Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease

Cardiovascular disease is a major target for many experimental stem cell-based therapies and mesenchymal stem cells (MSCs) are widely used in these therapies. Transplantation of MSCs to treat cardiac disease has always been predicated on the hypothesis that these cells would engraft, differentiate and replace damaged cardiac tissues. However, experimental or clinical observations so far have failed to demonstrate a therapeutically relevant level of transplanted MSC engraftment or differentiation. Instead, they indicate that transplanted MSCs secrete factors to reduce tissue injury and/or enhance tissue repair. Here we review the evidences supporting this hypothesis including the recent identification of exosome as a therapeutic agent in MSC secretion. In particular, we will discuss the potential and practicality of using this relatively novel entity as a therapeutic modality for the treatment of cardiac disease, particularly acute myocardial infarction.

Stem cells in the treatment of acute myocardial infarction

Acute myocardial infarction (AMI) is the primary cause of disease-related death in the world [1–3]. It is characterized by the disruption of blood supply to the heart muscle cells, which lead to myocardial infarction or death of cardiomyocytes. Reperfusion therapy or the restoration of blood flow by thrombolytic therapy, bypass surgery or percutaneous coronary intervention (PCI) is currently the mainstay of treatment for AMI and is responsible for the significant reduction in AMI mortality [4]. The efficacy of reperfusion therapy has led to increasing survival of patients with severe AMI who would not otherwise survive. However, many (23%) of these survivors progress to fatal heart failure within 30 days [5]. This phenomenon of an increasing number of severe AMI survivors contributes to an ever growing epidemic of heart failures [6–8].

Heart failure is characterized by dilatation and hypertrophy with fibrosis within the myocardium. The progression of an AMI survivor to heart failure is a multifactorial process that has been hypothesized to include the development of myocardial stunning and hibernation, remodeling and chronic neuroendocrine activation [9], and is dependent on the extent of the AMI suffered by the patient [10–15]. The development of reperfusion therapy and its subsequent improvements have significantly increased the salvage of ischemic myocardium from infarction and reduced infarct size, but further substantive improvement to reperfusion therapy is likely to require adjunctive therapies. Although it was recognized as early as 1960 that reperfusion of severely ischemic tissue causes lethal injury [16], the concept that reperfusion causes de novo lethal injury became more widely accepted only when infarct size was shown to be reduced by interventions applied at the onset of reperfusion (reviewed in [10]). Such interventions, also known as postconditioning, involve ischemic conditioning or application of pharmacological agents before the onset of reperfusion, and have demonstrated some protection against reperfusion injury in animals and in small clinical trials. However, none of these agents have proven to be efficacious in large clinical trials and this has led to speculations that reducing reperfusion injury may not be tractable to pharmaceutical interventions [17].

With the emergence of stem cells as potential therapeutic agents, attempts to use stem cells to reduce infarct size and enhance cardiac function in animal models and patients have increased exponentially. To date, stem cell therapy for the heart accounts for a third of publications in the regenerative medicine field [18]. Mummery et al. have recently reviewed the use of both adult and embryonic stem cells, such as bone marrow-derived stem cells, which include hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), endogenous cardiac progenitor cells (CPCs), human embryonic stem cells (hESCs), and human induced pluripotent stem cells (iPSCs) [19].
induced pluripotent stem cells, and hESC-derived cardiomyocytes [18]. The use of bone marrow-derived stem cells such as HSCs and MSCs to repair cardiac tissues was predicated on the hypothesis that these cells could differentiate into cardiomyocytes and supporting cell types. However, careful rodent experimentation has demonstrated that few of the transplanted bone marrow cells engraf and survive, and fewer cells differentiate into cardiomyocytes or supporting cells [18]. In spite of this, transplantation of bone marrow stem cells improves some cardiac functions in animal models and patients, and this has been attributed to a paracrine effect [18]. Although the presence of CPCs in fetal hearts is well established, the presence of CPCs in postnatal or adult heart remains controversial, and the possibility that the so-called CPCs from postnatal hearts are bone marrow cells has remained unresolved. Transplanted cardiomyocytes isolated from in vitro differentiation of hESCs and induced pluripotent stem cells could engraft in the heart to form a syncitium with each other, but not with the recipient heart. This failure to couple with the recipient cardiomyocytes could cause arrhythmia, a potentially fatal condition.

