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REVIEW

Therapeutic Efficacy of Cellular Transplantation for Cardiac Repair

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ABSTRACT

Cardiovascular problems have been known as largest cause of mortality in the world. It has been reported that only in U.S., 16.9 million deaths happen annually just because of coronary heart disease. Stem cells have shown a promising potential in regenerative medicine and more than 25,000 clinical trials have been completed so far. Stem cell researchers have ultimate aspiration to have successful cardiac regeneration and are working for the successful cardiac phenotype adaptation of transplanted stem cells. Obstacles and potential stem cell therapeutic risks associated with their clinical applications such as poor survival of the donor cells after engraftment etc, have reduced the translational potential of this research. In this review, we have focused on the advancement in cellular therapeutic efficacy for cardiac regeneration. It has been proposed that some engineering techniques applied to enhance therapeutic potential of stem cells have shown promising potential for daily routine clinical practice. Pre-clinical and clinical data have been presented to justify this improvement. The mechanisms to engineer cells for induction or alteration in stem cells for targeted therapy need recommendations for future clinical work to have a state-of-art approach in cardiac regeneration.

Keywords: Stem cell therapy, Cardiovascular diseases, Targeted cell therapy, Genetically modified cells, Transplantation

Stem cells are proliferative cells having self-renewal ability. Because of their promising regenerative potential, these cells have been applied to treat number of diseases. According to the US clinical trials registry database (www.clinicaltrials.gov), near about 30,000 clinical studies regarding cell therapy has been registered and stem cells account for 5000 studies. A multitude of factors have been studied carefully like cell homing, viability, engraftment, safety profile, cellular delivery time and retention (Loughran *et al.*, 2013). Poor survival, very low homing, possible teratogenic effect, massive apoptosis of transplanted cells, low cellular retention and viability have

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questioned the effectiveness of stem cell therapy and controversies flawing its clinical potential are in discussion.

In spite of having such type of limitations, stem cell therapy has gain a monumental advances for a number of clinical problems like cardiac regeneration (Le Huu *et al.*, 2012). Stem cell-based cardiac regeneration is under studies for a wide range of diseases such as acute and chronic ischemic myocardial damage, cardiomyopathy ,etc. Technical developments in last decades to solve cellular transplantation problems have been seen and cells are engineered or modified for enhanced grafting and improved regeneration (Pendyala *et al.*, 2008). Cellular engineering or modifications have gained considerable attention and scientists could developed techniques for enhanced pluripotent behavior, increased stem cell homing and retention etc (Ruvinov *et al.*, 2012).

Cardiovascular Problems and Treatments

Cardiovascular diseases (CVD) are the causing 30% global deaths every year. So far, 17 million cases of coronary heart diseases are being registered in United States only, out of which 5.3 have lost their cardiac functions (Loughran *et al.*, 2013). The most common cause of cardiac failure is ischemic heart in which oxygen supply is limited and depletion of adenosine triphosphate (ATP) happens. This cardiac failure occurs in three major steps i) coronary occlusion, 2) angina pectoris and 3) coagulative necrosis (Soonpaa *et al.*, 1994). CVDs consist of group of diseses that have been shown in Figure 1. This figure also demonstrate US prevalence of each categorise.

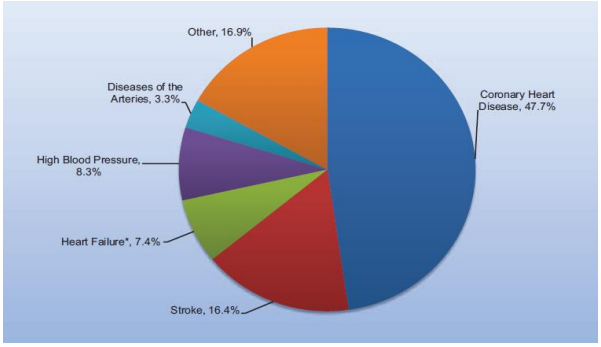

Figure 1. Percentage breakdown of deaths attributable to cardiovascular disease :United States- 2011 (Mozaffarian *et al.*, 2015)

On the other hand, an estimated 85.6 million American adults (>1 in 3) have ≥1 types of CVD. Of these, 43.7 million are estimated to be ≥60 years of age, but younger people may have this disease with lower chance. Figure 2 shows CVDs isn't specific to a gender or age.

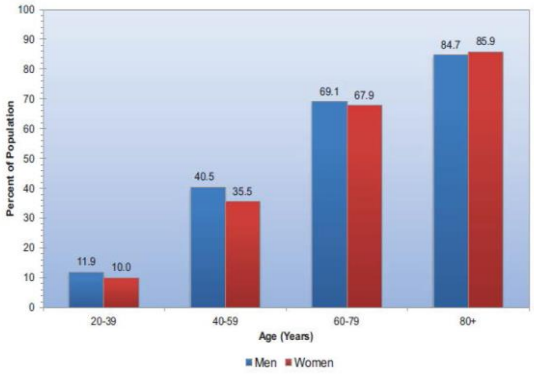

Figure 2. Incidence of cardiovascular disease (coronary heart disease, heart failure, stroke, or intermittent claudication; does not include hypertension alone) by age and sex (Mozaffarian *et al.*, 2015).

So we can say CVDs is the most known cause of the mortality in th word. Unfortunately, every year many humans dye due to CVDs (figure 3). Even in a comparative study has been reported that CVDs are more dangerous than cancer (figure 4)

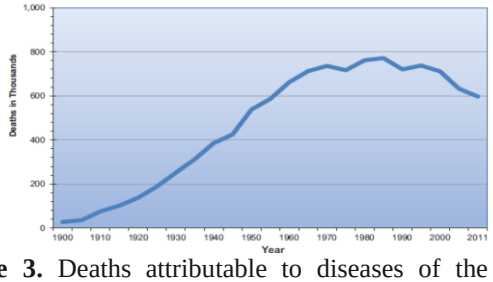

Figure 3. Deaths attributable to diseases of the heart (United States: 1900–2011) (Mozaffarian *et al.*, 2015).

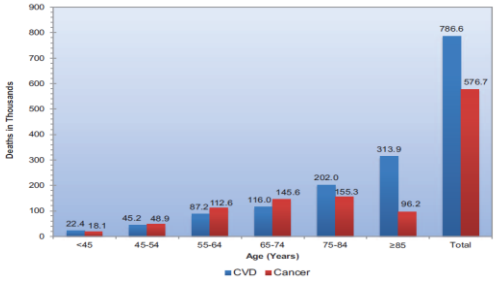

Figure 4. Cardiovascular disease (CVD) deaths versus cancer deaths by age (United States: 2011) (Mozaffarian *et al.*, 2015)

A heart is composed of cardiomyocytes which are constantly active, highly metabolic and regulate heart beats, comprising 80-90% of the heart volume and a number of supporting cells such as endothelial cells and smooth muscle cells creating a dense vascular network and fibroblasts responsible to generate a collagen-dense matrix (Lanza *et al.*, 2011).

