart thin sections showing the presence or absence of DAPI-positive nuclei (purple), cardiac alpha-myosin heavy chain (green), or sarcomeric alpha-actin (red). Nuclei and contractile proteins were not detected in decellularized constructs. Scale bars, 50 mm. Reprinted with permission from Ott et al. 2008. Copyright 2008 Nature Publishing Group

and functional features like extracellular matrices, can be seen. There are two main groups of tissue constructs manufactured for cardiac therapy destination: scaffolds (based on collagen, fibrin, matrix synthetic polymers, and decellularized heart) (Fig. 5.6) and scaffold-free (cell sheets and cell aggregation technologies). There is an increasing number of evidences that nanotissue engineering is a great improvement of cellular cardiomyoplasty (Bearzi et al. 2014; Cortes-Morichetti et al. 2007; Dai et al. 2009; Maureira et al. 2012; Ott et al. 2008; Sun et al. 2014).

Different models of 3D myocardial tissues have been designed by seeding cardiomyocytes into alginate, collagen, fibrin, or synthetic polymers, e.g., poly (glycolic acid) scaffolds. The transplantation of previously invented three-dimensional myocardial tissue using cardiomyocytes and 3D porous alginate scaffolds demonstrated some positive effects. Leor and colleagues have announced nearly fully vanishing scaffold and integration into the host organ with promising recovery of heart functions, such as the decrease of LV dilatation, the improvement of LV contractility, and increased neovascularization (Leor et al. 2000).

Certain structural modifications within the extracellular myocardial matrix have been observed in the infarcted regions of myocardium. The amount of collagen type I declines from 80 to 40%. It has been defined that the collagen matrix shows some characteristics in common with cardiac tissue; therefore, it was used as a supplying vehicle inhibiting the relocation of implanted MSCs (Fig. 5.7). Much of research so far has proven that a collagen tissue patch covered with MSCs inhibits the heart remodeling process and improves myocardial function (Chachques et al. 2007; Schussler et al. 2010; Vu et al. 2012).

The studies of a rat MI model revealed that autologous mesenchymal stem cells (MSCs) placed on a collagen-1 scaffold increased perfusion and reduced an infarct size with concomitant improvement in ventricular wall thickness and promotion of angiogenesis (Maureira et al. 2012). Another collagen model helped the vascular endothelial growth factor (VEGF) distribution in genetically modified skeletal myoblasts, which improved vascularization of the damaged myocardium (Lu et al. 2001). Human embryonic stem cells and human-induced pluripotent stem

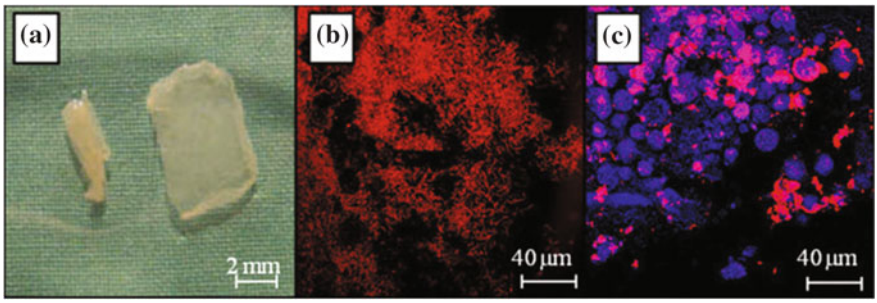


Fig. 5.7 MSC-patch characterization macroscopic view of 2 weeks in vitro cultured 3D-MSC patch (**a**), Collagen structure revealed by near-infrared and reflectance confocal microscopy (**b**), Multiphoton microscopic images of MSCs seeded in collagen patch showing positive staining for α-smooth muscle actinin (pink); the nucleus was counterstained by DAPI (blue) (**c**). Reprinted with permission from Maureira et al. 2012. Open Access—the Creative Commons Attribution License

cell-derived cardiomyocytes have also been used in a three-dimensional collagen matrix (Tulloch et al. 2011).