Despite our still evolving understanding of stem cell transplantation in treating cardiac disease, stem cell transplantation has already being tested in clinical trials. In a recent review of more than 20 clinical trials that primarily used adult stem cells, such as bone marrow stem cells, mobilized peripheral blood stem cells and skeletal myoblasts to treat heart disease [19], the trends favored such transplantations to treat cardiac disease when measured using clinical end points of death, recurrence of AMI or hospitalization for heart failure. The failure to elicit a more robust therapeutic response has been attributed to low engraftment of cells and poor survival of engrafted cells with an untested caveat that improved engraftment and survival will enhance the therapeutic efficacy. A general consensus from these clinical trials is that bone marrow- or blood-derived stem cells do not replenish lost cardiomyocytes or vascular cells to any meaningful extent. Instead, circumstantial evidence suggests that these stem cells secrete factors that exert a paracrine effect on the heart tissues [19].

**MSCs & the treatment of cardiovascular disease**

Among the stem cells currently being tested in clinical trials for the heart, MSCs are the most widely used stem cells. Part of the reason for this is their easy availability in accessible tissues, such as bone marrow aspirate and fat tissue [20], and their large capacity for ex vivo expansion [21]. MSCs are also known to have immunosuppressive properties [22] and, therefore, could be used in allogeneic transplantation. They are also reported to have highly plastic differentiation potential that included not only adipogenesis, osteogenesis and chondrogenesis [23–28], but also endothelial, cardiovascular [29], neurogenic [30–32] and neovascular differentiation [33–35]. MSCs transplantation in most animal models of AMI generally resulted in reduced infarct size, improved left ventricular ejection fraction, increased vascular density and myocardial perfusion [36–40]. In a recent Phase I, randomized, double-blind, placebo-controlled dose-escalation clinical trial, single infusion of allogeneic MSCs in patients with AMI was documented to be safe with some provisional indications that the MSC infusion improved outcomes with regard to cardiac arrhythmias, pulmonary function, left ventricular function and symptomatic global assessment [41].

Despite numerous studies on the transplantation of MSCs in patients and animal models, insight into the mechanistic issues underlying the effect of MSC transplantation remains vague. An often cited hypothesis is that transplanted MSCs differentiate into cardiomyocytes and supporting cell types to repair cardiac tissues. However, contrary to this differentiation hypothesis, most transplanted MSCs are entrapped in the lungs and the capillary beds of tissues other than the heart [42,43]. Furthermore, depending on the method of infusion, 6% or less of the transplanted MSCs persist in the heart 2 weeks after transplantation [44]. In addition, transplanted MSCs were observed to differentiate inefficiently into cardiomyocytes [45] while ventricular function was rapidly restored less than 72 h after transplantation [46]. All these observations are physically and temporally incompatible with the differentiation hypothesis and have thus prompted an alternative hypothesis that the transplanted MSCs mediate their therapeutic effect through secretion of paracrine factors that promote survival and tissue repair [47].

**Paracrine secretion of MSCs**
Paracrine secretion of MSCs was reported more than 15 years ago when Haynesworth et al. [48] reported that MSCs synthesize and secrete a broad spectrum of growth factors and cytokines such as VEGF, FGF, MCP-1, HGF, IGF-I, SDF-1 and thrombopoietin [49–53], which exert effects on cells in their vicinity. These factors have been postulated to promote arteriogenesis [51]; support the
stem cell crypt in the intestine [54]; protect against ischemic renal [49,50] and limb tissue injury [52]; support and maintain hematopoiesis [53]; and support the formation of megakaryocytes and proplatelets [55]. Many of these factors have also been demonstrated to exert beneficial effects on the heart, including neovascularization [56], attenuation of ventricular wall thinning [39] and increased angiogenesis [57,58].