Studies in regenerative medicine have proved that all organs of body can regenerate themselves and so as heart which has ability to recruit and mature the resident stem cells (Gonzalez *et al.*, 2008), but this regenerative potential is very limited and cannot repair damaged tissue resulting heart failure (Lyngbaek *et al.*, 2007; Urbanek *et al.*, 2005). It has been proposed that transplantation of exogenous cells/stem cells can enhance regenerative mechanism and compromised heart function can be improved.

Current gold standard CVD therapies are known as medical interventions such as pharmacological and surgical interventions. Pharmacological interventions such as b-blockers, diuretics, angiotensin-converting enzyme (ACE) inhibitors and surgical interventions such as mechanical ventricular assist devices (m-VADs) and heart transplantation are known as gold standard approaches to treat CVD (Chang and Towbin, 2006; Moffett and Chang, 2006). Pharmacological interventions may disturb hormonal system of patients so neurohormonal regulation is closely monitored during such a kind of chemical interventions (Chang and Towbin, 2006; Jefferies *et al.*, 2007).

All these therapeutic interventions are not sufficient to restore cardiac functions completely. Their effects are for short term and have a number of associated side effects. Although surgical implantation of m-VADs shown improved functioning but infection and blood clots are continuous complications. Organ availability and surgical expertise are the only successful approaches (Fukuda, 2001). Search for alternative approaches such as transplantation of modified stem cells are going on and number of successful clinical studies have been reported making it the next gold standard for CVD.

Except of cell/stem cell therapies, various treatment strategies have resulted in improved survival but mortality rate associated with cardiovascular diseases is rising on. According to American heart association, improved survival rate is obtain because of regular checkup and improvements in early diagnosis.

Cell Therapy in Cardiac Regeneration

Cellular therapy or stem cell therapy is the transplantation of stem cells to repair cardiac tissues. Among the innovative approaches which has received utmost aspiration to treat CVD (Moffett and Chang, 2006; Chang and Towbin, 2006), stem cells have potential to differentiate and adopt cardiac phenotype and regenerate damaged tissues, e.g. in case of ischemic myocardium, these cells participate in angiogenesis to restore regional blood flow**.** The potential of different types of stem cells e.g. Embryonic Stem Cells (ESCs), Skeletal Mmyoblasts (SMs), Blood and Bone marrow-derived cells, Resident Cardiac Sem cells (CSCs), Endothelial Progenitor Cells (EPCs) and induced Pluripotent Stem Cells (iPSCs) have been explored in cardiac regeneration (Passier *et al.*, 2008; Segers and Lee, 2008; Siepe *et al.*, 2008). Cells differ markedly in regard to their site of origin as well as characterized by surface markers, transcription factors, and expressed proteins (figure 5).

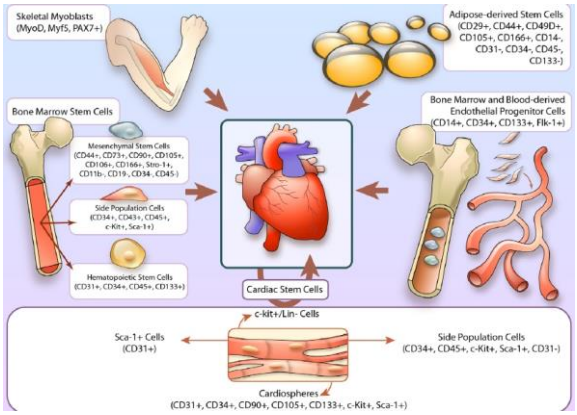

Figure 5. Sources of stem cells used for cardiac repair (Sanganalmath and Bolli, 2013).

Strategies in Cellular Modifications to Enhance Cellular Transplantation

The stem cell transplantations for treatment of heart failure have modest effect or no effect. In addition to problems in the best method of cell delivery, the treatment time, and patient selection, an important common issue the transplantation of stem cells faces

is that the survival rate in the host is very low. Because they are transplanted in an ischemia, hypoxia, and proapoptotic niche, most stem cells cannot survive after transplantation (Tang *et al.*, 2005b). Another reason is the amount of cells which migrate to regions of myocardial infarction is too small. Only 1.3% to 2.6% of the 18F-FDG-labeled stem cells which were intracoronary injected migrated to the myocardium 2 hours after injection, while the majority of the cells moved to the tissue outside heart muscle (Hofmann *et al.*, 2005). In order to enhance the therapeutic efficacy of cellular cardiomyoplasty, multiple remedial measures have been adopted.

Preconditioning

In order to improve the viability of stem cells after transplantation and counteract the hypoxia-induced apoptosis, effective protection strategy to use various methods of preconditioning of stem cells before transplanting the cells into the damaged myocardium. Pre-treatment with materials such as growth factors, cytokines or physical (i.e. heat shock or ischemic pre-conditioning) treatment of cells prior to transplantation makes them resistant to tissue ischemia which may otherwise adversely affect cell survival and lower the consequent functional benefits of cellular cardiomyoplasty (Sakakibara *et al.*, 2002; Müller-Ehmsen *et al.*, 2002)

Hypoxic Preconditioning: In this method the cell is cultivated under hypoxic conditions before transplantation (Mingliang *et al.*, 2011). It is reported that hypoxic preconditioning of cells increased the expression of various genes related to antioxidant and survival signals remarkably (Figure 6) (Forward, 2007). Hypoxic preconditioning has been found to be able to start the PI3K/AKT signaling pathway and enhance the stability of HIF-1 to increase the antiapoptotic ability of MSCs (figure 7,8). The functional benefit of H-MSC transplantation might be explained by several possibilities: (1) HP enhances the autocrine and paracrine signaling of MSCs, which reduces apoptosis of transplanted cells and endogenous cardiomyocytes; (2) the increased survival of H-MSCs provides better and longer trophic support for the reparative process; (3) the increase in survival of engrafted cells contributes to increased angiogenesis. These factors collectively promote tissue repair and may provide a simple but effective new strategy for clinical MSC transplantation therapy (Hu *et al.*, 2008).

