Global search for right cell type as a treatment modality for cardiovascular disease

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Introduction: Acute myocardial infarction is the primary cause of heart disease-related death in the world. Reperfusion therapy is currently the backbone of treatment for acute myocardial infarction albeit with many limitations. With the emergence of stem cells as potential therapeutic agents, attempts in using them to enhance cardiac function have increased exponentially. However, it has its own disadvantages, and we postulate that the primary drawback is choosing the right cell type and solving this may significantly contribute to ambitious goal of using stem cells in the regeneration medicine.

Areas covered: This article discusses several types of stem cells that have been proven to be likely candidates for treating cardiovascular diseases and their uses up to date, focusing on their biological characterization and potential usage in preclinical and clinical work.

Expert opinion: The research on cell therapies for cardiovascular disease is promising, but there is still much uncertainty surrounding the efficacy of these cells in clinical settings. With a wide range of cells available as potential treatment for cardiovascular diseases, one should avoid from being overzealous and extrapolating when reporting their data. Future studies should focus more in understanding the biological functions of the available cell lines.

Keywords: cardiac regeneration, cardiomyocytes, myocardial infarction, stem cells

1. Introduction

Out of the 57 million global deaths in 2008, 17 million are attributed to cardiovascular diseases with ischemic heart disease and congestive heart failure being the two leading causes (World Health Organization or WHO). Heart failure is the result of loss of cardiomyocytes and reduction in vessel numbers in infarcted area in response to acute myocardial infarction (AMI) [1,2]. Upon AMI, cardiomyocytes begin to die, and unless blood supply is restored in time, it will lead to either necrosis or apoptosis to the all-cardiac tissue connected by the infarcted blood vessels.

Current treatments using medication such as antihypertensives and antiarrhythmias or surgical intervention, which includes heart bypass and usage of stent, have been the norm to maintain or improve cardiac function, but the pitfall of these treatments is that it does not encourage cardiac regeneration and the development of a vascular network to support the myocytes and surrounding ischemic tissue [3]. For patients at the end stage of heart failure, heart transplant remains as a last resort but this is hampered by severe shortage of organ donors, potential for organ rejection and high medical costs. Latterly, stem cell therapy has been proposed as a promising approach for myocardial repair. A wide range of stem cells, particularly those from adult stem cells and progenitor cell types, including bone marrow mesenchymal stem cells (BM-MSCs), cardiac stem cells (CSCs), hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs) have been tested in experimental cardiac
damage models. In addition, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have also been studied extensively in cardio-related research especially in establishing homogenous cell population protocols and ways to remove undifferentiated cells that have the possibility to differentiate into tumor cells. Findings from a few clinical trials even suggested the administrations of stem cells to patients suffering from cardiovascular diseases, providing evidence that they are safe and have improved in cardiac function. With the abundance of fundamental research data, preclinical work as well as clinical studies, stem cells therapy seems to be making in ways into the as a promising mode of therapy in treating cardiovascular diseases. However, just like any other biological material intended for therapeutic uses, it has its own disadvantages, namely the choice of cells, method of administering and timing of delivery. We believe that by addressing the primary issue which is selecting the appropriate cell type, it can reveal significant findings that can be used to contribute to the utilization of stem cells in regulating the regeneration of tissue and organs.

Currently, arrays of stem cells ranging from embryonic, fetal and adult sources are suggested as potential treatment modality for all known diseases. However, based on our previous work, none of it is appropriate to treat all target diseases [4]. This is because properties of the stem cells are now based on its harvested site and may not be useful outside their normal function [5]. This leads to the establishment of tailor-made choice of stem cells for better clinical efficacy. Hence, in this article, we will attempt to identify advantages as well as the disadvantages for each of the said stem cells and how relevant they are in treating cardiovascular diseases.