In 2006, Gnecchi et al. demonstrated that intramyocardial injection of culture medium conditioned by MSCs overexpressing the Akt gene (Akt-MSCs) or Akt-MSCs reduced infarct size in a rodent model of AMI to the same extent [46]. This provided the first direct evidence that cellular secretion could be cardioprotective [46,59]. The authors subsequently attributed the cardioprotective effect of the conditioned medium to the culturing of the cells under hypoxia and the overexpression of AKT, which induced secretion of Sfrp2. siRNA mediated-silencing of Sfrp2 expression in Akt-MSCs abrogated the cytoprotective effect of their secretion [60].

Our group recently demonstrated that culture medium conditioned by human ESC-derived MSCs (hESC-MSCs) significantly reduced infarct size by approximately 50% in a pig and mouse model of myocardial ischemia/reperfusion (MI/R) injury when administered intravenously in a single bolus just before reperfusion [61]. However, these MSCs were derived from hESCs instead of rat bone marrow and were not genetically modified to overexpress Akt. The conditioned medium was prepared using a chemically defined medium without hypoxia treatment.

We further demonstrated through size fractionation studies that the active component was a large complex 50–200 nm in size. Using electron microscopy, ultracentrifugation studies, mass spectrometry and biochemical assays, we identified this complex as an exosome, a secreted bi-lipid membrane vesicle of endosomal origin (Figure 1). When purified by size exclusion using high-performance liquid chromatography, hESC-MSC exosomes also reduced infarct size, but at a tenth of the protein dosage used for conditioned medium [62]. We subsequently showed that exosomes constitute about 10% of the conditioned medium in terms of protein amount [Lai RC, Lim SK, Unpublished Data]. Therefore, the therapeutic activity in the hESC-MSC conditioned medium could be attributed primarily to the exosome [62]. The secretion of cardioprotective exosomes was not unique to hESC-MSCs and was also found to be produced under nonhypoxic culture conditions by MSCs derived from aborted fetal tissues [63]. Therefore, these observations suggest that the secretion of protective exosomes is a characteristic of MSCs and may be a reflection of the stromal support role of MSCs in maintaining a microenvironmental niche for other cells such as hematopoietic stem cells. The secretion of exosomes may also be a dominant function of MSCs. We recently observed that when GFP-labeled exosome-associated protein CD81 is expressed in hESC-MSCs (Figure 2A), they exhibit a punctate cytosolic distribution and these labeled proteins were secreted (Figure 2C). CD81 is a classical tetraspanin membrane protein usually found localized to the plasma membrane (as typified by their distribution in HEK 293 cells) (Figure 2B). The cellular distribution of the labeled CD81 in hESC-MSCs and its cellular secretion suggest that MSCs are prolific producers of exosomes, and that exosome, whose main function is to mediate intercellular communication (as discussed later), is also MSCs’ vehicle of choice for intercellular communication.

**What are exosomes?**

Exosomes are one of several groups of secreted vesicles, which also include microvesicles, ectosomes, membrane particles, exosome-like vesicles or apoptotic bodies (reviewed in [64]). Exosomes were first found to be secreted by sheep reticulocytes approximately 50 years ago [65,66]. They have since been shown to be secreted by many cell types, including B cells [67], dendritic cells [68], mast cells [69], T cells [70], platelets [71], Schwann cells [72], tumor cells [73] and sperm [74]. They are also found in physiological fluids such as normal urine [75], plasma [76] and bronchial lavage fluid [77].

