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# Stem Cells in Treatment of Coronary Heart Disease and Its Monitoring: Tissue Engineering and Clinical Evaluation

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Additional information is available at the end of the chapter

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## Abstract

Cardiovascular and coronary heart diseases involve molecular and tissue level damage of blood vessels and heart. Coronary Heart Disease and heart failure are the leading cause of mortality worldwide. Stem cell transplantation is emerging as a new treatment option. Stem cells are capable to reach and settle down at damaged cardiac tissue. This stem cell option also repairs the myocardial infarction area in heart or vascular territories and ultimately reduces the infarct-related mortality. Non-invasive cardiovascular imaging monitors the real-time status of cardiovascular remodeling or differentiated stem cell autografting. Cardiac magnetic resonance imaging (MRI) and bioluminescence are robust non-invasive monitoring techniques to visualize cardiovascular structure changes due to myocardial dysfunction or restorative myocardial recovery. The present chapter highlights the sources, types, delivery methods of stem cells in cardiovascular treatment, advantages and current limitations of stem cell monitoring, scopes of ultra-high field cardiac 900 MHz MRI and bioluminescence methods applied in stem cell transplantation, to translate stem cell molecular events into clinical success and evaluation of rejuvenation rate with future perspectives. *In conclusion*, right choice of stem cells, pluripotent stem cell delivery, transplantation and real-time monitoring of stem cell trafficking enhances the stem cell therapeutic efficacy in cardiac engraftment and differentiation.

**Keywords:** stem cell delivery, transplantation, magnetic resonance imaging, coronary disease, cardiac tissue engineering

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## 1. Introduction

Cardiovascular diseases (CVD) and coronary heart disease (CHD) are worldwide leading causes of present mortality as high as 32.8% [1]. Last 5-year American Heart Association

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(AHA) data show high prevalence of heart failure as high as 50% and mortality as high as 32.8%. Coronary artery bypass grafting (CABG) was routine interventional and surgical treatment to bring low mortality of coronary heart disease and now stem cell therapy is a new option. Still, major life threat is myocardial necrosis of myocardial tissue that cannot restore the original function of myocardium. Currently, stem cell research has opened vista in transplantation therapy, and its feasibility and effectiveness are well proven in animal experiments as well as in small-scale clinical trials [2–15].

The present chapter is divided into six sections. Section 1 introduces the evolution of stem cell therapy and its mechanism in regeneration and heart restoration. Section 2 introduces different stem cells and their purpose in repair and remodeling myocardium. Section 3 defines transplantation. Section 4 describes different modes of stem cell delivery. Section 5 highlights the purpose of rapid noninvasive real-time monitoring the myocardial repair and evaluation of heart territories. Section 6 reviews different clinical trials, available current nanotechnology and tissue engineering tools, and new approaches with future perspectives. A sketch of metabolic regulation during rejuvenation is presented for exploring new thoughts on secretory molecules regulating remodeling stem cells to explain regeneration of heart with possibility of better regeneration outcome. The chapter is written for interested physicians, surgeons, tissue engineers, scientists, and entrepreneurs.

### **1.1. Evolution of stem cell therapy: regeneration and healing**

History records two types of bone marrow cells (BMC): hematopoietic stem cells [red blood cells (RBC), white blood cells (WBC), lymphocytes, macrophages] and bone marrow stroma mesenchymal stem cells (osteogenic cells for bone formation, chondrogenic cells for cartilage formation, adipogenic cells for fat tissue, and myoblast cells for heart regeneration). Stroma mesenchymal cells are sources of stem cells. Now, stem cell treatment is emerging in heart regeneration and restoration by using intracoronary, intramyocardial, and epicardial injections.

Stem cell transplantation therapy was reported useful first time in recovering myocardial viability after myocardial infarction in ischemic heart disease [16]. Later, autologous intracoronary delivery of mononuclear bone marrow cells 5–9 days after percutaneous transluminal coronary angioplasty [(PTCA); performed within 12 hours of myocardial infarction) was successful in 10 patients. Patients showed improved wall motion [2]. These clinical trials showed angiogenesis, decreased perfusion defects, and improved ejection fraction by endocardial injection of bone marrow cells (BMC) directly in hibernating myocardium useful in heart failure patients. Later 'Myoblast Autologous Grafting in Ischemic Cardiomyopathy MAGIC-cell-5-combination cytokine clinical trial' using intracoronary blood stem cells or induced pluripotent stem cells along with granulocyte-colony-stimulating factor therapy recorded improved angiogenesis and cardiac function [17]. Currently, induced pluripotent stem cells bearing specific membrane surface marker proteins are emerging as potential engineered cells useful for constructing 3D matrices in cardiac repair.

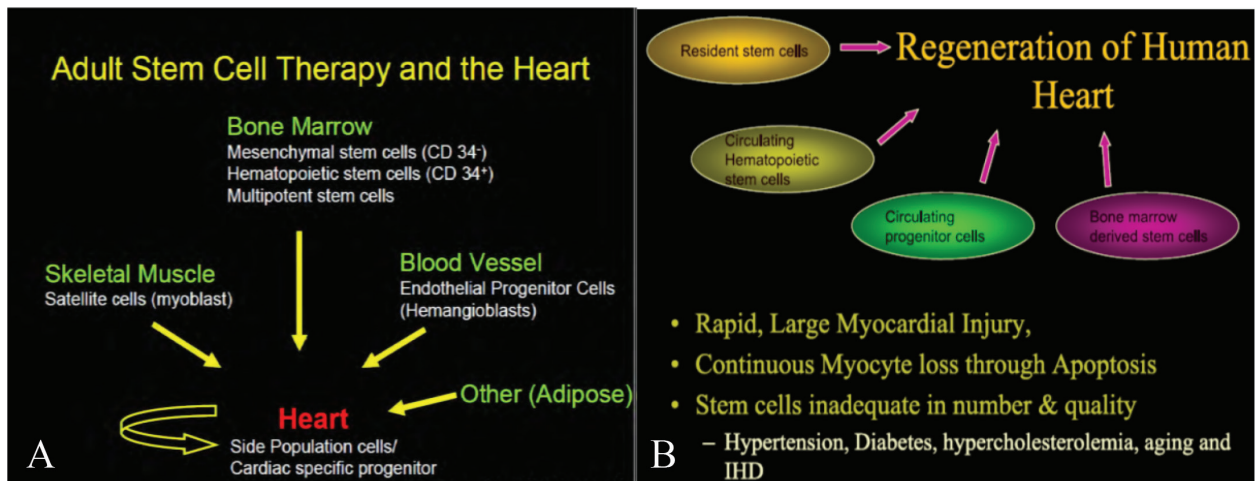
## 1.2. What should be the goal of stem cell therapy?

Stem cells are used as autograft (self-renewing, undifferentiated clonogenic) transplantation in myocardial repair or regeneration to bring tissue functionality back to normal for long-term survival in patients with permanent myocardial damage. Some stem cells are used as allograft (multipotent) daughter cells that give rise to multiple progenies [18]. The result of this asymmetric replication of stem cells is that after each division of stem cells, some progeny enters into the differentiation phase. Bone marrow cells and embryonic stem cells have differentiation plasticity and capacity. Goal is achieved by stimulating blood stem cells to cardiomyocytes providing a continuous supply of cardiac stem cells by trans-differentiation. Other exciting option is 'therapeutic cloning' means transplanted stem cells reprogram into induced pluripotent stem (iPS) cells at target organ. Still many issues are: (1) How many number of optimized stem cells needed when performing cell transplantation therapy?; (2) How survival time of transplanted cells can be best monitored?; (3) How do transplanted stem cells undergo differentiation into cardiomyocytes, smooth muscle cells, or endothelial cells?; (4) Do transplanted stem cells produce electrochemical coupling closer to normal myocardial tissue and normal cardiac cells and their functions really recover?; (5) What is the mechanism of cell transplantation in the treatment of myocardial perfusion and cardiac function after a short enhancement (myocardial cell regeneration or paracrine or other)? In nutshell, regeneration and healing of damaged cardiac tissue by myocardial repair is critical in survival. For interested readers, myocardial repair refers to the restoration of tissue architecture is shown in **Figure 1** and its remodeling of metabolic functions after injury is described in **Figure 7** in detail. Regeneration is defined as 100% myocardial repair and recovery.

Currently, cardiac stem cell therapy researchers are exploring new differentiation and surface protein expression markers. Main focus is to design stem cell therapy supports using different 3D extracellular matrices, polymeric scaffolds. Most intriguing advances are in innovative new methods of safe stem cell delivery with subsequent repair monitoring and follow up of stem cell therapy [19]. If incomplete repair, it leaves myocardial scar or fibrosis of collagen or necrosis after inflammation. After repair, myocardial recovery involves the proliferation of stem cells and interaction with native tissue cells to fill up myocardial mass and extra cellular matrix (remodeling) with its improved cellular paracrine function. The improved function is monitored by non-invasive MRI and bioluminescent techniques. Moreover, total success depends on choice of supporting engineered stem cell delivery method and monitoring the extent of repair or regenerating cardiac territories with its visualization by physiochemical methods during follow up of post-stem cell therapy benefits.