2. How can cell therapy be a suitable alternative?

Before divulging into the appropriate stem cell type for treatment of cardiovascular diseases, let us glance into possible mechanisms involved in stem cell therapy administered into an infarcted area. Typically, the left ventricular of the human heart contains ~5.8 × 10⁹ cardiomyocytes. Upon the onset of AMI, 25% of the cardiomyocytes will be lost [6,7]. Regeneration of the infarcted heart potentially requires a billion cardiomyocytes, along with other supporting cells such as endothelial and smooth vascular cells, for functional integration. Recent stem cell therapies have suggested a few mechanisms for which these may influence the cardiovascular regeneration; differentiation of stem cells into cardiomyocytes [8], fusion of stem cells with endogenous cells [9,10] and stem cells secretome signaling (Figure 1) [11]. However, the two former mechanisms are subject to controversy. Many argue that plasticity of the stem cells and the degree of cardiac regeneration from stem cells transdifferentiation have been described by some investigators, it has not been confirmed by others [12,13]. Further, low numbers of newly formed cardiomyocytes coupled with new evidence that what is actually taking place in hearts treated using stem cells are angiogenesis and arteriogenesis, not neovascularogenesis [14] leads to the notion that perhaps paracrine activity is the precise mechanism taking place in stem cells that were transplanted into infarcted hearts. In this regards, Raganath et al. [11], have vividly explained the paracrine activities of stem cells as well as ways to harness the stem cells secretome for the treatment of cardiovascular disease.

However, paracrine factors that are secreted by stem cells have little or no impact towards neovascularization but more towards angiogenesis and arteriogenesis, and the molecular process leading to the two latter categories are well orchestrated by proteins such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and angiopoietin. We have summarized in Table 1 other putative paracrine factors secreted by stem cells that are associated with cardiac development. The angiogenesis and arteriogenesis concepts are well supported by many cardiac related studies using various types of stem cells. For example, Aranha et al. have shown that hypoxic condition enhance expression of VEGF and induces endothelial cell proliferation and sprouting, which suggest the pivotal role of VEGF in revascularization [15]. In another study, Nagaya et al. administered intravenously expanded mesenchymal stem cells (MSCs) from bone marrow aspiration into rats with acute myocardial infarction and observed improved cardiac function through angiogenesis and myogenesis. The transplanted MSCs were preferentially attracted to the infarcted heart and the engrafted MSCs were positive for cardiac markers such as desmin, cardiac troponin T and connexin 43 [16]. A similar observation, excluding the arteriogenesis, was reported by Gandia et al. and this may be attributed by difference in cell source and the cell numbers used in their studies [17]. Moreover, stem cells supplemented with growth factors, such as the one conducted by Sun’s group, were able to increase capillary density and reduce infarct size in rat model of acute myocardial infarction [2]. At a canonical level,
the PI/3K-Akt signaling pathway is involved in fundamental cellular processes, including protein synthesis, proliferation and survival of cardiovascular system development. McDevitt et al. have shown that this pathway is a significant mediator in the proliferation of cardiomyocytes derived from human ESCs, and activation of PI3K and ERK related signaling cascades leads to increased survival of cardiomyocytes [18]. Studies also have shown that the PI/3K-Akt signaling pathway plays a critical role in angiogenesis [19]. Another important signaling pathway is the p38 mitogen-activated protein kinase (p38 MAPK) pathway, which mediates stem cells paracrine activities. Activation of this pathway can increase the production of VEGF, HGF and insulin-like growth factor-1 (IGF-1) [20]. Additionally, some transcription factors and receptors such as HAND2, GATA6, GATA 4 and KDR [17] are also linked to the angiogenesis pathway. It has been reported that loss of GATA6 blocks cardiac myocyte differentiation [21] whereas overexpression of GATA4 leads to increased production of IGF, HGF and VEGF [22]. Although the mechanistic studies conducted so far have provided some important knowledge, a more comprehensive understanding on the paracrine activities and signaling networks of stem cells are required.

3. Source for cardiomyocytes

The infarcted heart zone is characterized by hypoxia, acidosis, lack of substrates and accumulation of metabolites. Therefore, local microenvironment presents a challenge for the application of cell therapy. The optimal cell source should be easy to harvest, proliferative, nonimmunogenic, resistance to ischemia and have the ability to differentiate into mature, functional cardiomyocytes or at least be involved in angiogenesis and arteriogenesis process. Thus, to achieve cardiac regeneration, the flow of events can be broken down into a few steps, starting with the up-scaling of the desired cell type, the ability to differentiate into cardiomyocytes as well as withstanding harsh conditions and lastly, functional integration into the injured myocardium.