Compared with other secreted vesicles, exosomes have much better defined biophysical and biochemical properties (reviewed in [64]). They have a diameter of 40–100 nm, with a density in sucrose of 1.13–1.19 g/ml, and can be sedimented at 100,000 g. Their membranes are enriched in cholesterol, sphingomyelin and ceramide, and are known to contain lipid rafts. The presence of exposed phosphatidylserine was reported to be present on the membrane of some exosomes [78,79] and absent from others [80,81]. Exosomes contain both proteins and RNAs. Most exosomes have an evolutionarily conserved set of proteins, including tetraspanins (CD81, CD63 and CD9), Alix and Tsg101, but they also have unique tissue/cell type-specific proteins that reflect their cellular source. Mathivanan and Simpson have set up ExoCarta, a freely accessible web-based compendium of proteins and RNAs found in exosomes [82,201].
The functions of exosomes are not known, but they are believed to be important for intercellular communications. Exosomes were first documented in 1996 to mediate immune communication when it was observed that, when secreted by antigen-presenting cells (APCs), they bear functional peptide–MHC complexes [67]. This also provides the implication that exosomes could be used therapeutically. The therapeutic potential of exosomes was subsequently illustrated by the use of exosomes secreted by tumor peptide-pulsed dendritic cells to suppress tumor growth [68]. Ironically, exosomes are also implicated in tumorigenesis, with the observation that microvesicles mediate intercellular transfer of the oncogenic receptor EGFRvIII [83]. Exosomes have also been reported to have the potential to protect against tissue injury such as MI/R injury [62] or acute tubular injury [84].

In recent years, exosomes have also been implicated in neuronal communication or pathogenesis. For example, exosomes have been found to be released by neurons [85], astrocytes [86] and glial cells [87] to facilitate diverse functions that include removal of unwanted stress proteins [88] and amyloid fibril formation [89,90]. Exosomes containing α-synuclein have been demonstrated to cause cell death in neuronal cells, suggesting that exosomes may amplify and propagate Parkinson’s disease-related pathology [91,92]. It was also reported that, in Alzheimer’s disease,
β-amyloid is released in association with exosome [93]. More recently, oligodendrocytes were demonstrated to secrete exosomes to coordinate myelin membrane biogenesis [94]. Besides neuronal communication, exosomes secreted by cardiomyocyte progenitor cells were reported...
to stimulate the migration of the endothelial cells [95], while those secreted by the egg facilitate the fusion of the sperm and egg [96].

Exosomes have also been implicated as a vehicle for viral and bacterial infection (reviewed in [97]), including the assembly and release of HIV [98–100] and intercellular spreading of infectious prions in transmissible spongiform encephalopathies. The association of exosomes with disease or pathological conditions makes exosomes good sentinels for diseases. It was reported that the miRNA profile of circulating exosomes could be indicative or diagnostic of ovarian cancer [101]. Similarly, the proteins in the urinary exosome have been demonstrated to reflect acute kidney injury and are candidate diagnostic markers [102]. More recently, the function of exosomes as vehicles for intercellular communication has been exploited for the delivery of therapeutic siRNAs to the brain and to provide for alternative drug delivery systems [103].

**Exosome as an alternative therapeutic of MSCs?**

The paracrine hypothesis introduces a radically different dimension to the therapeutic applications of MSCs in regenerative medicine. By replacing transplantation of MSCs with administration of their secreted exosomes, many of the safety concerns and limitations associated with the transplantation of viable replicating cells could be mitigated. For example, the use of viable replicating cells as therapeutic agents carries the risk that the biological potency of the agent may persist or be amplified over time when the need has been resolved, and cannot be attenuated after treatment is terminated. This could lead to dire consequences, especially if treatment was terminated as a result of adverse outcomes. Although repeated direct endomyocardial transplantation of MSCs has been demonstrated to be relatively safe [104], intravascular administration could lead to occlusion in the distal microvasculature as a consequence of the relatively large cell size [105]. Transplantation of MSCs has been reported to induce proarrhythmic effects [106–108]. Their potential to differentiate into osteocytes and chondrocytes has also raised long-term safety concerns regarding ossification and/or calcification in tissues as reported in some animal studies [109].