## 1.3. Potential mechanisms

Basic mechanism is 'myocardial revascularization and regeneration' to combat little or no blood supply left to slowly dying heart after myocardial ischemia or infarct or hypoxia. If sufficient oxygen diffusion from endocardium and collateral vessels provide sufficient oxygen



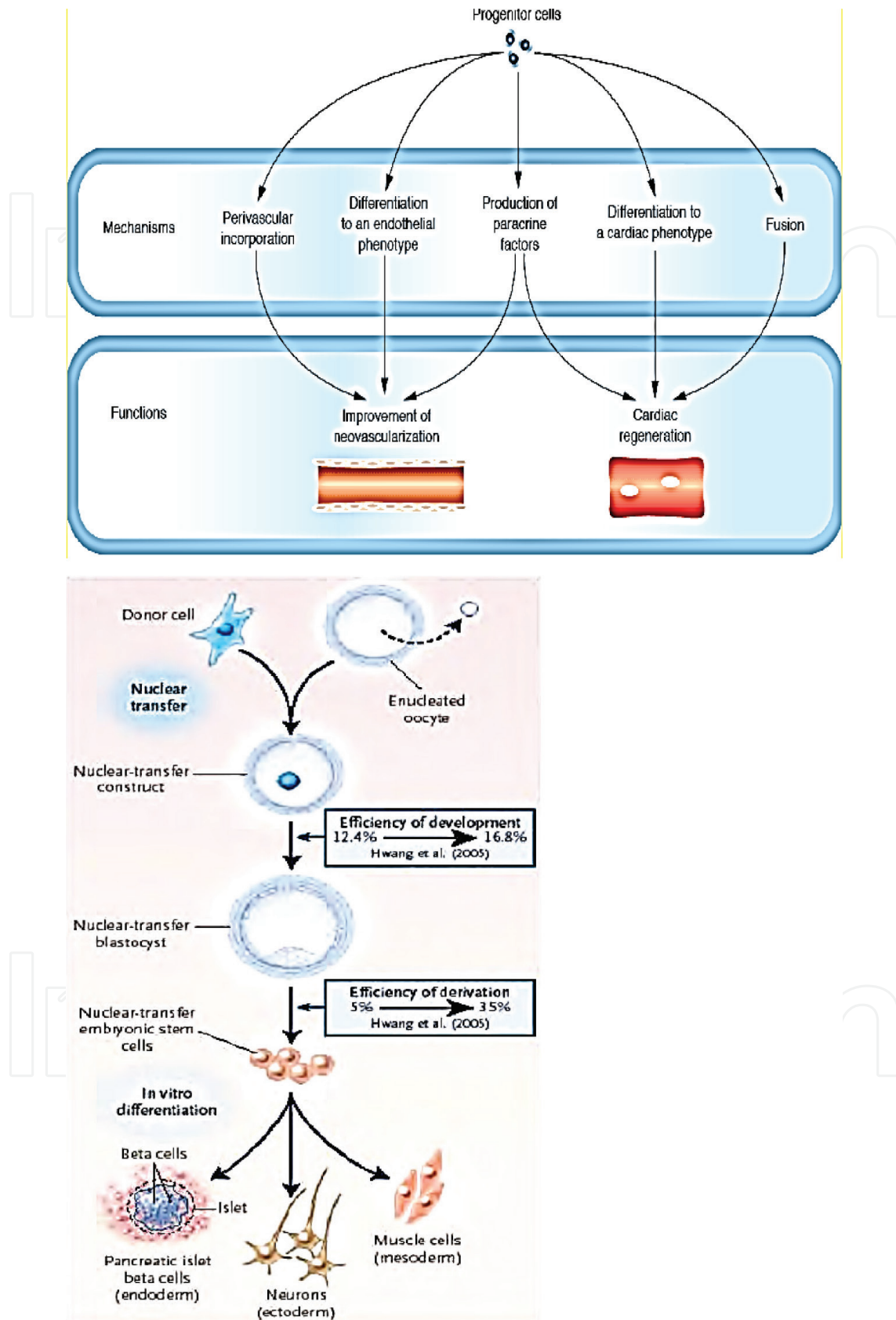
**Figure 1.** Different sources of adult stem cells are shown in heart regeneration in panel A and different conditions of myocardial injury are shown for the need of heart regeneration in panel B.

to preserve progenitor cells, cardiac repair is done by progenitor cell migration from healthy adjacent myocardium or from the blood circulation.

During regeneration, in fact, initially myoblasts, hemangioblasts, multipotent BMCs and adipocytes transform into cardiac specific progenitor cells. These resident stem cells, circulating hematopoietic cells, progenitor cells and BMCs collectively repair the dying heart by establishing revascularization and regeneration of heart as shown in **Figure 1**. These progenitor cells mainly differentiate into endothelial phenotype and cardiac phenotype to produce paracrine factors for perivascular incorporation and fusion to develop into myocytes and coronary vessels as shown in **Figure 2**. In this process, it requires specific transcription factors. In nutshell, bone marrow mononuclear stem cells, mesenchymal stem cells, endothelial stem cells, and hematopoietic stem cells undergo local neovascularization, neoangiogenesis and paracrine function to have positive effect on endogenous cell angiogenesis and energy metabolism by secretory molecules to inhibit myocyte apoptosis [20] as shown in **Figure 2**. As a result, heart left ventricle ejection fraction, arteriole, ventricular walls, end-diastolic and end-systolic ejection volumes, perfusion rate, contractility are improved with oxygen sufficiency. Revascularization and differentiation are mainly triggered by cycline dependent myocyte membrane surface proteins and remodeling factors as described in detail (see **Figure 7** in section 6).

#### 1.4. Cardiovascular tissue has progenitor differentiating cells to replenish dead or dying cells

Stem cells can be mobilized from bone marrow, fat tissue, or blood, and then cultured to produce large numbers of pluripotent stem cells to transplant into the area of heart injury. It can be explained by the concept of 'cardiac chimerism' that explains the role of putative stem cells and progenitor cells present in transplanted heart during regeneration from circulating stem cells. For example, human circulating endothelial progenitor cells from bone cells are rich in membrane surface proteins such as CD34, CD31, KDR, and c-kit positive myocardial



**Figure 2.** Panel on top: Different mechanisms are shown for differentiation of stem cells to improve revascularization and cardiac regeneration after stem cell therapy. Panel on bottom: Somatic nuclear transfer mechanism is shown for differentiated cells. Induced pluripotent cells have potential of cardiac repair and used in treatment.

differentiation proteins visible in myocardial cell biopsy or cultures [21]. Bone-derived endocardial progenitor cells also do cardiac repair of functional myocardium by declining angiogenic activity. Bearzi et al. reported chimeric heart containing human myocardium with myocytes, coronary arterioles, and capillaries formed in mice injected with human cardiac stem cells [22]. It also supported the view of human stem cell therapy of cardiomyopathy [22]. The following description introduces readers with stem cell types, sources, stem cell engineering, and clinical application in heart repair.

## 2. Stem cell types and regeneration

Human body has continuously dividing tissues, stable tissues, or permanent tissues. Hematopoietic cells in bone marrow continuously divide and readily regenerate. These regenerating matured cells are short-lived and continuously replenished by stem cells to maintain a constant equilibrium between replicating and dying mature cells, for example, skin and Gastrointestinal tract (GIT). Stable tissues with least replicating cells are heart, liver, kidney cells, endothelial cells, fibroblasts, and smooth muscle cells. Permanent cells are neuron and cardiac muscle cells. They can replicate but cannot terminally differentiate.

### 2.1. Sources of stem cells

- **Embryonic stem cells** originate from endoderm of embryo after fertilization. Endoderm cells produce 220 kinds of specialized cells during mammalian development by irreversible differentiation process [23]. Later, embryonic precursor cells differentiate into adult muscle or bone marrow cells, fat stem cells or multipotent cells.
- **iPS cells** are formed from regular adult cells by a “cocktail” of inducers or transcription factors so called “induced” pluripotent stem cells (iPS). These transform into the embryo-like state, without eggs or embryos. The iPS cells are pluripotent and make any type of tissue in human body because iPS cells can resemble genetically and immunologically matched with the recipient body. Now, transplantation of these cells into the desired organ offers regenerative therapy of that tissue. However, turning back the biological clock of adult cells to an embryonic state is myth or miraculous escape from aging “immortal divinity”. Interested readers may read comprehensive review on pluripotent cells [23, 24]. Yamanaka, 2007 introduced a combination of genes into adult cells changed their behavior as embryonic stem cell, hence called them ‘pluripotent’ stem cells. In fact, four gene trans-acting factors Oct3/4, Sox2, c-Myc, and Klf4 in adult myocyte cells possibly transformed them pluripotent stem cells [24]. Yu et al. 2007 reported the delivery of trans-acting factors Oct4, NANOG, Sox2, and LIN28 sufficient to reprogram a human somatic fibroblast cell into pluripotent cell bearing same telomerase and surface markers as embryonic cells [25]. Now, cellular programming by somatic nuclear transfer or cloning enables iPS cells behaving like embryonic cells [25]. Cloning develops embryo by the injection of new DNA material from an adult stem cell to an egg cell whose DNA is removed. This enucleated oocyte is the best source of pluripotent stem cells [24] as shown in **Figure 2**. The said engineered

egg rejuvenates the DNA of adult donor cells means restores telomere length without DNA loss during advancing age. This hypothetical idea poses ethical questions. On the other side of coin, iPS cells may treat or correct harmful mutations or diseases such as sickle cell hemoglobin.