Cell transplantation has already been used clinically in heart tissue repair with the usage of skeletal myoblasts since more than a decade ago [23]. Subsequently, other sources such as unfractionated bone marrow cells (BMCs) provide an easily accessible source of HSCs, MSCs and EPCs. In a search on the ClinicalTrials.gov website [http://clinicaltrials.gov/], in which the keywords “cardiomyocytes” and “stem cells” were used, 13 clinical trials were shortlisted.
Table 1. Putative paracrine factors secretes by adult stem cells.

| Putative secreted factor                        | Abbreviation | Proposed function                                      |
|------------------------------------------------|--------------|--------------------------------------------------------|
| Adrenomedullin                                 | ADM          | Cytoprotection                                         |
| Angio-associated migratory protein             | AAMP         | Angiogenesis                                            |
| Angiogenin                                     | ANG          | Angiogenesis, cell proliferation                        |
| Angiopoietin-1                                 | AGPT1        | Cell migration, vessel stabilization                    |
| Bone morphogenic protein-2                    | BMP2         | Cell differentiation, growth                           |
| Bone morphogenic protein-6                     | BMP6         | Cell differentiation, growth                           |
| Connective tissue growth factor                | CTGF         | Angiogenesis, cell growth                              |
| Endothelin-1                                   | EDN1         | Cell proliferation, cell proliferation, inflammatory response |
| Fibroblast growth factor-2                    | FGF2         | Cell proliferation, and migration                      |
| Fibroblast growth factor-7                     | FGF7         | Vessel maturation, cell proliferation                   |
| Hepatocyte growth factor                       | HGF          | Cytoprotection, angiogenesis, cell migration            |
| Insulin-like growth factor-1                   | IGF-1        | Cytoprotection, angiogenesis, cell migration            |
| Interleukin-1                                  | IL-1         | VEGF induction                                         |
| Interleukin-6                                  | IL-6         | VEGF induction                                         |
| Kit ligand/Stem cell factor                    | KITLG (SCF)  | Cell proliferation and migration                        |
| Leukemia inhibitor factor                      | LIF          | Cell proliferation, cytoprotection                      |
| Macrophage migration inhibitory factor         | MIF          | Cell proliferation, inflammatory response              |
| Matrix metalloproteinase-1                    | MMP1         | Loosens matrix, tubule formation                        |
| Matrix metalloproteinase-2                    | MMP2         | Loosens matrix, tubule formation                        |
| Matrix metalloproteinase-9                    | MMP9         | Loosens matrix                                         |
| Macrophage-specific colony-stimulating factor  | MCSF         | Monocyte proliferation/migration                        |
| Plasminogen activator                          | PA           | Degradating matrix molecules                           |
| Platelet-derived growth factor                 | PDGF         | Cell proliferation, cell migration                      |
| Pleiotrophin                                   | PTN          | Cell proliferation                                     |
| Secreted frizzled-related protein-1            | SFRP1        | Development                                             |
| Secreted frizzled-related protein-2            | SFRP2        | Development                                             |
| Stem cell derived factor-1                     | SDF-1        | Progenitor cell homing                                 |
| Thymosin-β4                                    | TMSB4        | Cell migration, cytok protection                        |
| Tissues inhibitor of metalloproteinase-1       | TIMP-1       | Cell migration                                          |
| Tissues inhibitor of metalloproteinase-2       | TIMP-2       | Cell migration                                          |
| Transforming growth factor-β                   | TGF-β        | Vessel maturation, cell proliferation                   |
| Tumor necrosis factor-α                        | TNF-α        | Degrade matrix molecules, cell proliferation            |
| Vascular endothelial growth factor             | VEGF         | Cytoprotection, proliferation, migration, angiogenesis  |

This table adapted from Massimiliano Gneccchi, Maria Miroutsou and Victor J. Dzau book chapter entitled Stem Cell Therapy for Heart Repair: The Paracrine Paradigm.

Table 2: 11 of these were using either autologous or allogenic bone marrow mesenchymal stem cells (BM-MSCs), whereas the remaining two trials investigated the effects of autologous derived CSCs in cardiac tissue repair. In this article, we will be discussing the two latter trials. One trial involves transplanted CSCs in patients with ischemic cardiomyopathy (SCIPIO) [24] and another one uses intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS) [25].

The settings were autologous wherein the cells were isolated and expanded prior to transplantation. A range of 1 million and 12.5 to 25 million cells were infused in intracoronary in SCIPIO and CADUCEUS, respectively. The only failure found in SCIPIO trial as compared to CADUCEUS is the insufficient amount of cells in a patient with cardiac amyloidosis. This failure may be due to bacterial contamination, cytogenetic abnormality and the inability to achieve minimal cell dose for infusion within the specified interval of up to 90 days after myocardial infarction.