Besides mitigating the risks associated with cell transplantation, exosomes can also circumvent some of the challenges associated with the use of small soluble biological factors such as growth factors, chemokines, cytokines, transcription factors, genes and RNAs [110]. The delivery of these factors to the right cell type and, in the case of those factors that work intra-cellularly, the delivery into the right cellular compartments, while maintaining the stability, integrity and biological potency of these factors during manufacture, storage and subsequent administration remains a costly challenge. As a bi-lipid membrane vesicle, exosomes not only have the capacity to carry a large cargo load, but also protect the contents from degradative enzymes or chemicals. For example, protein and RNA in MSC exosomes were protected from degradation by trypsin and RNase as long as the lipid membrane was not compromised [62,111]. We also found that storage without potentially toxic cryopreservatives at -20°C for 6 months did not compromise the cardioprotective effects of MSC exosomes or their biochemical activities [Lai RC, Lim SK, Unpublished Data].

Exosomes are known to bear numerous membrane proteins that have binding affinity to other ligands on cell membranes or the extracellular matrix, such as transferrin receptor, tumor necrosis factor receptors, lactadherin, integrins and tetraspanin proteins (e.g., CD9, CD63 and CD81) [82]. These membrane bound molecules provide a potential mechanism for the homing of exosomes to a specific tissue or microenvironment. For example, integrins on exosomes could home exosomes to cardiomyocytes that express ICAM1, a ligand of integrins after MI/R injury [112], or to VCAM-1 on endothelial cells [113]. Tetraspanin proteins, which function primarily to mediate cellular penetration, invasion and fusion events [114], could facilitate cellular uptake of exosomes by specific cell types.

Exosomes may also facilitate the uptake of therapeutic proteins or RNAs into injured cells. Although cellular uptake of exosomes has been demonstrated to occur through endocytosis, phagocytosis and membrane fusion [115–117], the mechanism by which these processes are regulated remains to be determined. It was observed that the efficiency of exosome uptake correlated directly with intracellular and microenvironmental acidity [117]. This may be a mechanism by which MSC exosomes exert their cardioprotective effects on ischemic cardiomyocytes that have a low intracellular pH [118].

Despite being smaller than a cell, exosomes are relatively complex biological entities that contain a range of biological molecules, including proteins and RNA, making them an ideal...
therapeutic candidate to treat complex injuries such as MI/R injury. It is well established that MI/R injury occurs paradoxically in response to a therapy that is highly effective in resolving the disease precipitating problem of no flow and ischemia. During MI/R injury, the restoration of blood and oxygen to ischemic myocardium paradoxically exacerbates the ischemia-induced cellular insults. This is because the biochemical cascades required for cell survival that are initiated by cells during no flow and ischemia [119] are not compatible with the rapid restoration of flow and oxygen supply, and at the same time, cells cannot alter their biochemical activities expeditiously enough to adapt to this restoration. This latter phenomenon was best evidenced by the reduction of MI/R through postreperfusion conditioning or postconditioning where cells were exposed to repeated short nonlethal cycles of reperfusion/ischemia to facilitate biochemical adaptation to reperfusion [120–131].

We postulate that with their complex cargo, exosomes would have adequate potential to participate in a wide spectrum of biochemical and cellular activities, and simultaneously target and correct the various ischemia-induced cascades, and prevent occurrence of the paradoxical reactions induced by reperfusion. In addition, many of the proteins in the exosomes are enzymes. Since enzyme activities are catalytic rather than stoichiometric, and are dictated by their microenvironment (e.g., substrate concentration or pH), the enzyme-based therapeutic activities of exosomes could be activated or attenuated according to the release of injury-associated substrates, which in turn, is proportional to the severity of disease-precipitating microenvironment. Resolution of the disease-precipitating microenvironment would reduce the release of injury-associated substrates and also the activity of exosome enzymes. Consequently, the efficacy of exosome-based therapeutics could be highly responsive to, but also limited by, the disease-precipitating microenvironment.

Together, the features discussed here render exosomes a highly efficacious therapeutic in neutralizing the complexity of MI/R and an effective adjuvant to complement current reperfusion therapy.