- **Cardiac stem cells** are composed of four types including: resident stem cells, circulating hematopoietic cells, circulating progenitor cells, bone marrow cells. These all cells have significant role in cardiac regeneration after myocardial infarction. Urbanek et al. 2005 reported high number of activated stem cells (myocytes, smooth muscle cells, and embryonic cells) formed after cell regeneration in acute myocardial infarcts over chronic infarcts. Poor cell regeneration caused predisposition to chronic congestive heart failure [19]. Answer lies in telomere attrition, leading to decreased telomerase levels in chronic infarct and higher telomerase activity in acute infarcts. Telomerase enzyme is a marker showing growth potential of myocytes, endothelial cells, and smooth muscle cell lineages. Telomerase protects the DNA at the end of a chromosome during mitosis. Autologous transplantation raises hope of increasing telomerase activity to correct end stage cardiomyopathy.
- **Mesenchymal stem cells** are nonhematopoietic cells in adult bone marrow and adipose tissues. These differentiate or modify *in vitro* to adopt phenotypic characters of cardiomyocytes and vascular cells by mesenchymal stem cell allogeneic therapy or cardiac repair by paracrine function [4].
- **Allogeneic stem cells** are “off the shelf” mesenchymal stem cell products from bone marrow of healthy donor. These are useful in therapy phase I trials as they target the myocardial injury site due to the presence of several stromal cell-derived factor-1 (SDF-1), major histocompatibility antigen class 2 molecules, and phenotypes CD145+, CD166+, and CD45– protein markers. These cells can differentiate into bone, tendon, fat, and muscle tissues. These cells also secrete immunosuppressive cytokines. Moreover, these cells can be administered by intravenous route. These stem cells also target and differentiate into cardiac myocytes and blood vessels [26].

### 3. Ideal stem cell transplantation to treat cardiovascular diseases

In stem cell transplantation methods, ideally adult stem cells, embryonic stem cells (ESC), or induced pluripotent stem cells (iPSC) are locally fixed at dying myocardial tissue sites. However, major challenge is to monitor them timely and confirm the real-time improvement in dying or recovering myocardial tissue physiology efficiently to treat the ischemic heart disease. In other words, capability of MR imaging and monitoring heart metabolism visualize the anterior wall in acute myocardial infarction patients to detect improved myocardial perfusion and myocardial recovery status. Real-time cell imaging also confirms the efficacy of injected bone marrow stem cells (BMC cells) in the recovery of myocardial fragility and viability without any increase in left ventricular ejection fraction (LVEF) [27]. In initial experimental study, the success of embryonic stem cell transplantation in rat myocardial ischemia model showed



significant recovery as reduced left ventricular expansion and reduced area of myocardial infarction after 3–6 weeks. However, in this recovery process, stem cell transcription factors such as Oct3/4, Sox2, Klf4, and c-Myc transformed the embryonic stem cells into induced pluripotent stem cells or iPSCs [28]. These pluripotent cells form regenerative myocardial tissue, smooth muscle, or endothelial vascular cells *in situ* to repair myocardial infarction in heart or increased ventricular wall thickness and electrical stability [29]. Recently, different clinical centers claim their success differently to transplant pluripotent stem cells in remodeling myocardial muscle or endothelial vascular cells [10, 28–35]. In fact, stem cell treatment centers follow the strategy that pluripotent stem cells may be stable rather than terminally differentiated as meta-analysis of randomized controlled clinical trials on stem cell therapy also indicated clearly that intracoronary adult bone marrow stem cells improve left ventricular function and reduce the risk of recurrent heart failure soon after acute myocardial infarction (AMI) [36]. **Table 1** shows the major stem cell types commonly used in medical practice using autograft or allograft transplantation in myocardial repair. Mainly adult stem cells, embryonic stem cells (ESC), or induced pluripotent stem cells (iPSC) are choice.

Allogenic origin of stem cells	Fate of stem cells	Autologous origin of adult stem cells	Fate of stem cells
Embryonic stem cells	Fetal cardiomyocytes	Mesenchymal stem cells	Endothelial progenitor cells
Adipose derived stem cells	Umbilical cord derived cells	Endothelial progenitor cells	Multipotent adult progenitor cells
Resident cardiac stem cells	Fetal cardiomyocytes	Endothelial progenitor cells	Induced pluripotent stem cells
Skeletal myoblast cells	Bone marrow mononuclear CD34 <sup>+</sup>		

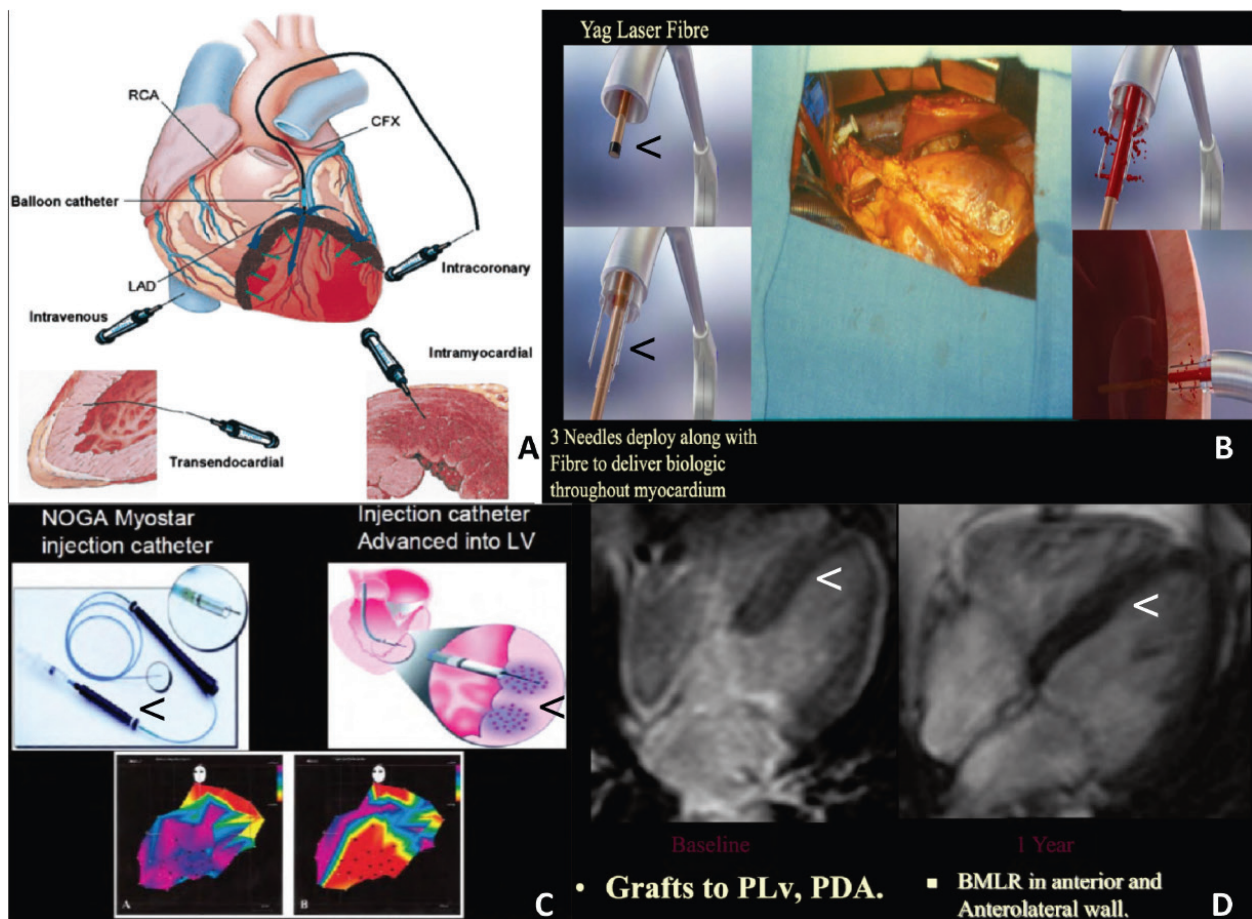
**Table 1.** Potential applications of different stem cell types for cardiomyocytes in heart transplantation for myocardial repair.

## 4. Delivery protocols of stem cell therapy

Each protocol differs in cell retention and regeneration rates depending upon method and site of injection, i.e., intracoronary, intramyocardial, transendocardial, or via coronary sinus delivery (see **Figure 3**), time of delivery, inflammatory response. Other factor is timing of administration rapid or slow injection rates. The early administration of cells facilitates better retention of stem cells or rejuvenating homing signals evidenced in TIME trial [6], while a long delay may cause scar formation in the LateTIME trial [7] as highlighted in **Tables 2** and **3**.

### 4.1. Intracoronary stem cell therapy

It is done by cell transplantation through transc coronary passage of cells at infarct site along with a standard percutaneous transluminal coronary angioplasty (PTCA) procedure or coronary artery bypass grafting (CABG) procedure, with the use of an over-the-wire balloon



**Figure 3.** Different delivery sites of stem cell injections are shown in panel A. Yag laser with three needles is shown for BM Laser Repair procedure to deliver stem cells and rejuvenation molecules in panel B. NOGA Myostar catheter is shown for delivery of stem cells in left ventricle in panel C. The evaluation of heart recovery as improved anterolateral wall after stem cell therapy by MRI is shown in panel D.

with central lumen placed at a desired position (see **Figure 3**). It allows intracoronary cells to “home-in” or retention of stem cells by extravasation of BMC to the infarcted area in the presence of chemokines and adhesion molecules, SDF-1, and beta-2-integrin factors induced by ischemic cell injury [32, 35–37].