Fibrin patches created using both fibrinogen and thrombin with either MSCs or hESC-VCs (human embryonic stem cell-derived vascular cells) have been studied in a porcine myocardial infarction model (Liu et al. 2004; Xiong et al. 2013). Sun and colleagues also performed a test in a rat MI model, discovering that fibrin seeded with adipose-derived mesenchymal stem cells (ADMSCs) showed an improvement of left ventricle (LV) activity and reduction of LV remodeling when compared with the ADMSCs only (Sun et al. 2014).

Polyglycolic acid (PGA) (Carrier et al. 1999), epsilon-caprolactone/L-lactide (PCLA) (Matsubayashi et al. 2003; Mazo et al. 2008), poly (glycerol-sebacate) (PGS) (Marsano et al. 2010), and polyglycolic acid cloth (PGAC) (Fukuhara et al. 2005) are synthetic polymers, which have been tested in cardiac tissue engineering. The results of research with a different population of cells (BMCs, MSCs, vascular smooth muscle cells (SMCs), cardiomyocytes) conducted in various animal models have been promising for future cardiac applications. The latest results received in a mouse MI model, with both a poly (ethylene glycol)–fibrinogen (PF) scaffold and two kinds of iPSCs: MiPS (iPS cells created to secrete matrix metalloproteinase 9-MMP9) and PiPS (iPS cells made to secrete placental growth factor-PlGF), revealed an improvement in revascularization and hemodynamic parameters compared with native cells alone (Bearzi et al. 2014).

The most important benefit of three-dimensional scaffolds is its easy construction, but an emphasis has been put on the connection between the stiffness of the material and the retractile ability of the construct. It has been said that the cells stretched on scaffolds had a more mature phenotype, e.g., extended elongation, improved gap junction expression, and greater contractility. Improved survival and engraftment of implanted cells within a stretched construct have been demonstrated in a rat MI model (Mihic et al. 2014).

5.5 Whole Heart Reconstruction

Different types of putative application in the cardiac regeneration are the 3D extracellular matrix scaffolds. This model applies decellularized animal organs with a combined vascular system and extracellular matrix, e.g., three-dimensional myocardial tissue was created by reseeding cardiomyocytes into decellularized rat heart (Ott et al. 2008). The presence of structural and functional molecules in the extracellular matrix such as glycosaminoglycans, collagen, elastin, fibronectin, laminin, and vitronectin has been documented. Furthermore, it has been reported that the cardiac extracellular matrix (ECM) features, which change with time after MI, may have an influence on cardiac differentiation of implanted stem cells. The 3D extracellular matrix scaffolds as a stencil for organ reconstruction applying recellularization is believed to enhance the function and phenotype of the cells. Scaffold material obtained by decellularization is to be tested to ensure that it maintains, e.g., its integrity, bioactivity, vascular, lymphatic, and neuronal systems. Since ECM is considered to be biodegradable and does not cause an undesirable response from the host immune system, it seems to meet the requirements of the perfect biomaterial for the tissue engineering. It has been reported that the growth factors from ECM degradation, such as bFGF, VEGF, positively affect the ability for recruitment and proliferation of the cells seeded in the bioscaffold (Crapo et al. 2012; Reing et al. 2009).

The latest reports have announced that there is a chance that the 3D extracellular matrix scaffolds may support the heart muscle tissue that could recover physiological cardiac functions. Many ECM scaffold preparations (including the method of decellularization and implantation) have been examined to improve the release of growth factors and to stop the immune rejection due to cell transplantation. The outcomes of this novel research demonstrated great improvement in therapeutic effects when MSCs had been preconditioned with transforming growth factor-β (TGF-β) (Godier-Furnemont et al. 2011).