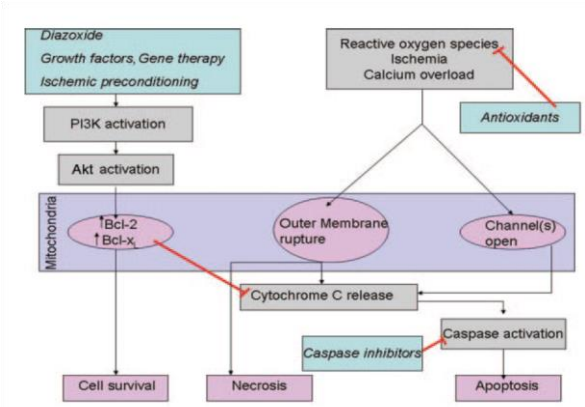

Figure 6. Mechanism of transplanted cell death and cell survival myocyte (Forward, 2007).

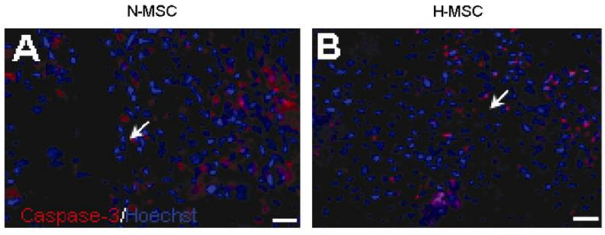

Figure 7. Effect of hypoxic preconditioning on implanted cell appotosis 1 to 3 days after myocardial infarction. A and B, Co-labeling of activated caspase-3 (red) and Hoechst (blue arrows) indicates apoptosis of graft cells in the ischemic heart 24 hours after myocardial infarction. Hypoxic preconditioning significantly reduced cell apoptosis of transplanted MSCs in the ischemic heart. In this expriment MSCs were labeled with Hoechst 33342 (blue) before transplantation. Cell apoptosis was identified with activated caspase-3 (Hu *et al.*, 2008).

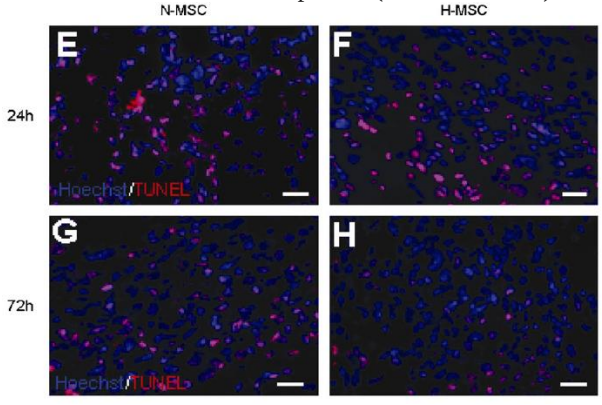

Figure 8. Effect of hypoxic preconditioning on implanted cell death 1 to 3 days after myocardial infarction. Cell death was identified with TUNEL staining. Co-labeling of

TUNEL (red) and Hoechst (blue) 24 hours and 72 hours after myocardial infarction in N-MSC and HMSC transplantation group, respectively. Hypoxic preconditioning significantly reduced cell death (Hu *et al.*, 2008).

Preconditioning with Growth Factors: By using stromal cell-derived factor-1 (SDF-1α) to precondition MSCs, Pasha et al. found that SDF-1 preconditioning through SDF/CXCR4 activated multiple signaling pathways, including the PI3K/AKT signaling pathway. The cumulative effect of PC on myocyte regeneration, angiogenesis, and cell survival in the ischaemic myocardium resulted in reduced infarct size and LV remodelling. Thus this novel, cell-based therapeutic approach has the potential in minimizing the adverse effects of ischaemia on cell death and cardiac remodelling. In this study *in vitro* data showed via using MSCs with SDF-1 preconditioning for the treatment of myocardial infarction in rats, it is found that the viability of MSCs which were transplanted significantly increased, and they got a better effect of myocardial repair. *In vivo* data in preconditioned group showed a robust cell proliferation, reduction in infarct size and fibrosis, and significant improvement in cardiac function (figure 9). Effects of SDF-1 PC were abrogated by CXCR4 antagonist (Pasha *et al.*, 2007).

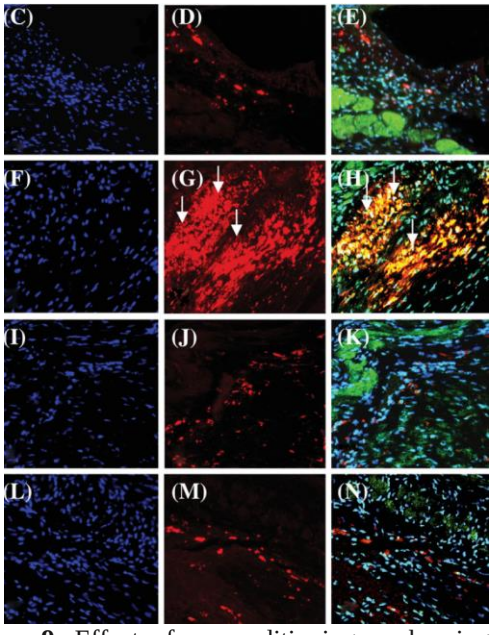

Figure 9. Effect of preconditioning on homing and proliferation of mesenchymal stem cells (MSCs) in infarcted myocardium. Representative immunofluorescen micrographs of hearts transplanted with PKH26 labelled MSCs. DAPI was used to identify nuclei. PKH26 labelling is in red; and a-sarcomeric actin is in green, yellow labelling indicates cardiomyocytes derived from PKH26 positive cells . A limited number of PKH26 labelled MSCs were observed in control group (C–E). On the other hand, extensive homing and proliferation of the PKH26 positive MSCs and cardiomyocytes derived from PKH26 labelled MSCs in infarct and peri-infarct regions were observed in SDF-1 preconditioned group (arrow) (F–H). PKH26 labelled cardiomyocytes appeared yellow when colocalized with a-sarcomeric actin staining (arrow) and were seen along the native myocytes (H). AMD group (I–K); SDF-1þAMD group (L–N). (Pasha *et al.*, 2007).