Coronary infusion of cells is performed four to six times, with 3-minute sequential balloon inflations followed by 3-minute rest periods, to create a “stop flow” situation for maximal retention period to come into contact with the microcirculation of the infarct-related artery. It maximizes the migration and retention of cells into the infarct and peri-infarct tissues for successful transplantation. After transplantation, baseline and post procedure LV angiograms are monitored for 24 hours, with cardiac markers checked at every 6 and 12 hours. Injection of stem cells into a contralateral artery may increase retention in ischemic area if there are well-formed collaterals. Imaging studies further confirm the success of contralateral stem cell injections to increase the retention of cells in occluded artery territories. The crucial issues are: retention of cells, improved ejection fraction, improved regional wall LV function, microvascular plugging, biodistribution, homing to myocardium, proapoptotic factors in the ischemic myocardium, CD34+ cells [35, 38].

Study name	Published	n	Days	Primary outcome	Imaging modality
A. Proven stem cell treatment and evaluation					
TOPCARE-AMI	2002	59	4–5.5	-Global LVEF improved 51–60% in 3 months	-SPECT, echo, MRI
BOOST	2004	60	5–6.3	-Global LVEF improved in large infarcts after 6 months(18 m follow up)	-Cardiac MRI
REPAIR-AMI	2006	187	3–6	-LVEF improved 2.5% in 4 months	-LV angiography
ASTAMI	2006	97	6–7	-No change in global LVEF in 1 year	-SPECT, MRI, echo
LEUVEN-AMI	2006	66	1	-No change in LVEF in 4 months but regional contractility improved & infarct size less	-Cardiac MRI, echo
FINCELL	2008	77	3	-LVEF improved 5% in 6 months but global LVEF same after 4 months	-Cardiac MRI, echo
HEBE	2010	200	3–8	-LVEF improved 6% in 6 months	-Cardiac MRI
B. Other clinical stem cell trials using different stem cell types					
Autologous BMNCs	CABG + SC	5	1y	>5 days old MI	Improved perfusion
Autologous BMNCs	PTCA + SC	13	3m	5–9 days post MI	Better perfusion, wall motion, less infarct size
BMNCs+EPCs	PTCA + SC	23	4m	<3 days post MI	Better LVEF, EDV, perfusion, % contractile function
BMCs+AC133	CABG + SC	12	3–9 m	0–3 m post MI	Better EF, better perfusion
BMNCs	EMM + SC	8	3 m	severe IHD	Improved perfusion, angina, contractile function
Autogous BMNCs	EMM + SC	14	2 m	CHF	Improved LVEF, perfusion, contractile function
Myoblasts	CABG + SC	10	11 m	CHF	Improved EVEF, contractile function
Autologous skeletal myoblasts	CABG + SC	12	12 m	Old MI + ischemic CAD	improved LVDF, regional contractility
Autologous skeletal myoblasts	IM SC injection at LVAD site	5	6 m	IHD	improved LVEF, wall thicker at the injection site
Autologous BMCs	IC infusion + PTCA	30	6 m	<5 days post MI	Improved LVEF, contractile function
Autologous BMSCs	IC infusion + 18 days post PTCA	34	3–6 m	10 days post MI	Better perfusion, high EDV, ESV, wall movement, LVEF
Autologous blood SCs + inj G-CSF	IC infusion + PTCA	10	6 m	>48 hours AMI(old)	Higher stent restenosis in G-CSF group

**Table 2.** Randomized control trials showing administration of pluripotent stem cells with primary outcome of improved cardiac mass by monitoring improvement in left ventricle ejection function by imaging.

Clinical trial	Administration	Engineered tissue construct used	Reference
Hirsch et al. 2011 HEBE clinical trial	Intracoronary	No change in LVEF in 4 months followup	[12]
Roncalli et al. 2011 BONAMI Trial	Intracoronary	Pluripotent cells	[13]
Traverse et al. 2011 LateTIME Trial	Intracoronary	Pluripotent cells	[7]
Bolli et al. 2011 SCIPPIO Trial	Intracoronary	Pluripotent cells	[5]
Makkar et al. 2012 CADUCEUS Trial	Intracoronary	Pluripotent cells	[89]
Zhao et al. 2013	Intracoronary	Pluripotent cells	[90]
Kurbonov et al. 2013	Intracoronary	Engineered stem cells	[91]
Forcillo et al. 2013	Via CABG+i.m.	Stem cells	[92]
Assmann et al. 2013	Via CABG+epicardial	Engineered stem cells	[93]
Nasseri et al. 2014	i.m	Stem cells	[94]
Brickwedel et al. 2014	Via CABG	Engineered stem cells	[95]
Hong et al. 2014	Intracoronary + retrograde coronary sinus	Engineered stem cells	[96]
Hao et al. 2015	Intracoronary	Stem cells	[97]
Chang et al. 2015	Intracoronary	Stem cells	[98]
Gao et al. 2015	Intracoronary	Stem cell engineering	[99]
Fiarresga et al. 2015	Intracoronary	Stem cell engineering	[100]
Helseth et al. 2015	Intracoronary	Stem cell engineering	[101]
Eirin et al. 2015	Intrarenal	Pluripotent cells	[102]
Lee et al. 2015	Intracoronary	Engineered stem cells	[103]
Tseliou et al. 2016	Intracoronary	Stem cell engineering	[104]
Hasan et al. 2016	Intracoronary	Stem cell engineering	[105]
Xiao et al. 2017	Intracoronary	Stem cell engineering	[106]
Gao et al. 2017	Intracoronary	Pluripotent cells in 3D scaffold	[107]

**Table 3.** A chronology of clinical trials using different stem cell delivery and engineered constructs.

*How success in heart recovery after heart transplantation is assessed?* After heart recovery, improvement in cardiac functions is the success key. Important cardiac parameters are improved ejection fraction (LVEF), improved contractile function, improved regional wall thickness reduction or improved LV function, Ejection Diastolic and Ejection Systolic Volumes, improved perfusion along with decreased adverse perfusion defects, all these events within less than a week as shown in **Table 2** and **Figure 3** (see panel D). In support, several randomized trials clearly shown that administration of intracoronary autologous bone marrow nuclear cells in patients soon after myocardial infarction improved the ejection fraction within 5 days [8, 39] shown in **Table 2** and illustrated in **Figure 3** (see panel D). Other randomized

trials showed clear evidence of improved regional wall LV function [9]. The Repair-AMI trial showed a significant decrease in major adverse events [10]. However, several clinical trial and pilot studies have failed to demonstrate that bone marrow nuclear cells really improve LV function in the setting of acute myocardial infarction because of empirical calibration or lack of preclinical results [7, 11–13, 31, 40]. Other critical issue is successful cardiac recovery or revived myocardial function rapidly and fast as much as possible. In the previous studies, most of the autologous bone marrow mononuclear cell implantations were performed within week following ST elevation myocardial infarction event. Specific mention here is the evidence of most favorable cardiac recovery effect on LV function obtained on the fifth day after delivery of stem cells in small cohort of patients in the Repair-AMI trial [10] as shown in **Figure 3**.

In the light of above, it is very important that timing of ‘appropriate stem cell conditioned delivery’ in right manner soon after myocardial infarction may have an influence on stem cell treatment as highlighted in **Figure 3** (see panel C). This timing and delivery issue has debated over the stem cell choice, delivery mode of stem cells, and timing of stem cell implantation after acute myocardial infarction. Two factors need attention here for successful implantation and its action on recovery of myocardium: (1) Release rate of circulating progenitor mononuclear cells from bone marrow within hours of acute myocardial infarction [27, 41, 42]; (2) Release of enormous hematopoietic stem cells, endothelial progenitor stem cells, mesenchymal stem cells, and a very small number of embryonic-like pluripotent cells with cardio-rejuvenating properties [43]. Moreover, other factors are also determinant in the success of cardiac rejuvenation such as inadequate cell count, improper processing, and timing of stem cell administration.

Important concern in regard to negative findings is timing of stem cell administration. The National Heart Lung and Blood Institute sponsored Cardiovascular Cell Therapy Research Network reported two prospective clinical trials, TIME [6] and LateTIME [7]. The TIME trial was proposed to compare the effects of bone marrow mononuclear source cells delivered at 3–7 days in patients with predominantly ST elevation myocardial infarction. The LateTIME trial proved the hypothesis that delayed delivery of autologous bone marrow cells at 2–3 weeks following acute myocardial infarction may improve global LV systolic function. LateTIME trial calibrated the cell count and processing issues but did not show any detectable improvement in LV function over a period of 2 years [44]. For interested readers, intramyocardial stem cell therapy protocol is described in following section.

#### **4.2. Intramyocardial stem cell therapy protocol**

In open heart surgery, direct visualization of the heart is a preferred method as an endocardial approach during supervised intramyocardial injection of stem cells. Using endocardial approach for intramyocardial stem cell therapy, a transmural injection of stem cells is guided by LV electromechanical mapping with NOGA™ software (Biologics Delivery Systems, Diamond Bar, CA) to deliver stem cells in target infarct area [15] as shown in **Figure 3**. For instance, in routine stem cells are injected into nonviable myocardium soon after an observed

low cardiac output, by an 8Fr MYOSTAR™ catheter (Biologics Delivery Systems) with nitinol tubing and retractable needle set up at a depth of 4.5–6 mm inside cardiac tissue and placed at an appropriate angle 45° under fluoroscopy observation as shown in **Figure 3**. Volumes of approximated 0.3 cc of stem cells are injected by manually advancing the needle initially at several different space volumes of 1 cm<sup>3</sup> in areas of thinned myocardium (<0.5 mm<sup>2</sup> by MRI). Without motion, still patient is kept under observation. Later, patient is monitored for 18–24 hours attached with cardiac life support device and recovery, and myocardial viability is monitored by continuous real-time LV angiography. First time, Federal Drug Agency (FDA) approved the protocol of autologous BMC stem cells as milestone showing salvaged hibernating myocardium with improved angiogenesis, 75% decreased perfusion defects, and improved 20–29% ejection fraction [17]. Now, improved protocols in clinical trials are in practice throughout the US and Europe as shown in **Tables 2** and clinical trials in recent 5 years shown in **Table 3**.