5.6 Tissue Engineering

5.6.1 Injectable Systems

The main problem of cell seeding regards the irregular distribution of cells within the scaffold. The inflexible components of the matrix isolate cardiomyocytes from each other. Therefore, a semiliquid matrix shows significant benefits over the inflexible materials, which have presented some troubles with the continuation of the myocardial structure, synchronization of the contraction, creation of vascular system, and signaling transfer. This issue has been solved by Zimmermann and colleagues, who created a combination of the cells with the soluble hydrogel of collagen type I and extracellular matrix protein (Matrigel). An electrical connection

with the host myocardium and improvement of heart function without arrhythmia has been observed (Zimmermann et al. 2002). Others who injected both stem cells and Matrigel into an infarcted heart reported improved heart geometry and function (Kofidis et al. 2005). Injectable scaffolds are considered to present some extra features, e.g., easy and minimal invasive delivery to the damaged myocardium. Matrigel is obtained from mouse sarcoma cells; therefore, the restriction of its application in clinical therapy stems from the fact that Matrigel is not tissue-specific, and it can be also associated with the high risk of tumor formation (Albini et al. 1992).

Different injectable materials studied for cardiac regenerative therapy are hydrogels based on N-isopropylacrylamide or poly (ethylene glycol) (PEG) (Rizzi et al. 2006). Some eventual obstacles of injectable materials have been observed, and they include poor mechanical support for the MI regions and the risk of blocking the blood circulation in the recipient organ.

Interesting studies have recently been reported by team of Black and colleagues. They created a hydrogel platform based on naturally derived silk fibroin containing cardiac tissue-derived extracellular matrix (cECM). These hydrogels demonstrated variable mechanical features and an adjustable rate of stiffness, which provided enhancement for cardiac fibroblast cell growth and viability throughout the in vitro culture. A cECM application increased the expression of integrins, showing an integration of cardiac fibroblasts with the cECM in the hydrogel and provoking an endogenous cell influx and cell ingrowth. These results revealed the possibility for a novel approach to more physiological constructs with a strong ability to replace healthy and injured tissue which can be also used for cardiac repair therapy (Stoppel et al. 2016).

5.6.2 Cell Sheet Engineering

Problems with scaffolds like the acute immune system response during their biodegradation and unwanted cell migration have brought about a new three-dimensional technological development. In opposition to 3D biodegradable scaffolds, a new technology, called cell sheet engineering (without scaffolds), has begun due to the invention of poly (N-isopropyl acrylamide) (PIPAAm) (Okano et al. 1993).

Shimizu et al. (2003) demonstrated that the layered cardiomyocyte sheets in vivo showed a prolonged survival rate, macroscopic pulsation, and typical construction for native heart tissue. The implantation of "layered cardiomyocyte" has been tested with cardiomyocytes from various sources (ESCs, iPSCs), and promising results were obtained (Lee et al. 2012; Stevens et al. 2009).

Sekiya and colleagues reported that cardiac cell sheets induced gene expression associated with angiogenesis; therefore, their implantation caused a neovascularization of the myocardium (Sekiya et al. 2006). Studies performed in a rat model demonstrated that the resistance and growth of transplanted myocardial cell sheets

were maintained for at least a year. Their effect on the cardiac stem cell therapy included the improvement of retractility, angiogenesis, and the reduction of fibrosis (Shimizu et al. 2006).

Improvement in LV wall thickness and a decrease of fibrosis and necrosis in the postinfarction scar region was noticed due to the cardiomyocyte sheets transplantation in a myocardial infarction in a rat model (Miyagawa et al. 2005). Also, similar results have been reported for other applied cell types: adipose-derived MSCs, mESC-derived cardiac cells, endothelial and skeletal muscle cells (Eschenhagen 2011; Masumoto et al. 2012; Miyagawa et al. 2010; Miyahara et al. 2006; Sekine et al. 2008). Furthermore, it has been described that the effect of MSCs sheets in the enhancement of the cardiac function is also associated with their paracrine activity.

Cardiomyocytes derived from pluripotent stem cells have been examined with optimistic results in both cell sheet and cell aggregation engineering. This assumes that new scaffold-free human myocardial patches may make significant progress in cardiac tissue engineering.