Both of these trials showed promising results, with no adverse side effect related to the treatment. In SCIPIO, it was shown to be highly efficacious in restoring left ventricular systolic function up to 1 year after treatment. Strategies to trace the fate of the injected cells may be the next move and better results might be obtained with repeated doses, as the cells can be frozen for subsequent use, as suggested in SCIPIO [24]. In CADUCEUS, there were reduction in scar mass, increase in viable heart mass, regional contractility and regional systolic wall thickening. However, changes in end-diastolic volume, end-systolic volume and left ventricular ejection fraction did not differ significantly between groups within the 6-month period. The underlying mechanism of the improved result is unknown for both trials and may be attributed to differentiation of the injected cells, activation of resident cardiac stem cells or paracrine factors that stimulate or activate endogenous regenerative pathway [25]. Both of these studies cannot be compared to each other due to different target population and different assessments endpoints. Nevertheless, these studies bring hope that cardiac tissue loss can be treated in a holistic manner.
| Clinical trial identification | Status | Conditions | Intervention/study phase | Patients enrolled | Route of injection | Sponsor and collaborator |
|-----------------------------|--------|------------|--------------------------|------------------|--------------------|---------------------------|
| NCT 01087996               | Completed | Chronic ischemic left ventricular dysfunction and heart failure secondary to myocardial infarction | Autologous and allogeneic mesenchymal stem cells/Phase I and Phase II | 30 | Transendocardial | University of Miami; National Heart, Lung and Blood Institute (NHLBI); The EMMES Corp. |
| NCT 00893360               | Completed | Recent myocardial infarction; ventricular dysfunction | Autologous cardiosphere-derived stem cell/Phase I | 31 | Intracoronary | Cedars-Sinai Medical Center; National Institutes of Health (NIH); NHLBI; Johns Hopkins University; The EMMES Corp. |
| NCT 00548613               | Completed | Coronary artery disease; coronary arteriosclerosis; coronary atherosclerosis; coronary disease | MESENDO/Phase I | 20 | Intracoronary or intramyocardial | TCA Cellular Therapy |
| NCT 01392105               | Completed | Acute myocardial infarction | Mesenchymal stem cell/Phase II and Phase III | 80 | Intracoronary | Yonsei University; FCB-Pharmicell Co Ltd |
| NCT 00768066               | Completed | Left ventricular dysfunction | Autologous human mesenchymal cells and autologous human bone marrow cells/Phase I and Phase II | 67 | Transendocardial | University of Miami, The EMMES Corp. |
| NCT 00474461               | Active, not recruiting | Coronary artery disease; congestive heart failure | Cardiac stem cell/Phase I | 40 | Intracoronary | University of Louisville, Brigham and Women’s Hospital; Jewish Hospital and St. Mary’s Healthcare |
| NCT 00313339               | Active, not recruiting | Myocardial infarction; coronary artery disease | Bone marrow derived autologous CD34+Phase I | 40 | Intracoronary | Emory University; Texas Heart Institute |
| NCT 00950274               | Recruiting   | Myocardial ischemia; coronary artery disease | CD133+ autologous bone marrow stem cell/Phase III | 142 | Intramyocardial | Miltenyi Biotec GmbH; German Federal Ministry of Education and Research |
| NCT 01442129               | Recruiting   | Heart failure; cardiomyopathy; ventricular dysfunction | Mesenchymal precursor cell/Phase II | 30 | Intramyocardial | Mount Sinai School of Medicine; Deborah Ascheim; NHLBI |
| NCT 01392625               | Recruiting   | Nonischemic dilated cardiomyopathy | Autologous and allogeneic mesenchymal stem cells/Phase I and Phase II | 36 | Transendocardial | University of Miami; NHLBI |
| NCT 01720888               | Recruiting   | Ischemic dilated cardiomyopathy | Bone marrow-derived Mesenchymal stem cells/Phase II | 80 | Intracoronary | National University of Malaysia; Cytopeutics Pte. Ltd. |
| NCT 01734356               | Recruiting   | Inherited arrhythmias and valvulopathies | Skin biopsies | 20 | - | Nantes University Hospital |
| NCT 01865981               | Enrolling by invitation | Hereditary cardiac arrhythmias | Eletrophysiology of iPS-derived cardiomyocytes | 5 | - | University of Dundee |
instead of the conventional therapy, thus giving it a better chance to advance to clinical stage.