Translating hESC-MSC exosomes into therapeutics

The translation of cardioprotective MSC exosomes into a therapeutic agent presents several unique challenges. The first major challenge would be to manufacture Good Manufacturing Practices (GMP) grade exosomes from non-autologous cell sources. Although exosomes as therapeutics have already been tested as a form of cancer vaccine in the clinic [132–134], these tests were limited to exosomes produced during short-term ex vivo culture of autologous dendritic cells. These exosomes, also known as dexosomes, were found to be safe in the small clinical trials [132]. Unfortunately, the manufacture of these exosomes cannot provide guidance for the large-scale GMP production of exosomes from nonautologous cell sources such as exosomes from hESC-MSCs. This manufacturing process faces many unique challenges, including ethical, legal, technical and regulatory/safety issues.

The use of hESCs for the derivation of MSCs presents both ethical and legal challenges. While ethical objections to the derivation and use of hESCs have initially hindered hESC research, they have abated. Instead, the use and applications of hESCs is now being hindered by complex and widespread patenting in some countries [135] and the ban on stem cell-related patents in other countries [136]. To encourage the development of hESC-based therapeutic applications, the need for freedom to use and share hESC resources and knowledge must be balanced with a need to incentivize commercial development of stem cells by protecting the intellectual property generated from research and development efforts. Unfortunately, this balance has not yet been reached.

One of the major technical hurdles to the use of hESC-MSCs is their large but finite expansion capacity, resulting in the need for constant costly re-derivation from hESC and re-validation of each of the derived cell batch. Therefore, a robust scalable and highly renewable cell source will be central to the development of a commercially viable manufacturing process for the production of MSC exosomes in sufficient quantity and quality to support clinical testing or applications. To address this issue, we demonstrated that immortalization of the ESC-MSC by Myc did not compromise the quality or yield of exosomes [137]. Therefore, this provides a potentially inexhaustible cell source for MSC exosome production. The translation of MSC exosomes into clinical applications is also complicated by the relative novelty of exosomes with few precedents in the regulatory and safety space of biopharmaceuticals. This will require the formulation of new standards for manufacture, safety and quality control.
Future perspective

The discovery of stem cells and their potential in regenerative medicine has evoked much excitement and hope in treating some of today’s most intractable diseases, including cardiac disease. However, much of the euphoria has dissipated as animal experimentation revealed and identified potential problems in translating the use of stem cells to treat cardiac disease. Although the reproducible large-scale preparation of homogenous clinically compliant ‘normal’ healthy cells has been a major preoccupation in the development of stem cell-based therapies in general, this has proven not to be an impediment in the development of such therapies for cardiac disease, as evidenced by the large number of stem cell-based clinical trials that are already being conducted. Instead, the problems facing stem cell-based therapies for cardiac disease are potentially more insidious. At present, most of the stem cells used in clinical trials are MSCs and bone marrow mononuclear cells that are generally considered to be safe. However, despite eliciting a sometimes positive therapeutic response, these cells often do not integrate or persist in the heart tissues. By contrast, the use of myogenic cells, such as

Executive summary

Stem cells in the treatment of acute myocardial infarction

- Advances in reperfusion therapy have increased survival of patients with severe acute myocardial infarction and contributed to a growing epidemic of heart failure.
- As reperfusion therapy itself causes lethal injury and has been demonstrated to be intractable to pharmaceutical intervention, stem cells are being scrutinized as alternative therapeutic agents.
- Attempts using stem cells to treat heart disease have generated mixed outcomes.
- Transplantation of bone marrow stem cells generally improved cardiac functions with little evidence of engraftment and differentiation of the transplanted stem cells.
- Effects of stem cell transplantation have been attributed to secretion of paracrine factors by the transplanted stem cells.

Mesenchymal stem cells & the treatment of cardiovascular disease

- Animal studies and early clinical trials demonstrated that mesenchymal stem cell (MSC) transplantation improved cardiac function after myocardial infarction.
- Inefficient MSC engraftment and differentiation, and their rapid cardioprotective effects suggested that MSCs act via a secretion-based paracrine effect rather than a cell replacement effect.