5.7 Concluding Remarks

Continued important elements of stem cell therapy should have been taken into consideration in order to increase the pro-regenerative impact of stem cells toward cardiac tissue, among others: the timing, cell dosage, and delivery techniques not described in this section. However, the other organs already profited from cellular therapies and medical use of stem cells shortly will become a future for biomedical technologies, overall.

Acknowledgements We should like to acknowledge a financial support from the following projects: National Centre for Research and Development, Poland; grant no. STRATEGMED1/233624/5/NCBR/2014, grant no. PBS3/A7/27/2015; National Science Centre, Poland; grant no. 2014/13/B/NZ3/04646.

References

Albini A, Melchiori A, Garofalo A, Noonan DM, Basolo F, Taraboletti G, Chader GJ, Gavazzi R (1992) Matrigel promotes retinoblastoma cell growth in vitro and in vivo. Int J Cancer 52:234–240

Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP (2013) Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3 K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res 10:301–312

Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 362:697–703

Baas T (2014) A big heart. SciBX 7:1–2

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

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

Bearzi C, Rota M, Hosoda T, Tillmanns J, Nascimbene A, De Angelis A, Yasuzawa-Amano S, Trofimova I, Siggins RW, Lecapitaine N, Cascapera S, Beltrami AP, D'Alessandro DA, Zias E, Quaini F, Urbanek K, Michler RE, Bolli R, Kajstura J, Leri A, Anversa P (2007) Human cardiac stem cells. Proc Natl Acad Sci USA 104:14068–14073

Bearzi C, Gargioli C, Baci D, Fortunato O, Shapira-Schweitzer K, Kossover O, Latronico MV, Seliktar D, Condorelli G, Rizzi R (2014) PlGF–MMP9-engineered iPS cells supported on a PEG–fibrinogen hydrogel scaffold possesses an enhanced capacity to repair damaged myocardium. Cell Death Dis 5:e1053

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

Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P (2011) Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): Initial results of a randomised phase 1 trial. Lancet 378:1847–1857

Carè A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Høydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G (2007) MicroRNA-133 controls cardiac hypertrophy. Nat Med 13:613–618

Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, Freed LE, Vunjak-Novakovic G (1999) Cardiac tissue engineering: Cell seeding, cultivation parameters, and tissue construct characterization. Biotechnol Bioeng 64:580–589

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

Chachques JC, Trainini JC, Lago N, Masoli OH, Barisani JL, Cortes-Morichetti M, Schussler O, Carpentier A (2007) Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM clinical trial): One year follow-up. Cell Transplant 16:927–934

Chakravarty T, Makkar RR, Ascheim DD, Traverse JH, Schatz R, DeMaria A, Francis GS, Povsic TJ, Smith RR, Lima JA, Pogoda JM, Marbán L, Henry TD (2017) ALLogeneic Heart Stem Cells to Achive Myocardial Regeneration (ALLSTAR) trial: rationale and design. Cell Transplant 26(2):205–214

Climent AM, Sanz-Ruiz R, Fernández-Santos MA, Arranz AV, Fernández-Avilés F (2016) General Overview of the 13th TECAM Conference. Circ Res 119:409–413

Cortes-Morichetti M, Frati G, Schussler O, Duong Van Huyen JP, Lauret E, Genovese JA, Carpentier AF, Chachques JC (2007) Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration. Tissue Eng 13:2681–2687

Crapo PM, Medberry CJ, Reing JE, Tottey S, van der Merwe Y, Jones KE, Badylak SF (2012) Biologic scaffolds composed of central nervous system extracellular matrix. Biomaterials 33:3539–3547

Dai W, Hale SL, Kay GL, Jyrala AJ, Kloner RA (2009) Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology. Regen Med 4:387–395

Eng G, Lee BW, Protas L, Gagliardi M, Brown K, Kass RS, Keller G, Robinson RB, Vunjak-Novakovic G (2016) Autonomous beating rate adaptation in human stem cell-derived cardiomyocytes. Nat Commun 7:1–10

