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Cellular cardiomyoplasty: routes of cell delivery and retention

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1. ABSTRACT

Experimental and clinical studies have proven the feasibility of cellular cardiomyoplasty in treating the damaged myocardium following ischemic injury. Over the years, this field has exploded with different investigators trying different routes of cell delivery ranging from direct cell injection into the heart to peripheral intravenous delivery utilizing the various signaling mechanisms known. These different routes have resulted in a wide range of retention and engraftment of cells in the target tissues. In this review, we will explore the different modalities of cell delivery, the pros and cons of each route and the cellular retention and therapeutic efficacy of these routes. We will then look into the different theories that try to explain the observed retention and engraftment of cells in the target tissues. Finally, we will discuss various methods that can improve cellular retention and engraftment and hence better improvement in myocardial function.

2. INTRODUCTION

During the past several years, there have been many experimental and clinical studies on progenitor/stem cell therapies for myocardial damage and heart failure. In view of continuous and forceful contraction of the heart, effective delivery of the therapeutic cells effectively into the target tissue has been quite challenging. Here, we will review the routes of cell delivery to the heart and explore the advantages and limitations of each mode of delivery. This will be followed by evidence of cell retention and engraftment and methods of improvement of the methodologies used. Finally, we will look at factors that decrease cell retention and how they could be inhibited.

3. CELL DELIVERY

Physiological mobilization of bone marrow stem cells (BMSCs) occurs after Acute Myocardial Infarction

(AMI) (1-3). This mobilization, although limited, is a natural process by which the body attempts to heal the dead cardiac cells by replacing them with multipotent BMSCs that are capable of differentiating into cardiac cells. Therefore, attempts to deliver more cells to the heart would augment this natural process and, in theory, amplify the healing response. Thus, different methods were developed in order to deliver the cells to the heart in a more controlled fashion. The optimal route of cell delivery is currently still under investigation. It should be able to deliver the maximum cell quantity with the best precision to the targeted site. It should also be easily reproducible, safe to the patient, with minimal unwanted adverse effects.

3.1. Intramyocardial injection

Direct Intramyocardial injection (IM) is one of the earliest routes of cell delivery that have been developed. It can be achieved either through direct injection into the epicardium or endocardium (4-7).

This route has a number of advantages. It allows the delivery of large number of cells to the targeted myocardium. Large cells such as Marrow Stromal Cells (MSCs) and myoblasts can be delivered without fear of coronary occlusion. Direct epicardial injection allows the visualization of the myocardium and targets the cell delivery precisely to scar areas and border zone of infarct areas. This visualization also allows avoiding damage to structures such as the coronary arteries whereas some of the other methods are blind to this point. When coupled with other planned open surgical procedures, this route has proven to be very useful with negligible added risk to the patient.

However, such a procedure is not without any limitations and disadvantages. Direct injection into ischemic and scarred myocardium creates islands of cells with limited blood supply that may lead to poor survival of the cells (5). This technique may not be safe in acute myocardial ischemia, where the myocardium is fragile and sensitive; thus direct injection may result in ventricular arrhythmias or perforation of the myocardium. In addition, epicardial injection may be difficult in reaching all areas of the heart, the septum being an important example. Also, direct IM route by itself causes mechanical damage of the cells and this subsequently provokes an acute inflammatory response in the host tissue that may translate into lower cell survival (8).

Due to the limitations of the direct epicardial injections in this population; namely the high risk of performing an open procedure in the absence of the need for concomitant cardiac surgical procedure, catheter based techniques were developed for cell delivery via the endocardium. This route, compared with the direct epicardial injection, has a number of advantages. It allows the delivery of large number of cells specifically into the myocardium without the need for a major surgical procedure. It also allows the delivery of multiple injections over time as it carries low procedure related risks. This route has proven very useful in no-option patients with advanced coronary artery disease. This method has also proven very beneficial in cases of chronic ischemia, when the patient is excluded from any revascularization (5, 6), or in diffuse pathology of the myocardium such as in viral cardiomyopathy, where diffuse cell delivery into the entire ventricular wall is desirable. Transcatheter techniques require mapping of the ischemic areas in order to achieve accurate delivery. Thus, this approach results in prolonged procedure times that may not be tolerated by sick patients (5, 6).

As in the development of other biotherapeutics, early studies in cardiac cell therapy were conducted in animal models. These animal studies have examined extensively the different aspects of the epicardial route and have become the foundation for subsequent studies using other methods (4, 7, 10-17). The ease of delivering cells using this route in animals is probably why it initially gained great popularity. These studies have proven the feasibility of this route, possible clinical safety and benefit and have led to subsequent use in phase I and II clinical trials (18-21). This was also true for the endocardial route in which animal studies have also been applied to clinical trials (4-7, 22).

In a small clinical trial by Tse *et al.* (6), eight patient with stable angina refractory to maximum medical therapy and not candidates for any revascularization procedure were enrolled in the study. Mononuclear bone marrow cells were first harvested from the BM. Using an electromechanical mapping of the left ventricle for ischemic areas, the cells were delivered through direct injection into the endocardium using a catheter. There were no procedure related complications such as ventricular or atrial arrhythmias, myocardial ischemia, pericardial effusion or bleeding. Although there was no improvement in the ventricular function of the treated patients, patients reported improvement in the anginal class and reduction in the use of antianginal treatment. Areas injected with BM cells showed significant improvement in target wall thickness, perfusion, and motion as assessed by MRI.

These results were supported in another study by Fuchs *et al.* (5), who studied ten patients with chronic ischemic heart disease all not candidates for any revascularization procedure. Fuchs used a cell delivery technique similar to Tse *et al.* There were no procedure related complications and at three months treated patients showed improved Canadian Cardiovascular Society angina score, as well as reduction in the stress induced ischemia within the injected areas.