Factors such as basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF)-1 have been used to pre-condition MSCs, and enhance their reparative effect (fiqure 10) (Bartunek *et al.*, 2007). Additional paracrine factors that are secreted, and have beneficial effects are vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β, secreted frizzled-related protein (SFRP)-1 and SFRP-2 (Gnecchi *et al.*, 2008). The effects of these, and other paracrine factors, extend beyond their cardioprotective effects, and include favorable effects on cardiac metabolism, contractility, regeneration and neovascularization

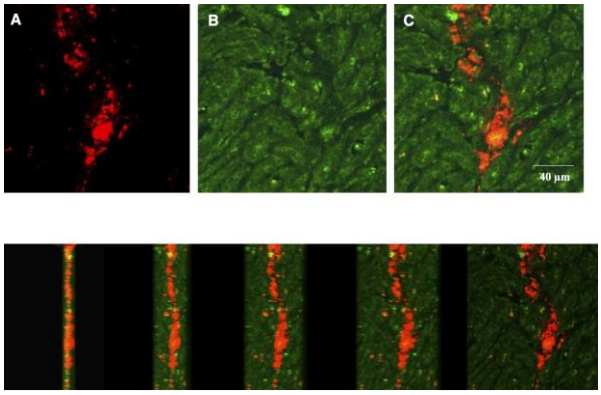

Figure 10. Confocal microscopy image of the tissue analysis 12 weeks after the injection. A : shows injected cells labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) (red color), and B shows staining for the cardiac-specific marker troponin (green color) Bottom: the staining in Fig. 3, panel I, is shown at various view angles to document the colocalization between DiI-labeled cells and cardiac markers (Bartunek *et al.*, 2007).

Combination Drug Therapy: Administration of drugs combination therapy can also improve the viability of MSCs, too. Statins used to reduce blood lipids in the past were considered to have good protective action. Yang et al. used simvastatin to conduct the combination therapy, and Xu et al. conducted the combination therapy with lovastatin. They both got remarkable curative effects. Then they thought that simvastatin and lovastatin could have a cytoprotective effect by inhibiting the mitochondrial apoptotic pathway to activate the signaling pathway of PI3K/Akt and ERK1/2 (Yang *et al.*, 2009; Xu *et al.*, 2008). In addition, Zhang et al. found that Chinese herbs Berberine can inhibit the hypoxia-induced apoptosis in vitro. The mechanism is also related to the inhibition of the signaling pathway of mitochondrial apoptosis (Zhang *et al.*, 2009).

Genetically Modified Cells: Obstacles inherent to the multipotent and proliferative nature of stem cells and the potential risks associated with the application of exogenous cells into the heart may reduce the translational potential of this research. The use of genetic engineering to induce or alter specific protein expression such as cytoprotective or angiogeneic genes in stem cells has already facilitated research in this field and may, additionally, offer a potential route for designing more efficient cell sources for cardiac repair. The uniqueness of the combined cell and gene therapy approach is attributed to the fact that it facilitates an inexpensive, safe, rapid, and site specific transgene delivery in vivo which may in turn, intricately influence the effectiveness of cellular cardiomyoplasty (Kirkton and Bursac, 2008). We will describe the applications of genetic engineering to improve the isolation, selection, and differentiation of stem cells prior to implantation as well as strategies to promote the retention, mobilization, survival, angiogenesis, anti-inflammatory and integration after implantation.

Enhancing the Selection of Cardiogenic Stem Cells: *Problem:* Pluripotent nature of stem cells have diverse regenerative capabilities, So specific applications of these cells require defined differentiation. For example, bone marrow-derived mesenchymal stem cells (MSCs) possess the capability to become muscle, fat, bone, or cartilage, but only the first of these our cell types repopulating the injured heart would be beneficial (Breitbach *et al.*, 2007). Furthermore, the injection of highly proliferative uncommitted embryonic stem cells may lead to tumor formation (Nussbaum *et al.*, 2007)
Solution: Selecting and isolating a pure population of stem cells exhibiting cardiogenic characteristics is crucial to the efficacy and safety of cardiac cell therapies. For example, selection for early mesodermal (Brachyury or T) and cardiovascular transcription factors (Isl1 and Nkx2-5) in mouse embryonic stem cells enables the simultaneous derivation of multiple heart cell types, including cardiomyocytes and smooth muscle cells (Wu *et al.*, 2006) or cardiomyocytes, smooth muscle, and endothelial cells (Kattman *et al.*, 2006; Moretti *et al.*, 2006). Specific cardiac muscle cells, such as ventricular myocytes, can be isolated using vectors incorporating the myosin light chain-2v promoter (Müller *et al.*, 2000), whereas atrial and nodal cells may be preferentially isolated under the control of the ubiquitous cardiac alpha myosin heavy chain promoter (Kolossov *et al.*, 2005).

Promoting Cardiogenic Differentiation of Donor Cells: *Problem*: If you solve problem 1 you still require a very large amounts of SCs to obtain sufficient quantity of cells.
Solution: Increase efficiency of SCs differentiations to Cardiomyocytes or to introduce cardiogenic potential in noncardiac cells.
For example, human MSCs genetically engineered to express myocardin, a dynamic cardiomyogenic transcription factor, attained a cardiac phenotype with an efficiency of 90– 100% when implanted into infarcted mouse heart. As a result, left ventricular function was increased, and detrimental ventricular remodeling was reduced, when compared with the use of control MSCs (Grauss *et al.*, 2008). Furthermore, human myocardial scar fibroblasts engineered to overexpress the myocardin gene attained cardiac gap junctions and became capable of transmitting action potentials and repairing conduction blocks within cardiac cocultures (van Tuyn *et al.*, 2007). Similarly, forced expression of the muscle-specific transcription factor, MyoD, in primary rat cardiac fibroblasts resulted in the formation of multinucleated myotubes, yielding a

potentially abundant myogenic source for cardiac cell therapies (Etzion *et al.*, 2002).

Facilitating Identification, Retention, and Mobilization of Implanted Cells: *Problems*: a) Lack of cell retention at site of injury. b) Tendency delivered cells to travel to another places such as liver or spleen rather than mobilize at infraction sites. C) Donor cell fair to remain at injury sites.
Solution: Facilitating Retention, and mobilization or migration of implanted cells to injury sites. For example, the retention of rat MSCs at the site of injection was improved three fold when the cells were genetically engineered to express tissue trans glutaminase (tTG), a fibronectin receptor that promotes integrin-mediated cell adhesion . The resulting improvement of cardiac function highlighted the fact that cell implantation therapies are unlikely to succeed if the donor cells fail to remain at the injury site (Song *et al.*, 2007). The damaged tissue expressed specific receptor or ligand, to guide corresponding stem cells to move and adhere to the injury. SDF-1/CX2CR4 is currently known to promote the homing of MSCs in on the injured tissue (Penn, 2009). SDF-1/CXCR4 cannot only promote the transplanted MSCs to migrate to the damaged tissue, but also inhibit apoptosis of MSCs, increase the survival rate and proliferation of MSCs (Yin *et al.*, 2011), and promote the homing efficiency of MSCs from many aspects.
The migration of MSCs from an intravenous rat tail injection to the site of myocardial infarction was increased more than twice when cells were retrovirally infected to overexpress CXCR4, a stromal-derived factor-1 (SDF-1) receptor (Cheng *et al.*, 2008). The release of SDF-1 from ischemic myocardial tissue has been shown to attract the circulating progenitor cells expressing CXCR4 to migrate to the injury site and, after binding to SDF-1, participate in endogenous tissue repair (Tang *et al.*, 2005a). As a result, the accumulation of genetically engineered MSCs within the infarct site translated into significant recovery of left ventricular function, whereas the rats injected with control MSCs exhibited no functional improvement.