Eschenhagen T (2011) The beat goes on: human heart muscle from pluripotent stem cells. Circ Res 109:2–4

Frangogiannis NG, Smith CW, Entman ML (2002) The inflammatory response in myocardial infarction. Cardiovasc Res 53:31–47

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

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

Godier-Furnémont AF, Martens TP, Koeckert MS, Wan L, Parks J, Arai K, Zhang G, Hudson B, Homma S, Vunjak-Novakovic G (2011) Composite scaffold provides a cell delivery platform for cardiovascular repair. Proc Natl Acad Sci USA 108:7974–7979

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

Hagège AA, Marolleau JP, Vilquin JT, Alhéritière A, Peyrard S, Duboc D, Abergel E, Messas E, Mousseaux E, Schwartz K, Desnos M, Menasché P (2006) Skeletal myoblast transplantation in ischemic heart failure: Long- term follow-up of the first phase I cohort of patients. Circulation 114:108–113

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

Hu X, Yu SP, Fraser JL, Lu Z, Ogle ME, Wang JA, Wei L (2008) Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. J Thorac Cardiovasc Surg 135:799–808

Hua P, Tao J, Liu JY, Yang SR (2014) Cell transplantation into ischemic myocardium using mesenchymal stem cells transfected by vascular endothelial growth factor. Int J Clin Exp Pathol 7:7782–7788

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

Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA (2008a) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 26:795–797

Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA (2008b) Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 26:1269–1275

Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC (2010) A nonviral minicircle vector for deriving human iPS cells. Nat Methods 7:197–199

Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K (2009) Virus free induction of pluripotency and subsequent excision of reprogramming factors. Nature 458:771–775

Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4:472–476

Kim SW, Lee DW, Yu LH, Zhang HZ, Kim CE, Kim JM, Park TH, Cha KS, Seo SY, Roh MS, Lee KC, Jung JS, Kim MH (2012) Mesenchymal stem cells overexpressing GCP-2 improve heart function through enhanced angiogenic properties in a myocardial infarction model. Cardiovasc Res 95:495–506

Kofidis T, Lebl DR, Martinez EC, Hoyt G, Tanaka M, Robbins RC (2005) Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. Circulation 112:173–177

Kuppusamy KT, Jones DC, Sperber H, Madan A, Fischer KA, Rodriguez ML, Pabon L, Zhu WZ, Tulloch NL, Yang X, Sniadecki NJ, Laflamme MA, Ruzzo WL, Murry CE, Ruohola-Baker H (2015) Let-7 family of microRNA is required for maturation and adult-like metabolism in stem cell-derived cardiomyocytes. Proc Natl Acad Sci USA 112(21):E2785–E2794

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

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

Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, Granot Y, Cohen S (2000) Bioengineered cardiac grafts: a new approach to repair the infarcted myocardium? Circulation 102:56–61

Li Q, Verma IM (2002) NF-kappaB regulation in the immune system. Nat Rev Immunol 2:725–734

Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, Piechaczek C, Moebius JM, Lützow K, Lendlein A, Stamm C, Li RK, Steinhoff G (2007) Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells 25:2118–2127

Li TS, Cheng K, Malliaras K, Matsushita N, Sun B, Marbán L, Zhang Y, Marbán E (2011a) Expansion of human cardiac stem cells in physiological oxygen improves cell production efficiency and potency for myocardial repair. Cardiovasc Res 89:157–165

Li Q, Guo Y, Ou Q, Chen N, Wu WJ, Yuan F, O'Brien E, Wang T, Luo L, Hunt GN, Zhu X, Bolli R (2011b) Intracoronary administration of cardiac stem cells in mice: a new, improved technique for cell therapy in murine models. Basic Res Cardiol 106:849–864

Lin CJ, Lin CY, Chen CH, Zhou B, Chang CP (2012) Partitioning the heart: mechanisms of cardiac septation and valve development. Development 139:3277–3299

Linke A, Müller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Böhm M, Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, Anversa P (2005) Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function. Proc Natl Acad Sci USA 102:8966–8971