#### **4.3. Retrograde coronary sinus injection**

It is other approach to deliver potentially therapeutic stem cells in coronary sinus. A double lumen catheter attached with a larger proximal and a smaller distal balloons is used for delivery of cells in distal lumen. The stem cells are injected and their transport is confirmed angiographically in the mid- to distal interventricular vein that runs parallel to the left anterior descending artery, as shown in **Figure 3** panel A.

#### **4.4. Intravenous delivery**

This approach depends upon the intravenous access site as shown in **Figure 3** panel A. The cells get trapped in the lungs, liver, and spleen, so that only a small number may enter in coronary circulation, and myocardial homing is minimal [15]. Myocardial homing depends on the expression of adhesion molecules, cytokines, and homing receptors. In following sections, growing interest of real-time noninvasive monitoring of pre- and post-cardiac recovery of myocardium tissue by advanced 900 MHz MRI methods in preclinical studies and real-time stem cell behavior are discussed. 900 MHz MRI facility is available only in laboratory at our place in the light of less known facts, limitations and challenges to use this facility.

### **5. Need of noninvasive in vivo monitoring stem cells in preclinical studies**

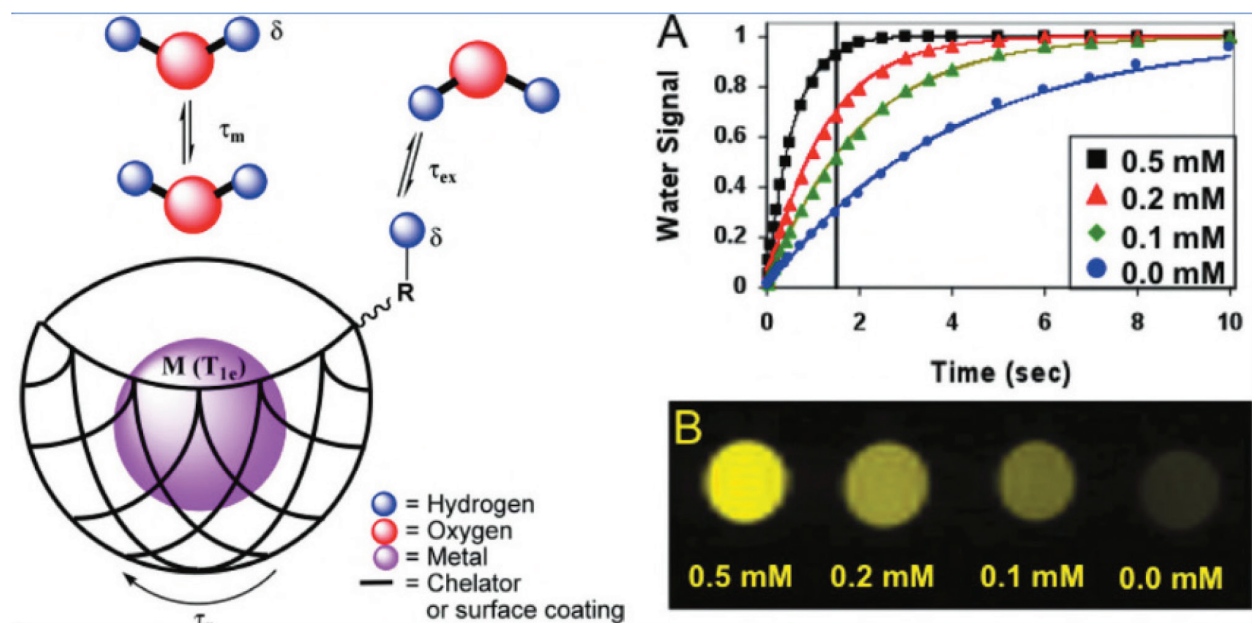
Molecular events by imaging methods offer excellent opportunity to visualize and track stem cell behavior in vivo to evaluate their efficacy of cardiac cell recovery or therapy in preclinical studies. Monitoring the settled home-in rejuvenated stem cells functioning well at cardiac infarct site is based on the fact that active myocardial metabolite protons and water relaxation dynamics is characteristic while <sup>31</sup>P MR peaks predict the settled stem cell physiology [45]. Ultrasound imaging, positron emission tomography/single photon emission computed

tomography (PET/ SPECT), magnetic resonance imaging (MRI), optical imaging, and CT imaging are routine molecular imaging techniques. Magnetic resonance of odd-numbered protons in cardiac tissue molecules with resonant radiofrequency in high magnetic field generates the physiological MR cardiac MRI fingerprint as most promising in clinical transformation to provide the structural-functional information of resettled cardiac mass with superior resolution and high sensitivity relatively safer and without radiation [45, 46].

Other major challenges are visualizing myocardial functionality and real-time monitoring the status of transplanted stem cell behavior within native tissue as true representative of altered or improved visible myocardial territories or metabolic recovery. For this purpose, the smart imaging contrast agents or contrast labeling of stem cells offer to visualize the behavior of transplanted stem cells in tissue in situ. Different techniques of cell MRI, bioluminescence, chemiluminescence, myofibril scanning, and DNA end-labeling are routine methods to track myocardial functionality, viability, and fragility [47, 49]. Recently, nanoparticle-labeled stem cells have been developed to achieve dephasing susceptibility contrast and monitoring the stem cell behavior, physiological changes and molecular events by 900 MHz MR imaging stem cells [50].

### 5.1. MRI contrast labeling of stem cells: source of contrast in images

Tracking of transplanted stem cells and their behavior in native tissue is done using stem cell MRI contrast agents such as gadolinium (Gd) chelating agents (Gd-DTPA) and manganese chloride ( $MnCl_2$ ) [51–53]. Mostly, these image contrast agents provide longitudinal relaxation constant (T1) enhanced positive contrast effects of stem cell originated proton nuclear magnetic resonance (NMR) relaxation as shown in **Figure 4**. Recently, several stem cell specific iron oxide paramagnetic/super paramagnetic contrast agents emerged as negative contrast agents.



**Figure 4.** The metal (M) is encapsulated in chelator coat (Gd-DTPA in dextran coated SPIO). The water accessibility ( $\tau_m$ ), rotational tumbling time ( $\tau_r$ ), electron spin state  $T_{1e}$  of superparamagnetic metal, chemical exchange rate ( $\tau_{ex}$ ), and MR frequency ( $\delta$ ) are shown as source of contrast. On right: Distinct longitudinal relaxation T1 constants as graphs at different contrast agent concentrations are shown in panel A and respective T1 images are shown in panel B. Reproduced from [52] with permission.

Iron oxide nanoparticles produce strong transverse relaxation constant/dephased transverse relaxation constant ( $T_2/T_2^*$ ) ratio as negative contrast effect due to dephasing effect [54–56].

## 5.2. Iron oxide particle stem cell labeling

Super paramagnetic iron oxide nanoparticles (SPION) are family of paramagnetic/ superparamagnetic contrast agents. It consists of a ferrite (maghemite or magnetite) core and a polymer coating. Depending on the diameter size (including both metal core and polymer coat), the nanoparticles can be divided into the SPION (diameter size 60–150 nm), USPIO (diameter size 10–40 nm), and MION (diameter size 10–30 nm) categories [57]. Ferucarbotran (Resovist®) and ferumoxides (Endorem or Feridex®) are MRI enhancement contrast agents approved by FDA for clinical diagnosis of liver tumors and metastatic lymph nodes. High concentrations of ferromagnetic material can shorten both the  $T_1/T_2$  constants as well as the effect of  $T_2^*$ , resulting in a significant reduction in MR relaxation with higher biological safety of stem cells [48, 58–60].

## 5.3. Positively charged polymer transfection agents

Most of these labeled stem cells do not internalize SPIONs and they need endocytosis polymer boosters. Positively charged polymer transfection agents (TAs) or polycations enhance the endocytosis across negatively charged membrane surface. So, they can be coated on the surface of magnetic iron oxide particles to boost SPION endocytosis or stem cells nonspecifically uptake these SPION particles through the negatively charged membrane surface. At present, composites of SPION and polycation TAs are the most commonly used methods to enhance the endocytosis of iron oxide particles [49, 60–62].

- Negative charge on stem cell membrane surface does not permit ferric oxide particles to attach them with the stem cells. To accomplish it, iron oxide particle surface modifications can enhance cellular endocytosis. In this direction, several surface modification approaches of polycation binding, incubation with hematopoietic cells, monoclonal antibody-antigen binding, receptor binding, magnetoelectric perforation and others are used in but these are still in infancy.
- Positive charged polycation TAs macromolecules such as polylysine or protamine sulfate are used in making SPION/TA composites with strong positive/negative interaction or cationic polymer material coating [63]. These SPION/TA composites easily adhere to the surface of stem cell membranes and persuade the phagocytosis of iron oxide particles without aggregation of SPION particles [51]. Ferumoxides (Feridex) with USPIO (MION-46 L) and added polycationic TAs have been in use to raise the concentration of intracellular SPION particles [32]. After 4–48-hour incubation with 25  $\mu\text{g}$  Fe/mL TA-(USPIO), target stem cells demonstrated a significant reduction in  $T_2$  signal intensities due to dephasing effect [52]. Ferumoxides mixed with protamine sulfate (50:3)  $\mu\text{g}/\text{ml}$  offers an optimized protocol [53].
- Overnight incubation of the human mesenchymal stem cells with hematopoietic  $\text{CD}34^+$  stem cells and specific mammalian cells, increases the iron content in the stem cells 1.47–17.31 pg/cell [64].