Encouraging Survival of Implanted Cells and Regeneration of Host Tissue: *Problem*: Low survival in hostile ischemic or inflamed microenvironments. *Solution*: Overexpression of antiapoptotic or proangiogenic gene
Also genetically engineered expression, hypoxia inducible factor 1(HIF-1) (Dai *et al.*, 2007), fibroblast growth factor-2 (FGF-2) (Song *et al.*, 2005), within implanted bone marrow-derived stem cells has yielded up to fivefold greater survival relative to that of control cells.
In an experiments MSCs genetically engineered by the spermine-dextran complex with plasmid DNA of adrenomedullin (AM**),** and secreted a large amount of AM, an anti-apoptotic and angiogenic peptide. Transplantation of AM gene-engineered MSCs improved cardiac function after myocardial infarction significantly more than MSCs alone. Thus, this genetic engineering technology using the non-viral spermine-dextran is a promising strategy to improve MSC therapy for ischemic heart disease (Jo *et al.*, 2007).
Tang et al. modified MSCs with heme oxygenase -1 (HO-1), and found tolerance of modified MSCs to hypoxia significantly increased, and the survival rate in the ischemic heart also significantly increased. Seven days after the transplantation, survival rate of HO-1-modified MSCs was as 5 times as that of the control group in vivo. With the modified MSCs transplantation therapy of myocardial infarction, cardiac function was further improved. These genes enhance the viability of MSCs from such perspectives as anti-apoptotic, promoting survivals, antioxidant protection, and so forth (Tang *et al.*, 2005b).
Genetic modification of donor cells for over expression of the cell survival signaling mediators significantly prevent their apoptosis after transplantation (figure 11). Akt and its downstream mediator Bcl.xl have a central role in cell survival signaling (Huang *et al.*, 2003) their gene transfer exerted a powerful cardio-protection after transientischemia, inhibited cardiomyocyte death and improved function of the surviving cardiomyocytes (Lim *et al.*, 2006; Huang *et al.*, 2003).

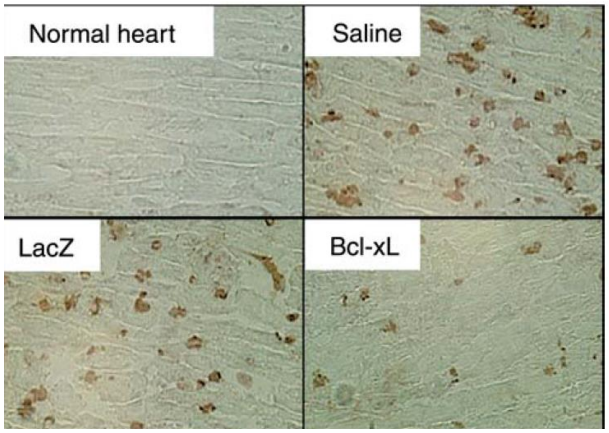

Figure 11. Apoptotic nuclei in the myocardium after global I/R were detected by TUNEL staining. Normal heart: the heart injected with saline before I/R; saline: the heart injected with saline after I/R; LacZ: the heart transduced with LacZ after I/R; and Bcl-xL: the heart transduced with Bcl-xL after I/R. a few apoptotic cardiomyocytes were displayed in the specimens from the Bcl-xL-overexpressed heart (Huang *et al.*, 2003).

Li et al applied anti-apoptotic gene Bcl-2 to modify MSCs and found that the anti-apoptotic ability of modified MSCs increased; they can also promote VEGF secretion under the hypoxic condition; the number of surviving cells after transplantation in vivo significantly increased, and improvement in cardiac function appeared significantly (Li *et al.*, 2007). For instance, MSCs transfected to overexpress Akt seem to confer a possible myocardial protective function (Matsui *et al.*, 2001; Mangi *et al.*, 2003; Lim *et al.*, 2006; Noiseux *et al.*, 2004). Akt-transduced MSCs secrete a number of proteins in response to hypoxia, including the recently described Hypoxia and Akt induced Stem cell Factor (HASF). This has been shown to be an important mediator of cardioprotection following ischemic injury (Huang *et al.*, 2014). These studies indicate improved ejection fraction and reduced infarct size with the administration of the Akt-overexpressing MSCs over that seen with injection of control MSCs. Furthermore, MSCs engineered to express combinations of gene products such as Akt and Ang1 are also showing promise in animal

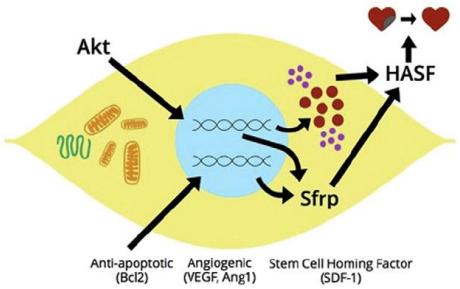

Figure 12. Genetic modification of MSCs.Various factors have been transfected into MSCs, including anti-apoptotic, angiogenic and stem cell homing factors, as well as Akt. A potentially important factor, secreted frizzled related peptide is produced, as are many other intermediaries, which result in the secretion of Hypoxia and Akt induced Stem cell Factor, an important mediator of the reparative process.HASF:Hypoxia and Akt induced Stemcell Factor; Sfrp: secreted frizzled related peptide; VEGF: vascular endothelial growth factor; SDF-1: stem cell derived factor-1 (Kim *et al.*, 2015).

Lim et al. modified MSCs with Akt and found that Akt-MSCs tolerated more about hypoxia-induced apoptosis. And after hypoxia, extracellular signal regulated ERK activation, VEGF expression increased, and survival of Akt-MSCs increased after transplantation. Compared with MSCs transplantation alone, it further repaired the damaged myocardium and mimproved cardiac function. Through enhancing the MSCs's ability of antihypoxia, it can also play the role of increasing the survival rate of the transplanted cells (Lim *et al.*, 2006).