Liu J, Hu Q, Wang Z, Xu C, Wang X, Gong G, Mansoor A, Lee J, Hou M, Zeng L, Zhang JR, Jerosch-Herold M, Guo T, Bache RJ, Zhang J (2004) Autologous stem cell transplantation for myocardial repair. Am J Physiol Heart Circ Physiol 287:501–511

Liu XB, Chen H, Chen HQ, Zhu MF, Hu XY, Wang YP, Jiang Z, Xu YC, Xiang MX, Wang JA (2012) Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation. J Zhejiang Univ Sci B 13:616–623

Liu XB, Wang JA, Ji XY, Yu SP, Wei L (2014) Preconditioning of bone marrow mesenchymal stem cells by prolyl hydroxylase inhibition enhances cell survival and angiogenesis in vitro and after transplantation into the ischemic heart of rats. Stem Cell Res Ther 5:111

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

Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E (2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 379:895–904

Marsano A, Maidhof R, Wan LQ, Wang Y, Gao J, Tandon N, Vunjak-Novakovic G (2010) Scaffold stiffness affects the contractile function of three-dimensional engineered cardiac constructs. Biotechnol Prog 26:1382–1390

Masumoto H, Matsuo T, Yamamizu K, Uosaki H, Narazaki G, Katayama S, Marui A, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK (2012) Pluripotent stem cell-engineered cell sheets reassembled with defined cardiovascular populations ameliorate reduction in infarct heart function through cardiomyocyte-mediated neovascularization. Stem Cells 30:1196–1205

Matsubayashi K, Fedak PW, Mickle DA, Weisel RD, Ozawa T, Li RK (2003) Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. Circulation 108:219–225

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

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

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

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

Mihic A, Li J, Miyagi Y, Gagliardi M, Li SH, Zu J, Weisel RD, Keller G, Li RK (2014) The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes. Biomaterials 35:2798–2808

Mikkelsen TS, Hanna J, Zhang X, Ku M, Wernig M, Schorderet P, Bernstein BE, Jaenisch R, Lander ES, Meissner A (2008) Dissecting direct reprogramming through integrative genomic analysis. Nature 454:49–55

Miyagawa S, Sawa Y, Sakakida S, Taketani S, Kondoh H, Memon IA, Imanishi Y, Shimizu T, Okano T, Matsuda H (2005) Tissue cardiomyoplasty using bioengineerred contractile cardiomyocyte sheets to repair damaged myocardium: their integration with recipient myocardium. Transplantation 80:1586–1595

Miyagawa S, Saito A, Sakaguchi T, Yoshikawa Y, Yamauchi T, Imanishi Y, Kawaguchi N, Teramoto N, Matsuura N, Iida H, Shimizu T, Okano T, Sawa Y (2010) Impaired myocardium regeneration with skeletal cell sheets-a preclinical trial for tissue-engineered regeneration therapy. Transplantation 90:364–372

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

Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, Nishikawa S, Tanemura M, Mimori K, Tanaka F, Saito T, Nishimura J, Takemasa I, Mizushima T, Ikeda M et al (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem 8:633–638

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

Nowbar AN, Mielewczik M, Karavassilis M, Dehbi HM, Shun-Shin MJ, Jones S, Howard JP, Cole GD, Francis DP (2014) Discrepancies in autologous bone marrow stem cell trails and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. BMJ 348:g2688

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

Onai Y, Suzuki J, Maejima Y, Haraguchi G, Muto S, Itai A, Isobe M (2007) Inhibition of NF-kappa B improves left ventricular remodeling and cardiac dysfunction after myocardial infarction. Am J Physiol Heart Circ Physiol 292:530–538

Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA (2008) Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 14:213–221

Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, Kadota K, Roth SL, Giardina P, Viale A, Leslie C, Bushman FD, Studer L, Sadelain M (2011) Genomic safe harbors permit high β-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol 29:73–78