Most of the current clinical trials make used of the BM-MSCs as a cell source, and this is due to the position of these cells which are highly characterized, but the question is that do other cell types have the same capability? In this context, we have reviewed some of the famous types of cells that are always associated as good source in regenerative medicine.

4. Embryonic stem cells

Embryonic stem cells (ESCs) originate from the inner cell mass of preimplanted embryo blastocysts with the capacity to generate any type cells from the body [26]. ESCs can differentiate into cardiomyocytes via formation of embryoid bodies [27]. Most of the research works in ESCs were invested to elucidate the mechanism of cardiomyocytes formation. Such efforts include coculturing ESCs with END-2 cells, visceral endoderm-like cells to induce the cardiomyocytes [28].

Lately, a shift towards using serum-free medium has been occurring in studied involving cardiomyocytes originating from ESCs. Serum-free media is shown to improve differentiation efficacy. In addition, it also reduces potential risk of contamination from transmission of viral or prionic disease that can become antigenic substrates on transplanted cells, causing immunological reactions. This conforms to the 3R concept of refinement, reduction and replacement of demand for animal-based product. All these steps bring us closer to clinical applications in the future. Moreover, fetal bovine serum is a complex and ill-defined animal-based product which could lead to inconsistencies in stem cell culture work [19,29]. The most recent trend in culturing ESCs under serum-free conditions uses several cytokines as supplements, including Activin A and bone morphogenetic protein 4 (BMP 4). Lafflame et al. used Activin A and BMP 4 to direct the differentiation of human ESCs into cardiomyocytes, followed by Percoll gradient centrifugation to obtain the cardiomyocytes [30]. In another study, Passier et al. reported an increase in cardiomyocytes differentiation from human ESCs in serum-free cultures [29]. Yang et al. use combinations of Activin A, BMP 4, bFGF, VEGF and dickkopf homolog 1 in serum-free media, and was able to demonstrate human ESCs-derived embryoid bodies generating a KDR low/C-KIT (CD117) negative population that displays cardiac, endothelial and vascular smooth muscle potential in vitro as well as in vivo [31]. These will improve the efficiency of cardiomyocytes differentiation. In addition, a study by Min et al. was carried out by infusing mouse ESCs into a rat model of myocardial infarction. Engrafted ESCs that improved the heart function may have been the result of cardiomyocytes differentiated stem cells and the formation of blood vessels during the period of experiment [32].

Despite being a promising cell source in regenerative medicine, ESCs inherent pluripotency has tumorigenic potential in vivo. Further, the use of embryo also raises ethical concerns.

Transplantation of human ESCs into patients is also limited by potential human leukocyte antigen (HLA) incompatibility; in turn immunosuppressant therapy is needed to overcome rejection. Life-long immunosuppressive therapy, which can lead to infections and organ-based toxic side effects, such as nephropathy, might be required to prevent graft rejection. We still believe that ESCs hold a tremendous potential, and addressing key issues such as prior selection of more committed cells, avoiding pluripotent cells contamination in cell transplantation as well as genetic and epigenetic stability may help overcome the drawbacks.

5. Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are somatic cells that have been reprogrammed to a pluripotent state by the introduction of a group of transcription factors; Sry-related high-mobility-group (Sox)-2, Lin-28 homolog (Lin)-28, Cellular-myelocytomatosis viral oncogene homolog (c-Myc), Octamer (Oct)-3/4, Kruppel-like factors (Klf)-4 and Nanog [33]. The risk of insertional mutagenesis caused by viral integration into the genome is of particular concern because patients who have received gene-modified lymphoid cells have had aggressive leukemia as a result of this phenomenon. Viral oncogene used to engineer the cells, such as c-Myc also poses risks. Yamanaka and Yoshida suggested iPSCs as a potential source for cardiac regeneration if the reprogramming efficiency can be improved and safety issue are addressed before progressing to clinical application [34]. Taking into account the safety issues, several research works have successfully differentiated fibroblast iPSCs or cardiomyocytes without the introduction of harmful genes. For example, Efe et al. converted mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy without c-Myc, which proved that c-Myc was dispensable, and that the transduction of just three factors worked equally well [35]. They reasoned that providing the appropriate developmental cues after an initial epigenetic activation phase might allow us to hijack conventional reprogramming at this early unstable stage, and specifically shift the outcome towards cardiogenesis. Others have successfully obtained iPSCs via small molecules such as valproic acid and recombinant proteins [36]. The advantage of the iPSCs technology is that it can generate a patient’s specific stem cells. A classic example is that of a study conducted by Freund et al., wherein they successfully generated cardiomyocytes from the skin fibroblast of a patient with hereditary hemorrhagic telangiectasia [37]. Many studies have shown a positive outcome on the transplantation of iPSCs in infarcted area. This includes a study by Nelson et al. who reported that there were in situ regeneration of cardiac tissue and improvement in the postschemic cardiac function after intramyocardial delivery of mouse iPSCs [38]. On another study, Lin et al. developed a strategy to enrich endothelial cells, smooth muscle cells, and cardiomyocytes
has shown that intracoronary administration of CSCs ameliorates left ventricular remodeling in rodents with AMI. Subsequently, they also used pigs, a large and clinically relevant animal species to determine whether intracoronary CSCs are beneficial in the setting of an old MI. The intracoronary infusion of autologous CSCs improved regional and global left ventricular function and promotes cardiac regeneration in pigs with chronic myocardial infarction. These findings lay the groundwork for clinical trials of CSCs in patients undergoing coronary artery bypass grafting (CABG) surgery [24]. CSCs also advanced to clinical trials SCIPIO [24] and CADUCEUS [25], with both showings promising result. Nevertheless, invasive procedure and lack of tissue source restricted the usage of these cells.

Another excellent adult source is the MSCs. MSCs were first discovered in bone marrow. The MSCs population is rare in the bone marrow, accounting for only about 0.01% of bone marrow mononuclear cells. Hence, the MSCs require substantial culture expansion for weeks prior to most experimental applications. Because stem cells are both maintained and expanded in vitro before transplantation, culture conditions adhering to conditions for human administration must be used. To achieve adequate number of cells for clinical applications, in vitro expansion is inevitable. Therefore, a large amount of experimental work were emphasizing on understanding stem cell characteristics such as proliferation pathways [47], quality of the cells under in vitro culture conditions [48], expression pattern of Oct-4, Sox2 and c-Myc in the primary culture of cells [49] and culturing cells in serum free media.

MSCs have been identified in a variety of tissues including, human umbilical cord [50], adipose tissue [51] and dental pulp [52]. This indicates that MSCs are diversely distributed in human tissue, can be obtained from patient with relative ease, cultured and manipulated in vitro for cell therapy application. Because of the existence of different methods of isolation, expansion and characterizing MSCs, Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes at least three criteria: i) they must be plastic-adherent when maintained in standard culture conditions; ii) they must express C105, CD73 and CD90 and not CD45, CD34, CD14, CD11b, CD79 or CD19 and HLA-DR surface molecules and iii) they must be capable of differentiating into osteoblasts, adipocytes and chondroblasts when cultured in inductive media [53,54]. However, this set of criteria is not definitive as the expression of cell surface markers can be influenced by extrinsic factors such as those secreted by accessory cells in the initial passages, and it is important to note that in vitro expression of cell surface markers may not correlate with in vivo expression. Other markers that are generally accepted include CD44, CD71, Stro-1 and adhesion molecules such as CD 106, CD166 and CD29 [55].

A study carried out by Wang et al. reprogrammed MSCs in the Wharton’s Jelly of the human umbilical cord into...
capacity of three different tissue-specific MSCs from BM, the cells with VEGF and Human CD34+ hematopoietic differentiate into endothelial progenitor cells by supplying the cells with VEGF[57] and Human CD34+ hematopoietic stem cells can differentiate to cardiomyocytes[58]. Arminan et al. demonstrated that MSCs provide better results compared to hematopoietic precursors for treatment of myocardial infarction as MSCs are more effective in reducing infarct size and preventing of ventricular remodeling[59]. Arminan et al. characterized the differentiation capacity of three different tissue-specific MSCs from BM, adipose and dental pulp in vitro and supported the potential use of MSCs in cell-based cardiac therapies[60]. ADSCs and dental pulp stem cells (DPSCs) represent an interesting cell source as both sources contain an abundant population of MSCs. Cultivation period to expand the number of cells can be reduced, particularly for the ADSCs which that can be harvested within a single surgical procedure. This could be an advantageous approach as it reduces length of time and cost in in vitro culturing steps.