A distinctive approach of cell manipulation has been adopted by Tara et al. to prevent their apoptotic death under nutrient and oxidant stress. A super antiapoptotic protein FNK was developed and used for transduction of the cells. The novel protein FNK was previously constructed from an anti-apoptotic factor Bclxl by site-directed mutagenesis and was transduced into the cells using protein transfer domain (PTD) of HIV-Tat protein. They have explicitly demonstrated better survival of bone marrow mononuclear cells (BM-MNCs) treated with PTD-FNK under oxidant stress and serum starvation conditions in vitro and depicted higher engraftment efficiency in a rat hind-limb ischemia model (Tara *et al.*, 2007).

Improve Anti-Inflammatory: *Problem*: The nonspecific inflammation of the body is one important reason that causes loss of transplanted cells (Fan *et al.*, 2007). *Solution*: Genetically manipulation of tumor necrosis factors (TNF) as an important kind of inflammatory mediators. TNF act as a major factor to the mediated apoptosis of the receptor.

Bao et al. studied the anti-inflammatory and cardiac function improvement effects of TNF receptor- (TNFR-) modified MSCs transplantation in the treatment of myocardial infarction. Two weeks after transplantation of TNFG-transfected MSCs into the ischemic myocardium, they found that the expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 reduced, myocardial apoptosis decreased and the left ventricular function improved significantly (Bao *et al.*, 2008).

Promoting Angiogenesis: *Problem*: Insufficient angiogenesis at injury site. *Solution*: To promote angiogenesis effectively and improve myocardial blood flow may be one of the effective ways of the treatment of ischemic heart disease. Genes related to angiogenesis include VEGF, Ang-1, FGF-2, IGF, and hepatocyte growth factor (HGF) (Mingliang *et al.*, 2011).

Yang et al. used liposome-mediated method transfected pcDNA hVEGF to rat MSCs and used intramyocardial injections method to inject the myocardium of the rat two weeks after myocardial infarction. After four weeks, the results showed that the cardiac function, infarct size, and angiogenesis of the VEGF modified group were significantly better than the other groups, MSCs treatment group (Yang *et al.*, 2007). Also Yang et al. transplanted HGF-modified MSCs through the non-infarct-related coronary artery into pig heart. The results showed a significant increase in angiogenesis (Yang *et al.*, 2006).

Sun et al. intramyocardially injected human Ang1-modified MSCs (hAng1-MSCs) to treat rats acute myocardial infarction. The results showed that hAng1-MSCs could survive in the local and express hAng-1 mRNA and protein. Vascular density of hAng1-MSCs and MSCs group was significantly higher than PBS control group, ventricular remodeling and cardiac function were significantly improved. What is more, compared with MSCs, the increase of angiogenesis and arteriogenesis and the decrease of the infarct size and thickening of the left ventricular wall were more significant in hAng1-MSCs group (Sun *et al.*, 2007). Combining of cell transplantation with angiogenic growth factor gene delivery can improve the cell graft survival in the heart. A multimodal approach of bone marrow cell-based delivery of insulin-like growth factor (IGF) and VEGF transgenes resulted in reduced apoptosis of the transplanted cells and increse angiogenesis (Yau *et al.*, 2005).

Directly Improving Myocardial Function by Promoting Stem Cell Integration: *Problem*: the lack of gap junction formation beetween differntiated myotube and host tissue. This indicates importance of host donor electromechanical coupling. *Solution*: Implanted cells should be both myogenic and functionally integrate with in the heart syncytium

Initial clinical trials involving the implantation of autologous skeletal myoblasts were associated with arrhythmias, possibly owing to the lack of gap junction formation between differentiated myotubes and host tissue (Mummery *et al.*, 2010). Implanting skeletal myoblasts derived from transgenic mice that express connexin-43 via a skeletal muscle promoter eliminated the arrhythmias associated with the implantation of wild-type cells (Roell *et al.*, 2007). On the other hand, MSCs, which endogenously express gap junctions and functionally couple to host cardiomyocytes, exhibited significantly reduced arrhythmogenesis when compared with skeletalmyoblasts (Mills *et al.*, 2007).

Recently, implanted green fluorescent protein-labeled human embryonic stem cell-derived cardiomyocytes were shown to functionally integrate With in the myocardium and actively pace guinea pig ventricles after cryoablation of the atrioventricular (AV) node (Xue *et al.*, 2005).

Similarly, implanted human MSCs genetically engineered to express ‘‘funny’’ current (HCN2 gene) were able to pace, in a catecholamine-responsive manner, canine ventricles after AV node ablation. Mechanistically, the HCN2 expressing MSCs were not able to actively generate action potentials, but they produced a depolarizing current, which spread to couple cardiomyocytes, bringing them to the excitation threshold (Plotnikov *et al.*, 2007).

Gene Delivery to Cells

More recently, gene therapy approach is being employed for controlled gene expression in stem cells to influence their fate (Hough *et al.*, 2006). One of the most commonly intended therapeutic effect from transgene delivery to the heart is the overexpression of pro-angiogenic growth factors to achieve angiogenesis which alleviates myocardial ischemia via enhanced regional blood flow (Ylä-Herttuala and Martin, 2000). Likewise, delivery of the transgenes encoding for survival factors achieved cardiac protection and anti-apoptotic effects (Huang *et al.*, 2003; Mangi *et al.*, 2003). Moreover, gene therapy targeting the underlying biological processes affecting the injured cardiomyocytes, including modification of cellular contractile signaling by the normal function of the *β*-adrenergic receptor cascade (Tevaearai *et al.*, 2005; del Monte *et al.*, 2002) and regulatory pathways such as intracellular calcium signaling (Logeart *et al.*, 2006; Ennis *et al.*, 2002) present promising new objectives for biologic therapy of heart disease.

The success of an experimental or clinical gene therapy procedure is critically dependent on the efficiency of gene transfer and expression. By and large, gene delivery to the heart is achieved by using various gene delivery vectors to compensate for the poor penetrability of the naked DNA plasmid across the cell membrane which translates into a low level of transgene expression. The pattern of transgene expression varies with the choice of delivery vectors and its *in vivo* pharmacokinetics is influenced by various physical, chemical, and biological factors. The currently available gene transfer vectors are less than ideal in their characteristics for clinical applications. Hence, there is a need to design a vector which may overcome these limitations to accomplish optimal gene transfer with minimum undesired effects (Table 2). Of the commonly used vectors, replication deficient viral vectors based on RNA and DNA viruses have been extensively used for therapeutic gene delivery (Young *et al.*, 2006). Particular viral gene delivery systems have been derived from retrovirus, adenovirus, adeno-associated virus, poxvirus and herpes virus. Despite their distinctive advantages, the risks associated with their use have forced the researchers to design alternative gene delivery strategies including non-viral vectors. Regardless of their low transfection efficiency and short term expression, the use of non-viral gene delivery system is safe and flexible in terms of the plasmid DNA size which can be delivered (Müller *et al.*, 2007). Some of the frequently used non-viral vectors for gene delivery to the heart include lipopolyplexes, liposomes, and polaxamine nanospheres (Bull *et al.*, 2003; Fukuyama *et al.*, 2007).