- Monoclonal antibodies of pancreatic cancer specific antigen (PAP2a) fused with dextran-modified SPIONs show antigen-antibody reaction to target the iron oxide particles in pancreatic cancer cells and promote the receptor-mediated SPIO endocytosis [65].
- Iron oxide particle surfaces can be modified by specific target receptors such as vascular cell adhesion molecule-1 and membrane mucin A5. These nanoparticles target specific tissues or organs, but the presence of specific target receptors limits the application of modified nanoparticles in cell tracking [66, 67].

#### 5.4. Magnetolectric perforation method

It increases the efficiency of nanoparticle endocytosis. Toxicity testing of mesenchymal stem cells, neural stem cells and adipose cells in vitro all indicate safer use of magnetolectric perforation because of less cell incubation time and effective safer SPION contrast agent to target cells approved by the FDA [68, 69]. Still, stem cell transplantation biological safety considerations need attention and further research.

#### 5.5. Biosafety of iron oxide particle labeling on stem cells

Cell labeling with iron oxide requires intensive toxicity evaluation tests for every protocol and characterization of cell type before translating them in clinical application. Feridex<sup>®</sup>, Resovist<sup>®</sup>, and Endorem<sup>®</sup> are FDA-approved agents. These agents in stem cells are cleared by the reticulo-endothelial system. Peripheral blood mononuclear cells labeled in vitro with Ferumoxide<sup>®</sup> upon administrated these cells through intravenous injection in organs showed localized T<sub>2</sub>\*-weighted images and R<sub>2</sub>\* maps of cell migration at the tissue inflammation damage areas [65]. However, extensive experiments are needed to verify the bio-safety of paramagnetic SPION contrast agents.

#### 5.6. Sensitivity of in vivo MRI detection of labeled stem cells

The intracellular iron distribution in stem cells influences greatly the MRI detection signal from labeled stem cells. Several inherent factors are determinant of image quality such as MRI sequence selection, spatial resolution, magnetic field intensity, and surrounding stem cell or cardiac tissue heterogeneity to affect the molecule sensitive signal. Known factors are: (1) Higher intracellular iron content in cells shortens the relaxation time; (2) The T<sub>2</sub>\*-weighted image is highly sensitive for iron oxide particle labeling load; (3) Field inhomogeneity and surrounding tissues; and (4) MRI sensitivity can reach 3000 times that of T<sub>1</sub> weighting or 60 times that of T<sub>2</sub> weighting due to iron oxide-induced dephasing effect [54]. To nullify the iron-induced MRI signal sensitivity, specific techniques are chosen. T<sub>2</sub>\* sequence or steady-state free precession (SSFP) is a choice to detect SPION-labeled cells. However, the T<sub>2</sub>\* sequence gets artifact by intracellular magnetic field inhomogeneity and interference of the surrounding normal tissues at high magnetic field. Fast 3D gradient echo (GE) sequences balance this effect of T<sub>2</sub>\* sensitivity, spatial resolution within imaging time. At the present time, the best choice is gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP), to create a positive contrast of SPION free from T<sub>2</sub>\* artifacts and high sensitive and specific hyperintense signal of cell tracking even for smaller imaging voxel size in the high field MRI [55].

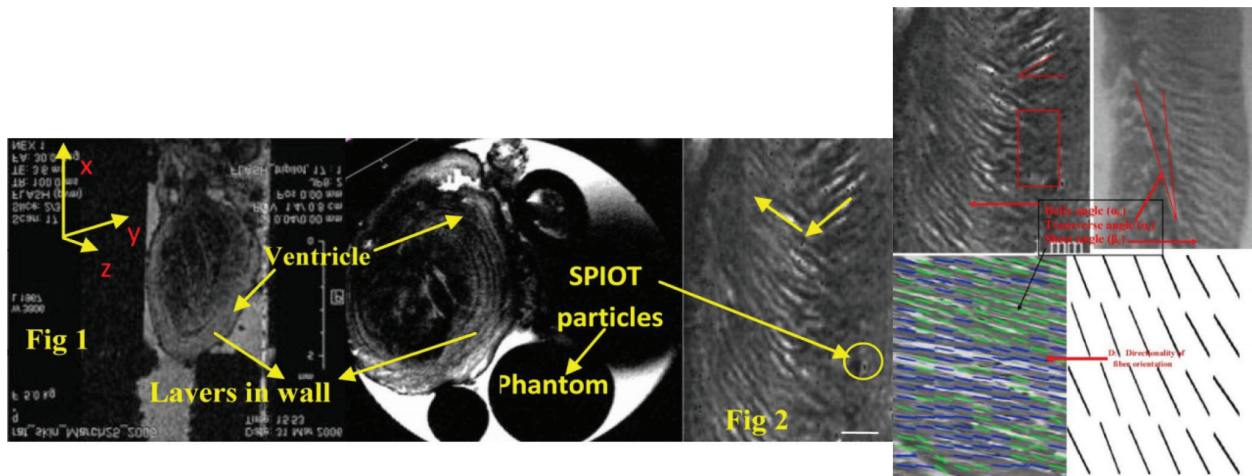
### 5.7. Limitations of tracking SPION labeling stem cells

SPION particle cell tracking method for cell labeling has some shortcomings. The MRI signal in preclinical or clinical studies is usually generated from surrounding tissue areas of noninterest cardiopulmonary junction [56]. The paramagnetic material usually accumulates in hemorrhagic infarction. So, hemoglobin shows false low signal intensity on T2\*-weighted image [70]. In case of death and rupture of transplanted cells, targeted SPIO nanoparticles can be trapped in surrounding tissue cells or reticuloendothelial cells. Subsequently, SPION are redistributed, deposited, or differentiated in extracellular environment to generate false positive signal. In author's opinion, direct iron oxide labeling is only suitable for short-term stem cell tracking in vivo or in vitro experiments. Other reason of false negative signal can be partial volume effects or low concentrations of cells in one imaging voxel. After every cell division, intracellular iron content remains half. So, every cell division evidences gradual reduction in cell detection sensitivity. The said fact was reported as MRI nonvisible heart cells after 6 weeks post-transplant stem cell administrated to the heart [71]. Despite these limitations and shortcomings, paramagnetic/superparamagnetic iron oxide particles are still highly popular in the field of stem cell tracking because of their high sensitivity.

### 5.8. Reporter gene labeling in stem cells

Reporter gene labeling is other method based on fusion of an MRI reporter gene to a target gene in stem cells. In transfection of a target stem cell, genes are incorporated into the cellular DNA via transgenic methods. These products of reporter genes are expressed in living stem cells and produces reporter gene expression as indirectly MRI visible in vivo. Transgenic gene labeling methods are highly valuable in long-term studies of labeled stem cell survival, proliferation, and differentiation in vivo. The MRI reporter gene expression can make two products of its expression in stem cells: (1) Intracellular enzymes including  $\beta$ -galactosidase, cytosine deaminase, creatinine kinase, tyrosinase, and arginine kinase [72]; (2) Ferritin or transferrin receptors [73]. Recently, a MRI reporter gene (a ferritin receptor) has emerged as a choice of robust contrast. Excessive expression of ferritin can increase iron uptake. Inside cells, redistribution of intracellular iron enhances transverse relaxation rates and reduces T2 relaxation constants. Recently, adenovirus-ferritin reporter gene injection into murine corpus striatum generated robust contrast on T2 and T2\*-weighted imaging within 5–39 days [74].

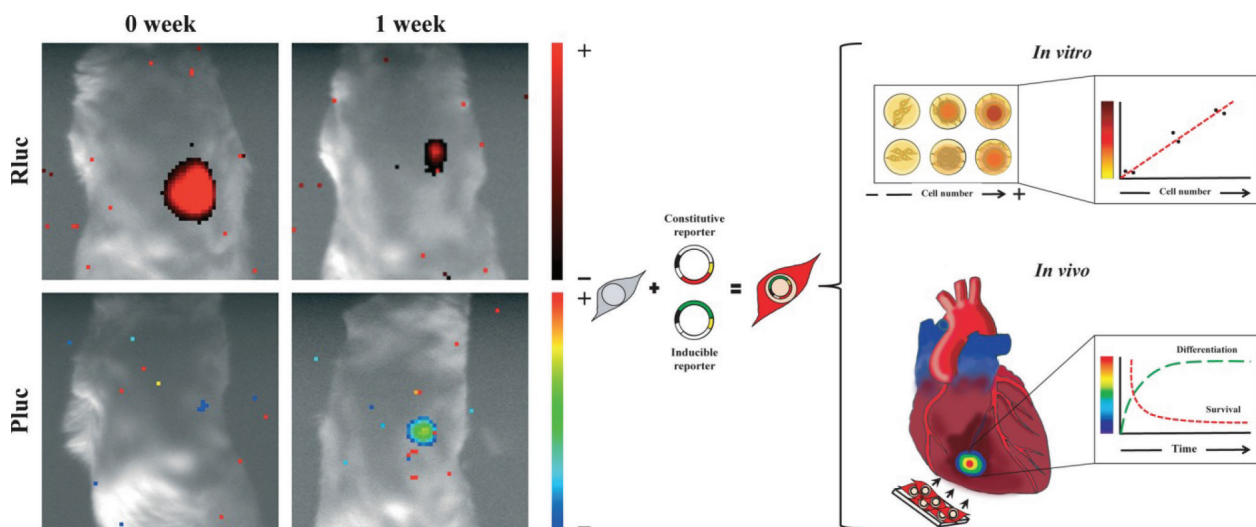
Clearly MRI reporter gene imaging is still a choice, but it cannot rule out the potential damage to cell proliferation and differentiation. Still open issues are the sources and safety of cells, issues relating to gene mutation and sensitivity [75]. Now, MR microimaging technology has advanced with available 900 MHz magnetic fields to visualize cardiovascular myofibrillar territories up to 30 micrometer resolution using SPION and SPOIT nanoparticle-enhanced relaxation susceptibility signal intensities of revived cardiac muscles enough to decipher the insight of stem cells as shown in **Figure 5**. Author developed mice beating heart microimages using antibody-coated nanoparticles to visualize cardiac muscle orientation angles as fingerprints of cardiac revival and rejuvenation [76]. It can be easily noticed that dying heart left ventricle wall clearly shows the damage sites with clear muscle mass with altered orientation of angle proportional to degree of distortion.