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

Ravichandran R, Venugopal JR, Mueller M, Sundarrajan S, Mukherjee S, Pliska D, Wintermantel E, Ramakrishna S (2013) Buckled structures and 5-azacytidine enhance cardiogenic differentiation of adipose-derived stem cells. Nanomed. (Lond) 8:1985–1997

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

Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL (2004) Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 109:1292–1298

Reing JE, Zhang L, Myers-Irvin J, Cordero KE, Freytes DO, Heber-Katz E, Bedelbaeva K, McIntosh D, Dewilde A, Braunhut SJ, Badylak SF (2009) Degradation products of extracellular matrix affect cell migration and proliferation. Tissue Eng Part A 15:605–614

Rizzi SC, Ehrbar M, Halstenberg S, Raeber GP, Schmoekel HG, Hagenmüller H, Müller R, Weber FE, Hubbell JA (2006) Recombinant protein-co-PEG networks as cell-adhesive and proteolytically degradable hydrogel matrixes part II: Biofunctional characteristics. Biomacromol 7:3019–3029

Roquin A (2006) Wilhelm His Jr (1863–1934)—The man behind the bundle. Heart Rhythm 3:480–483

Salomon C, Ryan J, Sobrevia L, Kobayashi M, Ashman K, Mitchell M, Rice GE (2013) Exosomal signaling during hypoxia mediates microvascular endothelial cell migration and vasculogenesis. PLoS ONE 8:e68451

Schussler O, Chachques JC, Mesana TG, Suuronen EJ, Lecarpentier Y, Ruel M (2010) 3-dimensional structures to enhance cell therapy and engineer contractile tissue. Asian Cardiovasc Thorac Ann 18:188–198

Sekine H, Shimizu T, Hobo K, Sekiya S, Yang J, Yamato M, Kurosawa H, Kobayashi E, Okano T (2008) Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation 118:145–152

Sekiya S, Shimizu T, Yamato M, Kikuchi A, Okano T (2006) Bioengineered cardiac cell sheet grafts have intrinsic angiogenic potential. Biochem Biophys Res Commun 341:573–582

Shimizu T, Yamato M, Kikuchi A, Okano T (2003) Cell sheet engineering for myocardial tissue reconstruction. Biomaterials 24:2309–2316

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

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

Stancovski I, Baltimore D (1997) NF-kappaB activation: the I kappaB kinase revealed? Cell 91:299–302

Stevens KR, Pabon L, Muskheli V, Murry CE (2009) Scaffold-free human cardiac tissue patch created from embryonic stem cells. Tissue Eng Part A 15:1211–1222

Stoppel WL, Gao AE, Greaney AM, Partlow BP, Bretherton RC, Kaplan DL, Black LD 3rd (2016) Elastic, silk-cardiac extracellular matrix hydrogels exhibit time-dependent stiffening that modulates cardiac fibroblast response. J Biomed Mater Res-Part A 104(12):3058–3072

Stroorvogel W (2012) Functional transfer of microRNA by exosomes. Blood 119:646–648

Sun CK, Zhen YY, Leu S, Tsai TH, Chang LT, Sheu JJ, Chen YL, Chua S, Chai HT, Lu HI, Chang HW, Lee FY, Yip HK (2014) Direct implantation versus platelet-rich fibrin-embedded adipose-derived mesenchymal stem cells in treating rat acute myocardial infarction. Int J Cardiol 173:410–423

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872

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

Tang XL, Rokosh G, Sanganalmath SK, Yuan F, Sato H, Mu J, Dai S, Li C, Chen N, Peng Y, Dawn B, Hunt G, Leri A, Kajstura J, Tiwari S et al (2010) Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. Circulation 121:293–305

Tulloch NL, Muskheli V, Razumova MV, Korte FS, Regnier M, Hauch KD, Pabon L, Reinecke H, Murry CE (2011) Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ Res 109:47–59

Vu DT, Martinez EC, Kofidis T (2012) Myocardial restoration: is it the cell or the architecture or both? Cardiol Res Pract 2012:240497