A polyethyleneimine (PEI)-based nanoparticle system have designed to deliver humanVEGF165 into human SkMs (Ye *et al.*, 2006). PEI has cationic nature and has strong DNA compaction capacity, effective DNA protection and with an intrinsic endosomolytic activity which contribute to superior gene transfection efficiency (Lungwitz *et al.*, 2005). It mis reported more than 11% transfection efficiency of SkMs with the peak level expression (25 ng/ml) on days 4–6 after transfection. At four weeks after transplantation into an experimental rat heart model of permanent coronary artery ligation, blood vessel density, and regional blood flow (ml/min/g) in the left ventricle were significantly higher in the transfected cell transplanted group of animals as compared with the controls. This results clearly signify the safety and effectiveness of the nanoparticle-based non-viral vectors for gene delivery to the heart.

Clinical Trials

Clinical trials have shown that cell engineering has a very positive outcome to treat disease indicating its future potential to be a recommended therapy (figure 13).

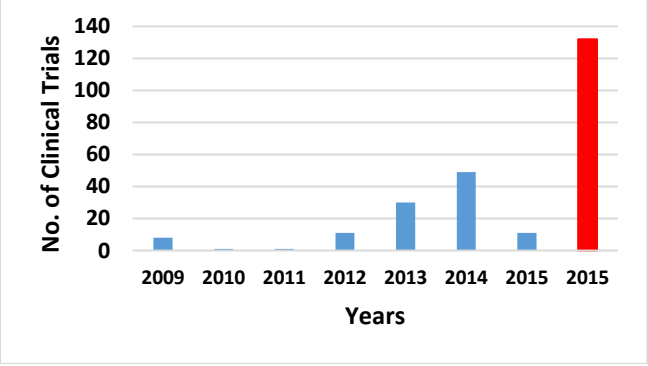

Figure 13. Number of clinical trials by engineered celss (www.clinicaltrials.gov)

Also it is reported variety of stem cell types have been used in preclinical studies and clinical trials in cardiac regeneration (figure 14).

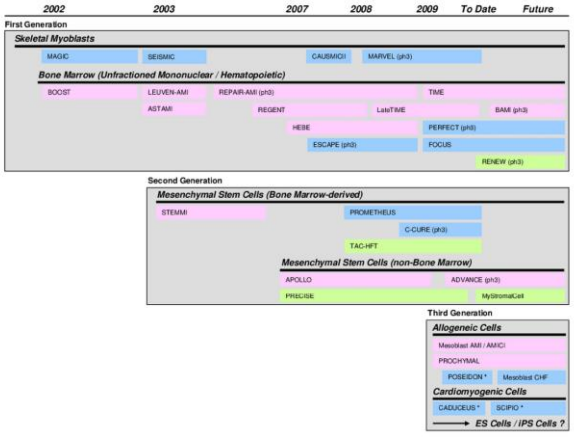

Figure 14. The use of various stem cell types in preclinical studies and clinical trials in cardiac regeneration. The main randomised controlled trials (RCTs) and some other key clinical trials in patients with an acute myocardial infarction (red), congestive heart failure (blue) and chronic myocardial ischaemia (green) are summarised. 'ph3' indicates a phase III trial and an asterisk denotes a non-RCT (Takashima et al., 2013)

Existing Challenges

Despite the progress of current technologies being employed in the building of new heart tissue, many challenges need to be overcome before human transplantation of engineered tissue can become a reality.

1. The choice of the cell type is domineering for ex vivo delivery of the therapeutic genes to the heart. If improvement of the systolic function is the ultimate goal, bone marrow cells should better be used as the carriers of the transgene as they can transdifferentiate to adopt cardiac phenotype. However, if improvement of the diastolic function is the prime consideration, SkMs may be the best choice to serve as the carriers of the transgene as their myogenic differentiation would exert scaffolding effect and prevent left ventricular dilatation. ESC-derived cardiomyocytes may provide an effective alternative as they will better integrate with the host myocardium as compared with the other cells, thus alleviating the problem of arrhythmia

2. Optimal number of cells, the route of administration and time of injection after infarction episode. The purification of donor cells for each patient and their propagation in vitro to get the required number is time consuming and expensive.

3. Transduction of autologous cells can also modify their immunogenicity thus prompting concerns about their acceptance by the host immune system. Cell encapsulation technology may provide an answer to this problem. Encapsulation of the genetically modified cells within a microporous polymer membrane will isolate the transplanted tissue from the host and allow the eventual retrieval of engineered cells (Rinsch *et al.*, 2001). Immuno isolation of the donor cells in this manner improves their protection from cell contact-mediated rejection by the host's immune system

4. Transfer of therapeutic gene to stem cells may be inefficient due to cell quiescence or because of the insufficient viral receptor density. A number of investigators have attempted to alter viral vectors to allow their entrance into target cells via more specific or more abundant receptors. A safer option may be to implicate nanotechnology for gene transfection into stem cells (Jeong *et al.*, 2006; Ye, 2006).

5. Transgene transfer should be without interference with their differentiation potential (Liu *et al.*, 2005).

6. It is obvious that a single growth factor (i.e. VEGF) will be less efficient to complete the biological process of angiogenesis alone. Therefore, the multiple growth factor delivery approach may be more pertinent in creating a sustainable angiogenic effect with clinically relevant outcome. The challenge is to find complementary partner for VEGF in angiogenesis. The angiopoietin family of growth factors and their receptors are critical regulators of vascular structure maturity (Markkanen *et al.*, 2005) and have an indispensable role in coronary artery development (Ward *et al.*, 2004)

7. However, adult cardiomyocytes are quite resistant to gene transfer. Both viral as well as non-viral gene delivery strategies have been developed and optimized for genetic modification of cardiomyocytes. Transfection efficiency to the level of 25%–30% has been achieved in cardiomyocytes with lipid vector-VEGF165 plasmid DNA complex. So we optimize gene transfer systems (Xu *et al.*, 2006).