**Figure 5.** Monitoring cardiac cells by 900 MHz MR microimaging on left panels. Axial image shows details of muscle fibers in ventricle wall (shown as arrows). Cardiac muscle fibers are shown on right panels with superparamagnetic iron oxide troponin nanoparticles (shown in circle) to indicate angles of muscle fiber orientation on right panels. On top right, change in muscle fiber orientation angle is shown before and after infarction (shown as vector directions).

### 5.9. Regenerating stem cell *in vivo* dual optical imaging

Cellular engraftment may be monitored by reporter gene construct (fluc-mrfp-ttk) visualization by optical methods such as bioluminescence (BLI), chemiluminescence combined with MRI, PET, fusion multimodal imaging (FMI), near infra-red (NIR), and radionuclide methods [77]. The dual-modality imaging has unique strength to monitor cell delivery, survival status, graft morphology, and impact on post-MI remodeling on same platform in less time [78]. Recently, application of BLI for tracking transplanted stem cells was reviewed on the association of stem cell viability with the therapeutic efficacy of stem cell evaluated in pre-clinical disease models of vascular disease [79]. Reporter gene technology with BLI provides



**Figure 6.** Noninvasive bioluminescence (BLI) monitoring of cardiac differentiation in the experimental model of acute myocardial infarction shows BLI images showing decrease in RLuc by CMV promoter and increase in PLuc by cardiac-specific cTnI promoter in adipose tissue-derived progenitor cells after myocardial implantation. BLI can finely quantify cardiac regeneration degree relative to the number of surviving cells under ischemic conditions. See Ref. [80].

valuable information about the location and functional status of regenerative cells implanted into numerous animal models of disease to define the effectiveness and underlying mechanisms of cardiac cell therapy. The light-emitting capability of BLI illustrates the insights of cardiac regeneration [80]. Recently, survival kinetics of induced pluripotent stem cell and engraftment of viable cells was monitored by BLI imaging by visualizing the retention of bioluminescent agents in adult stem cells as shown in **Figure 6** to monitor stem cells [34]. Efforts are still continuing for regenerating the heart and using myocardial stem cells in cardiovascular system in treatment of heart disease or remodeling [81]. Now, new trend of noninvasive in vivo MR imaging with spectroscopy is emerging to visualize cardiac muscle metabolites [81] and products of gene expression or imaging reporter gene induced inhomogeneity signal peaks from regenerating stem cells [82].

## 6. Future perspectives

Future research may focus on conversion of adult cells into iPS cells, and conversion of these iPS cells to relevant cell types to treat individual diseases. In near future, multimodal single platform bioluminescent/NIR/FRET optical cum MRI/CT/PET microimaging techniques will emerge to track the pluripotent stem cell sensitive superior detection methods by monitoring the distribution of molecular events in differentiating myocardial progenitor cells in less time. It remains to see in coming years if differentiating stem cells remain safe and stem cells are not affected by radionuclide, chelators, contrast agents, and electromagnetic radiations used to image these cells. To expose these stems cells in different preservative media solutions for storage without any effect on their capability to remodeling is also a challenge in tissue engineering art. Before transplantation and regrafting, it needs thorough investigation of perfect autograft and metabolic compatibility, myocardial contractility to remain viable longer. Larger double blinded placebo-controlled clinical trials are needed on trans-aortic or trans-septal approach to reach different zones of endocardial necrosis. In cases of intramyocardial or epicardial necrosis, epicardial approach should be compared with endocardial one. Brachial can be an alternate option for patients who have peripheral vascular disease with difficult femoral approach. Safer delivery of stem cells to the heart opens vista of transplantation of stem cells as tissue-engineered constructs.

Throughout life, every person experiences many injuries and recovers with time spontaneously by wound healing, organ recovery, or repair mechanisms without even realizing the past injuries in the first place. In this repair and wound healing process, proliferation of existing stem cells makes an individual capable of repairing or restoring the injured tissue(s). In fact, these pluripotent stem cells contain the genetic fingerprint or molecule metabolic blueprint as memory of tissue origin how a particular tissue cell was assembled from biomolecules and functionalized into physiochemical units of organs constructed to begin with from embryonic progenitor cells. If these pluripotent stem cells are maintained artificially in physiological cultures, rejuvenation potential of stem cells maintains all properties of bio-transforming and differentiating into organ cells. This potential offers an excellent opportunity of clinical applications. In fact, these restorative potentials in stem cells are possible due to simultaneous multiple functions of stem cells, such as self-renewal, multipotency, and paracrine functions. Of mention, paracrine secretion releases colony-stimulating factors,

growth factors, regulatory energy molecules, and stimulatory cytokines from a number of retained stem cells during regenerative processes at tissue sites as shown in recent clinical trials in **Table 3**. These secretory molecules lead to further mobilization of endogenous progenitor cells. We do not understand the complete sequence of underlying mechanisms of stem cell during regeneration and cardiac healing, even though everyone experiences the benefits of cardiac rejuvenation even without complete knowledge of origin of electrophysiology of heart, cardiomyocyte functions, and mechanism of molecular events.

The embryonic stem cells have excellent capacity to differentiate into virtually any type of tissue cells [83]. Presently, investigators and government agencies have intensified the detailed search for a similar cell lineage or stem cell rejuvenate database in adults [84]. However, many challenges remain to understand how these adult stem cells over-ride the complex tasks (failed heart in to beating heart again) to take up residence quickly when placed in just the right place to gain control and restore or correct the necessary cardiomyocyte shape to assume paracrine functions to perform their multiple plasticity functions in a complex different cellular environments (rejuvenation). Other major challenge is perfect retention of these cells after implantation via intracoronary, intramyocardial, and retrograde coronary sinus approach. In fact, a significant percent of stem cells leaves the heart soon after implantation and stem cell administration before they stick at damage site [84]. So, the clinical ramifications may be significant but they are limited. One fact is clear that remarkable universal nature of stem cells offers the exciting possibility of a universal stem cell transformation capability into any tissue cell or organ that can circulate throughout the body and reside wherever needed to promote regeneration or repair of local tissue if retention of stem cells is good. These stem cells have multiple functions and behave proangiogenic and proparacrine, thereby stem cells may consume or produce potentially detrimental substances as indicated in recent clinical trials shown in **Figure 7**, while stem cells may also survive in nontarget organs [85].

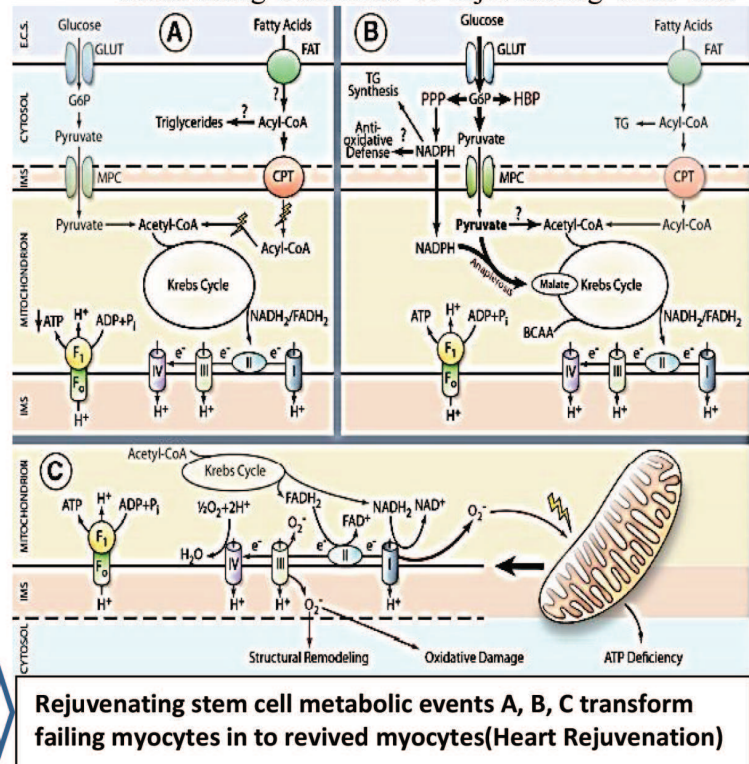
From clinical practice standpoint, the major hurdles to the clinical application and translational research in regard to adult stem cells are the limited small number of stem cells isolated from any adult tissue with successful propagation and harvesting of multipotent adult stem cells [86]. Other hurdle is the development of perfect “stem cell cocktails” to optimize the proliferation and of adult stem cells and differentiation in timely manner [30]. These hurdles indicate the urgent attention on supervised expansion of adult stem cells in cultures uniformly keeping stem cell intrinsic properties intact may be the answer to stable retention [87]. Although extensive cultures of human adult cells may suddenly change the intrinsic properties of stem cells in vivo [88], putting them unfit rendering them with no restoring capability to repair or reverse the injured or diseased tissue in prospective heart failure patients.