Another new approach would be using DPSCs. Gandia et al. tagged DPSCs with green fluorescent protein (GFP) and 1.5 × 10⁶ GFP-DPSCs were intramyocardially delivered into rats which suffered myocardial infarction induced by coronary artery ligation. The rats showed an improvement in cardiac function, accompanied by thickening of the anterior wall and reduction in infarct size. However, no histological evidence was seen for GFP-labeled ECs, SMCs, or cardiac muscle cells within the infarct. The improved heart function was attributed to the angiogenesis and not the differentiation of DPSCs to cardiomyocytes[17]. The prime pitfall of the adult stem cells is the inability of these cells to undergo neovascularization, which is far more important than angiogenesis.

7. Conclusion and challenges ahead

Previous research work have shown promising results by demonstrating the usage of various types of stem cells for myocardial infarction and go on to suggest that these findings could be translated into medical applications. A major obstacle of cell-based therapy is the survival of cells in the ischemia region. To address this issue, various strategies have been employed to enhance the therapeutic potential of the stem cells. Another major issue that needs to be looked into, apart from efficacy and safety, is that cells require strict regulations that agree with current good manufacturing practice (cGMP) in the upscaling procedure. Paying attention to all these issues will ensure a potent medical remedy for cardiovascular disease.

8. Expert opinion

All sources of stem cell including ESCs, adult stem cells and iPSCs were shown to induce cardiomyocytes in in vitro culture conditions as well as other remarkable outcomes such as alleviating cardiac function in animal models with heart injury (Table 2). However, the efficacies of these cells in clinical trials are still controversial, with many of these trials stuck in Phase I/II and hardly any making it to Phase III. Apart from high costs, from culturing and expanding cells in cGMP laboratory to running full-pledge clinical trials coupled with day-by-day stringent regulatory, we believe that many institutions that run these types of trials fail to understand the fundamental biology of these cells. This resulted in the use of their proprietary cells by most institutions to treat all known diseases, including cardiovascular. Based on our previous work[4], we strongly believe that there are no unique master stem cells especially from adult origin, to treat all diseases. Our notion is well echoed by other stem cell expert as well. Rao [5] in his editorial remark said that the utility of the adult cells depends on the tissue it came from and its functional properties. He then gave an example using the MSCs, which looks very promising in treating limb ischemia or graft versus host disease, as this appears to be their normal functions in the adult. However, the same cells have not been useful in forming neurons in the brain as this is not what they were programmed to do. In our opinion, a progenitor cell of a particular cell tissue is perhaps the right source to treat tissue-orientated diseases, and this can be obtained via differentiation of ESCs. These cells will then perhaps differentiate into the target cells upon transplantation or at least release molecule signals that are similar and needed by the host tissue. ESCs in general are more immature and can easily differentiate into progenitors cells as well. It is still possible to use progenitors cells for transplantation due to the lack of HLA markers. However, key issues with ESCs that need to be addressed, which are safety and ethical issues, causes doubt that these cells will go to clinical stage. Hence, in our opinion, it is worthwhile to invest in iPSCs in relation to adult stem cells or ESCs. The discovery of the iPSCs in 2006 has astounded the field of regenerative medicine by showing that personalized or individualized cell therapy may be possible, either by generating ESC-like pluripotent cells or by direct transformation of adult cells into desired progenitors. Many breakthrough discoveries are currently flourishing in the iPSCs field. Recently, the Japanese government has approved the first ever human iPSCs clinical trial for eye diseases. Further, the problem involving efficiency of generating iPSCs was addressed in a paper published in Nature[61], where scientists from the Weizmann Institute of Science, Israel, reported that by removing a single protein (Mbd3) from mice skin cells, they were able to produce iPSCs with near perfect efficiency. Our laboratory too has intensified the work towards generating quality functional cardiomyocytes via direct reprogramming method. We emphasize on basic fundamental knowledge to understand the heart
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Development and continue to improve current technology by generating more in situ learning data. Once bona fide cardiomyocytes can be generated, the next issue will be how, when and what dosage of stem cells are required to improve clinical outcomes. Randomized double-blinded and placebo-centralized clinical trial will be able to answer the above questions.

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Declaration of interest

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