Paracrine secretion of MSCs

- MSCs synthesize a broad spectrum of growth factors and cytokines that exert paracrine effects.
- Gnecchi et al. produced the first evidence that cellular secretion alone improved cardiac function in an animal model of acute myocardial infarction.
- Culture medium conditioned under nonhypoxic conditions by untransformed MSCs derived from human embryonic stem cells or aborted fetal tissues reduce infarct size in animal models of myocardial ischemia/reperfusion.
- Exosome is the primary mediator of MSCs’ paracrine effect.

What are exosomes?

- Exosomes are bi-lipid membrane vesicles secreted by many cell types into culture medium and other bodily fluids such as blood and urine.
- They function as mediators of intercellular communication.

Exosome as an alternative therapeutic for MSC?

- Exosome-based therapy circumvents some of the concerns and limitations in using viable replicating cells and does not compromise some of the advantages associated with using complex therapeutic agents such as cells.
- Exosomes are ideal therapeutic agents because their complex cargo of proteins and genetic materials has the diversity and biochemical potential to participate in multiple biochemical and cellular processes, an important attribute in the treatment of complex disease.
- Exosomes home to specific tissue or microenvironment.
- Their bi-lipid membranes can protect their biologically active cargo allowing for easier storage of exosomes, which allows a longer shelf-life and half-life in patients.
- Their biological activities are mainly enzyme-driven and, therefore, their effects are catalytic and not stoichiometric.
- Having enzyme-driven biological activities, they are dependent on the microenvironment (e.g., substrate concentration or pH) and could be activated or attenuated in proportion to the severity of disease-precipitating microenvironment.
- Exosome-based therapy cannot replace lost myocardium but can prevent or delay loss of myocardium.

Challenges for translating embryonic stem cell-MSC exosomes into therapeutics

- Ethical issues exist, especially with the derivation and use of human embryonic stem cells for generating MSCs.
- Legal issues include excessive intellectual property protection in some countries, which hinder research and development. A ban on embryonic stem cell-related intellectual property in other countries de-incentivize research and development.
- Technical limitations include the need for a robust scalable and highly renewable cell source embryonic stem cell-MSCs to support large scale, commercially viable manufacturing process.
- Exosomes are relatively novel biological entities with few precedents to establish safety and manufacturing guidance.
skeletal myoblasts, cardiac progenitors or stem cell-derived cardiomyocytes, to replace lost myocardium has been demonstrated to increase the risk of arrhythmias when the donor cells failed to couple with the host tissues, in early clinical trials and animal studies. Resolution of these problems would require the development of cell delivery or cell engraftment techniques that can facilitate proper mechanistic integration of the donor cells into the recipient tissues to enable coordinated heart functions. Other potential problems include problems that are generally universal in cell-based therapy, such as host rejection and risk of tumor formation. We anticipate that aside from the issue of proper integration of donor cells into the recipient heart, many of these problems will be resolved or partially resolved in the next 5–10 years. However, without the resolution of the poor coupling between donor and recipient cells, it is unlikely that cell-based therapy using stem cells to replace lost myocardium will evolve into a standard therapy for the treatment of cardiac disease.

Although paracrine secretion of stem cells provides an alternative approach for the development of stem cell-based therapies, it does not replace the need for cell-based therapy to replace lost myocardium. However, it may reduce cardiac injury and delay the loss of myocardium to the extent that replacement of lost myocardium does not become critical. The identification of exosomes as the cardioprotective factor in MSC secretion reduces the paracrine secretion to a single biological entity that is more amenable to the stringent criteria for clinically compliant preparation and use. As a bi-lipid membrane vesicle with many membrane-bound proteins and a diverse cargo, exosome represents an ideal therapeutic agent that has the potential to home to target tissues and treat complicated diseases such as MI/R injury. With the advance of new bioengineering and cellular modification techniques, engineering or modification of the exosome surface antigen and internal content will enable it to target other more complex diseases with even more specificity.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease


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Website

ExoCarta: Exosome protein and RNA database http://exocarta.ludwig.edu.au