Author offers his opinion that cardiac stem cell therapy in future will have an acceptable wide spectrum of preclinical and double blinded placebo-controlled clinical trials on trans-aortic or trans-septal approaches solving the issue of epicardial or endocardial necrosis in cardiovascular regenerative medicine as shown in **Table 3** with emphasis on intracoronary and retrograde coronary methods or possibly combined with 3D scaffold biomatrices delivery. In development of engineered and constructed scaffold, intensive investigations will introduce new rejuvenator secretory molecules in remodeling and metabolic regulation to provide insight of right choice and optimization for best cardiac repair. Researchers may explore

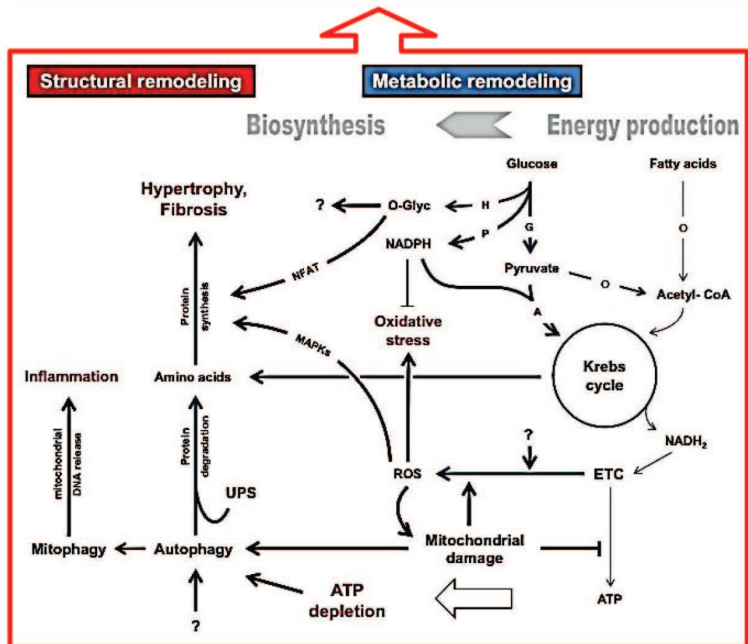
**Secretory molecules in remodeling**

- Cadherins (N-,P-,E- types)
- C-Jun+c-Fos = AP-1
- ckit(CD117)
- Epidermal Growth Factor Receptor
- Erythropoitin (wound healing)
- ERK
- Beta-Fibroblast Growth factor
- Gaunylin peptide for GMP
- Glycogen Synthase Kinase 3
- Heat Shock Proteins (Hsp60,70,90)
- Hepatocyte Growth Factor(HGF)
- HGF Receptor (Tyr Kinase activation)
- Human Epidermal Receptor/neu
- HMGB(High Mobility Group Box)
- Hypoxia Inducible Factors
- Intracellular cell Adhesion Molecule(ICAM)
- Insulin like Growth Factor Receptor
- Insulin Receptor (CD220)
- Insulin Receptor Substrate(MAP Kinase)
- Jun N-terminal kinases(JNKs)
- MAP Kinase Activated Protein Kinase 2
- Matrix Metalloprotease(MMPs) (Zn rich)
- Mutagen Activated Protein Kinase
- Osteocalcin
- Osteonectin (Glycoprotein)
- Osteopontin(OPN) (Sialoprotein)
- Osteoprotegerin(OPG)
- p38
- p53
- p70S6 Kinase (Ser/Thr Kinase)
- Platelet derived Growth Factors
- Poly(ADP-ribose) Polymerase (PARP)
- Protein C (Ser Protease)
- Chemokine(C-C motif) Ligand 5(RANTES)
- Rb
- Resistin
- SAA (Serum Amyloid A)
- SAPP-Alpha and Beta
- Selectins
- STAT
- Tau Proteins
- Transforming Growth Factors
- Thrombopoitin
- Thrombomodulin
- Thrombospondin 2(TSP2)
- TIMP (Metallopeptidase Inhibitor)
- TNF Receptor
- Vascular Cell Adhesion Molecule(VCAM)
- PI<sub>3</sub>Glycan Biosynthesis Class F Proteins(PIGF)

**Remodeling Functions of rejuvenating stem cell\***



**Rejuvenating stem cell metabolic events A, B, C transform failing myocytes in to revived myocytes(Heart Rejuvenation)**



**Figure 7.** Different secretory molecules are shown either synthesized or released from damaged myocardium from altered cardiomyocyte metabolic pathways (shown in left panel). The process of rejuvenation in stem cells is shown to correct the metabolic events (see at top on right) to lead repair, rejuvenation and restoration of cardiomyocyte viability with improved functions by remodeling in metabolic steps (shown in bottom at right).

more options of differentiated stem cell remodeling in addition to the engineered constructs, rejuvenative molecules and regenerative metabolic pathways highlighted in clinical trials shown in **Figure 7**. What secretory molecules and metabolic regulatory events are common in

differentiated stem cell remodeling?. During remodeling, substrates are transported across the extracellular membrane into the cytosol and are metabolized in various ways. For oxidation, the respective metabolic intermediates [e.g., pyruvate or acylcoenzyme A (CoA)] are transported across the inner mitochondrial membrane by specific transport systems. Once inside the mitochondrion, substrates are oxidized or carboxylated (anaplerosis) and fed into the Krebs cycle for the generation of reducing equivalents [reduced nicotinamide adenine dinucleotide (NADH)<sub>2</sub>; reduced flavin adenine dinucleotide (FADH)] and GTP. The reducing equivalents are used by the electron transport chain to generate a proton gradient, which in turn is used for the production of ATP. This principal functionality can be recovered in various ways during reverting heart failure (HF), thereby regaining ATP production or improving cellular function in other many ways. Researchers may explore more molecular options of remodeling in addition to the molecules and regenerative metabolic pathways shown in **Figure 7**.

Mainly two types of cardiovascular tissue biomaterials synthetic (polymer, ceramic, or metals) and biologic (cell-based, extracellular matrix-based, whole tissue) and hybrid biomaterials will be available. Advanced therapy medicinal products (ATMP), cardiomyocytes, ECM hydrogels and scaffolds, urinary bladder matrix (UBM) scaffolds, glycosaminoglycans (GAGs), collagen, fibronectin and laminin matrix, endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), poly-tetra fluoro ethylene (PTFE), cardiac patches, cell-seeded matrices, and pericytes seeding with biodegradable tissue engineering-based graft (PLLA/PCL, designed to be degraded in 3–5 years) are currently used methods for dynamic seeding with total BMCs or selected bone marrow aspirate mononuclear cells (BMMNCs). Electrospinning is routine to design scaffolds. For heart regeneration, cell delivery vehicle is implanted using smooth muscle cells (SMC), fibroblasts, endothelial progenitor cells, embryonic CD 34 stem cells, BM cells, tissue-engineered vascular grafts are becoming promised biomaterials. Vascular CorMatrix® patch, vascular grafts made of PG/PGA, PCL/PLA polymers offer clinical use [107–109]. The stem cell treatment will have a universal role in reversing the aging process, although a natural phenomenon. Naturally with the aging process, there is a continuous decline in stem cell number and their viability or physiochemical cardiac capability with time. Due to these facts, aging and heart diseases are interlinked and advancing age promotes organ diseases. Therefore, restorative repairing capability of stem cells may provide a renewable life, and a “fountain of youth” as evidenced by jelly fish rejuvenation.

## 7. Conclusion

The in vivo imaging techniques are useful in dynamic monitoring of cardiac stem cell therapy following myocardial infarction. Choice of stem cells and mode of delivery are very crucial in getting successful stem cell therapy positive outcome. Cardiovascular remodeling evaluation by MRI has merits because it is safe, sensitive, lacks radiation, provides good resolution, generates a real-time events’ blueprint or first-hand information of myocardial viability with functional information of cardiac territories and their physiochemical changes in cardiac functions during stem cell rejuvenating process and after myocardial repair. Present time, ultra-high magnetic field CMR possibly has preclinical prospects as in vivo noninvasive molecular

imaging or restorative monitoring reporter of rejuvenating stem cell genes to evaluate success of transplantation and cardiac repair. On the other side of coin, researchers are continuously developing new real-time physiological cum functional MRSI options to explore new stem cell molecular probes and smart MRS imaging sequences with improved MRI sensitive specific stem cell differentiation and rejuvenating detection by targeting energy metabolites, myocardial viability, and vital physiochemical molecules. Noninvasive monitoring is necessary and bioluminescence or other radionuclide methods may be alarming because the potential biological damage caused by radionuclide exposed reporter genes and bioluminescence induced immune responses is concern in differentiating stem cells. Seriously, all these issues need research to minimize artifacts within safe limits. With the help of stem cell imaging and monitoring, transplantation of stem cells sooner or later will be optimized for the effective long-lasting therapy of myocardial infarction and heart failure on some day.

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