Wang K, Zhao X, Kuang C, Qian D, Wang H, Jiang H, Deng M, Huang L (2012) Overexpression of SDF-1α enhanced migration and engraftment of cardiac stem cells and reduced infarcted size via CXCR4/PI3 K pathway. PLoS ONE 7:e43922

Wang L, Pasha Z, Wang S, Li N, Feng Y, Lu G, Millard RW, Ashraf M (2013) Protein kinase G1 overexpression increases stem cell survival and cardiac function after myocardial infarction. PLoS ONE 8:e60087

Wang H (2014) Small molecules enable cardiac reprograming of mouse fibroblast with a single factor. Oct4 Cell Rep March 13, 6(5):951–960

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

Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ (2010) Highly efficient reprogramming to pluripotency and directed differentation of human cells with synthetic modified mRNA. Cell Stem Cell 7:618–630

Williams AR, Hatzistergos KE, Addicott B, McCall F, Carvalho D, Suncion V, Morales AR, Da Silva J, Sussman MA, Heldman AW, Hare JM (2013) Enhanced effect of combining human cardiac stem cells and bone marrow mesenchymal stem cells to reduce infarct size and to restore cardiac function after myocardial infarction. Circulation 127:213–223

Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A (2009) PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 458:766–770

Xiong Q, Ye L, Zhang P, Lepley M, Tian J, Li J, Zhang L, Swingen C, Vaughan JT, Kaufman DS, Zhang J (2013) Functional consequences of human induced pluripotent stem cell therapy: myocardial ATP turnover rate in the in vivo swine heart with postinfarction remodeling. Circulation 127:997–1008

Xu R, Chen J, Cong X, Hu S, Chen X (2008) Lovastatin protects mesenchymal stem cells against hypoxia- and serum deprivation-induced apoptosis by activation of PI3 K/Akt and ERK1/2. J Cell Biochem 103:256–269

Yin Q, Jin P, Liu X, Wei H, Lin X, Chi C, Liu Y, Sun C, Wei Y (2011) SDF-1 inhibits hypoxia- and serum deprivation-induced apoptosis in mesenchymal stem cells through PI3 K/Akt and ERK1/2 signaling pathways. Mol Biol Rep 38:9–16

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

Yu B, Gong M, Wang Y, Millard RW, Pasha Z, Yang Y, Ashraf M, Xu M (2013) Cardiomyocyte protection by GATA-4 gene engineered mesenchymal stem cells is partially mediated by translocation of miR-221 in microvesicles. PLoS ONE 8:e73304

Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA (2009) Human induced pluropotent stem cells free of vectors and transgene sequences. Science 324:797–801

Zeng H, Li L, Chen JX (2012) Overexpression of angiopoietin-1 increases CD133 +/c-kit + cells and reduces myocardial apoptosis in db/db mouse infarcted hearts. PLoS ONE 7:e35905

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

Zhang Z, Li S, Cui M, Gao X, Sun D, Qin X, Narsinh K, Li C, Jia H, Li C, Han Y, Wang H, Cao F (2013) Rosuvastin enhances the therapeutic efficacy of adipose- derived mesenchymal stem cells for myocardial infarction via PI3 K/ Akt and MEK/ERK pathways. Basic Res Cardiol 108:333

Zhang Y, Cao N, Huang Y, Spencer CI, Fu JD, Yu C, Liu K, Nie B, Xu T, Li K, Xu S, Bruneau BG, Srivastava D, Ding S (2016) Expandable cardiovascular progenitor cells reprogrammed from fibroblasts. Cell Stem 18(3):368–381

Zhu XY, Zhang XZ, Xu L, Zhong XY, Ding Q, Chen YX (2009) Transplantation of adipose-derived stem cells overexpressing hHGF into cardiac tissue. Biochem Biophys Res Commun 379:1084–1090

Zimmermann WH, Schneiderbanger K, Schubert P et al (2002) Tissue engineering of a differentiated cardiac muscle construct. Circ Res 90:223–230