Table 1. Cells used for heart cell therapy

Cell type	Angiogenesis	Myogenesis	Therapeutic Potential	Potential Advantages	Disadvantages	References
Mesenchymal Stem Cells (MSCs)	+	+	1. Can be differentiated toward cardiomyocytes. 2. Can have clinical agents for cardiac	1. Allogeneic transplantation. 2. Multi lineage differentiation potential. 3. Low immune responce	1. Lineage restriction 2. Cases of bone or cartilage formation in the myocardium	(Dominici *et al.*, 2006; Heng *et al.*, 2009; Gálvez-Montón *et al.*, 2013; Pittenger *et al.*, 1999; da Silva Meirelles *et al.*, 2006; Shake *et al.*, 2002; Amado *et al.*, 2005; Miyahara *et al.*, 2006; Toma *et al.*, 2002)
Embryonic Stem Cells (ESCs)	+	+	1. Efficient cardiogenic differentiation 2. have beat spontaneously and rhythmically in vitro cell culture conditions 3. Have action potential	1. ESC-derived cardiomyocytes establish a renewable source of donor cardiomyocytes 2. Histological and electrophysiological integration with the host cardiomyocytes.	1. Isolation of committed ESCs from the undifferentiated ESCs in cell culture. 2. Teratogenicity of ESC-derived cardiomyocytes 3. Immunogenic and ethical problems	)Xu *et al.*, 2002; Kehat *et al.*, 2001; Kolossov *et al.*, 2006; Johkura *et al.*, 2003(
Cardiac Stem Cells	+	+	1. More effective in making new myocardium than stem cells from other organs including the bone marrow 1. Maintain normal homeostasis in the heart	1. Multipotent 2.Autologous 3.without ethical issues	1. Under 1%, CSCs are unable to completely remedy the massive loss of tissue after 2. Their location in heart is sjown be restricted to the right atrium in the adult heart:	(Beltrami *et al.*, 2003; Dawn *et al.*, 2005; Linke *et al.*, 2005; Malliaras *et al.*, 2013; Mollova *et al.*, 2013; Laugwitz *et al.*, 2007)
Cardiomyocytes	−	+	Cardiomyocytes provide an optimal cell type for heart cell therapy.	1. Robust growth kinetics 2. Simple isolation and expansion process	Difficulty in availability	(Wei *et al.*, 2005; Johkura *et al.*, 2003; Beltrami *et al.*, 2003)
Skeletal Myoblast	+	+	SkMs are inherently myogenic and get mobilized in response to muscle injury.	1. Commitment to a myogenic lineage without the potential drawbacks of undifferentiated stem cells,	1. Inability to electrically couple with the host cardiomyocytes	(Reinecke *et al.*, 2000; Léobon *et al.*, 2003; Babai *et al.*, 1990; Stagg *et al.*, 2006; Dib *et al.*, 2005;

				2. Autologous origin circumventing immune rejection 3. High proliferative potential 4. Resistance to ischemia	2. Risk of life-threatening arrhythmias. It is necessary to address these issues prior to clinical application	Haider *et al.*, 2004; Taylor *et al.*, 1998)
Endothelial Proginator Cells (EPCs)	-	+	Differentiate into new blood vessels (neovascularization).	1. Promote neovascularization by secreting proangiogenic growth factors 2. Stimulating re-endothelialization;	1. Non electrical integration 2. Non myocyte differentiation	)Gersh *et al.*, 2009; Orlic *et al.*, 2001; Marsboom and Janssens, 2008; Marsboom *et al.*, 2008(
Hematopoietic Stem Cell	+	–	Improve cardiac function and infarct size	Potential for vasculogenesis	Electrical integration? Myocyte differentiation? Mechanisms of benefit?	(Gersh *et al.*, 2009)
Adipose tissue-derived Stem Cells	+	+	1. Improves heart function and reduces infarction size in murine and rat models 2. High cardiomyogenic and vasculogenic potential	1. Easily isolated 2. High availability 3. Multipotent 4. Low immune response	Low survival	(Gálvez-Montón *et al.*, 2013; Bayes-Genis *et al.*, 2010)
iPSC	+	+	Can be driven into many different cell types	1.Creation of patient-specific cell types 2. Easy to expand 3. Good availability 4. Autologous	1. Tumorigenicity 2. Heterogeneity in final induced SC population 3.Technically challenging 4. Low efficiency of conversion	(Li *et al.*, 2009; Gálvez-Montón *et al.*, 2013)

Table 2. Vector systems used for transgene delivery into cells (Dimarakis, 2009)

Type of Vectors	**Delivery Approaches**	**Advantages**	**Limitations**
Naked plasmids	(a) Microinjection (b) Particle bombardment "gene gun" (c) Electroporation (d) Sonoporation (e) Laser irradiation		
Non-viral vectors	(a) Calcium phosphate (b) DEAE dextran (c) Cationic polyplexes (d) Cationic lipoplexes: Liposoma and Non-liposomal (e) Cationic bioplexes	• Ease of manipulation • low cost • Low transfection efficiency • Large scale production • Flexible in gene size • Insertion • Lack of safety concerns • Non-immunogenic	Short term expression
Viral vectors	(a) Retrovirus (b) Adenovirus (c) Adeno-associated virus (d) Lentivirus (e) Other virus	Higher transfection Efficiency	• Restricted size of gene delivery • Immune response • Lack of target specificity • Problems in large scale production • Safety concerns • Probability of long term • Integration with the host genome • Potentially tumorigenic

Conclusion

Cardiovascular diseases, including myocardial infarction, severe ischemic heart disease and chronic heart failure, currently have no cure. The discovery of stem cells capable of generating angiogenic and/or contractile cells holds out promise as new therapies. However, additional directions will focus on: (1) selection of the optimal cell types by which efficacy and safety are assured, (2) identification of the methods at which stem cells are most effective (3) determination of the modes of their delivery and direct targeting of the injured tissues.

So we should be cautious about the study and application of this technology. Improving the survival rate after stem cell transplantation and prompt homing of stem cells may be effective strategies for stem cell therapy. Further understanding of the genetic mechanisms that govern cardiogenic stem cell differentiation as well as the cellular properties that lead to safe host–donor integration and successful functional repair of damaged heart tissue will provide future opportunities for the use of genetic manipulations in cardiac stem cell research. The studies that use genetic engineering to enhance the selection, survival, and integration of stem cells in cardiac implantation therapies will be vital to the forward momentum of this field. The knowledge gained from these aspects plus large standardized clinical trials will eventually accomplish the stem cell therapy of cardiovascular diseases.

Conflict of Interest

I declare no conflict of interest with anybody or any organization regarding this review paper.

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Note: To clarify the real concept, the orignal pictures of the citing researchers have been shown and references have been given for all the pictures used in this manuscript.