Catheter endocardial technique has been proven to be clinically safe and feasible. Although the studies were phase I trials only, there was subjective and objective evidence of improvement. However, it is important to note that these studies were carried out on no-option patients. Further studies, with more liberal selection criteria, are needed in order to be able to generalize the results to wider patient population.

The catheters that have been developed for cell delivery try to simulate the direct surgical injection

techniques. There are currently different catheters available in the market but all have the same basic components (23). There is a core element, which is dedicated for the transport of cells. This terminates in the beveled injection needle distally. The rest of the catheter is composed of elements that are designed to support the core element and direct it towards the target sites. The catheters that have been used so far in trials are either integrated systems (Myostar™, MyoCath™), in which the core and support units are joined to form a single unit, or non-integrated systems (Stiletto™, Helix™), in which the core catheter is physically separate and can be inserted and removed separately from the support catheters. One piece integrated systems are easier to use but non-integrated systems are more versatile (23).

The pressure of syringe injection with these systems can be destabilizing and cause expulsion of the needle tip from the myocardial interface (24). In addition, needle site may provide an exit point for injected cells to escape. Currently, myocardial thickness of < 5mm is considered a contraindication for endocardial injection because of risk of perforation.

3.2. Intracoronary injection

This route is based on the principle that cells can be delivered at maximum concentration to the site of injury through the culprit coronary artery. The biological goal is thus to amplify cell trafficking to the ischemic myocardium, raising the natural process of cell homing that occur after ischemia (25, 26) to clinically beneficial levels. Cells can thus be delivered at the time of coronary intervention either in the setting of acute myocardial infarction or chronic myocardial ischemia. Once the culprit vessel has been dilated with balloon and stent, therapeutic cells can be infused homogenously to the target area. This method has the benefit of being minimally invasive and thus can be carried out in all spectrums of patients including those who are high risk for any surgical revascularization. The procedure takes less time compared with endocardial injection as it does not need mapping prior to cell delivery (5, 6) and is more site specific than trans-coronary-venous injection (27). In the hands of an experienced operator the delivery of the cells can be achieved in less than 1 hour.

Feasibility of this approach has been studied in a number of animal models and compared to other modes of cell delivery (14, 28, 29). Myocardial infarction in a swine model was followed with intra-coronary (IC) injection of MSCs and was compared to intravenous and endocardial cell delivery. The procedure proved feasible and cell delivery was comparable to the other methods (29). One advantage is that the cells will follow blood flow distribution and will reach well-perfused areas with enough oxygen and nutrient supply to support cell survival.

However, this route is limited by the fact that the injected cells can actually occlude the coronary vessels that are used to deliver them. Cell delivery is limited by cell size, volume, and concentration, thus it is not the best method to deliver large cells or cells at high concentrations which may form agglutination. As this may not appear to be a problem in freshly purified bone marrow cells, the risk of coronary occlusion is more likely when infusing *ex vivo* expanded cells that are larger in size and often more adhesive. Animal studies have demonstrated rising Troponin levels, markers of myocardial damage, and EKG changes (ST-segment elevation and T-wave inversion) after intra-coronary infusion of MSCs. The ST-segment rose as the dose of cells increased and histological analysis of the heart confirmed the presence of micro-infarction in the culprit areas (30). This may have significant functional implications although the study did not address the functional deterioration associated with these microinfarctions. This study (30) used low passage MSCs that are about 20 microns in diameter while freshly prepared MSCs are only about 10 microns in diameter, which might explain the infarcts that was not seen in other studies (31). In a similar study on swine, treated animals with umbilical cord blood stem cell (UCSC) had significantly larger infarct size on MRI at 5 weeks compared to the control group that only received medium. Histology of the heart showed microinfarctions from obstructed coronary arteries in the treated animals. However, there was no difference in global and regional LV function at 5 weeks between MI animals receiving UCSC or control (28). Inference of functional changes with treatment should be guarded as this study used UCSC, and the animals were treated only 1 week after the MI. It should be noted that cells expanded in culture have increased expression of adhesion molecules compared to freshly isolated cells, so that these cells are more likely to agglutinate or adhere to vascular endothelium throughout the coronaries and cause obstruction (32).

One study has observed that following IC MSC infusion, some animals exhibit decreased blood flow distal to the infusion site. However, no animals exhibited any adverse effects and all had normal recovery, surviving to the end point of the study (29). Some animal studies have demonstrated the occurrence of fatal ventricular arrhythmias in animals, which did not respond to cardioversion (14, 29).

In clinical trials, IC infusion was not associated with increase in Troponin T concentration levels or in-stent stenosis (33% in IC treatment group and 32% in control group at 6 months) in one study (31) but this safety profile was not reproducible in other studies (33-35) as patients exhibited increased in-stent stenosis.

Cells that are used for intra-coronary infusion must be capable of transendothelial migration to perivascular spaces. This depends on the expression of adhesion molecules on the endothelial surfaces that peaks during acute ischemia but may be absent later on (36, 37). In addition, in chronic ischemia, the ischemic areas of the myocardium are fed by collateral vessels that may not be easily accessible by intra-coronary infusion.

Studies have demonstrated that IC transfused cells remain in the area fed by the culprit vessels and are focused mainly in the border zone of the infarct (38-40). To note, intra-coronary infusion cannot be utilized to deliver

cells in areas where the feeding vessel is occluded beyond and cannot be balloon dilated. Areas of minimally perfused myocardium have demonstrated improvement when treated with cells delivered by other methods (6, 23).

Technically, cells can be delivered through coronary balloon angioplasty catheter. After the catheter has been placed in the coronary artery, the guide wire is withdrawn allowing the use of the central lumen as the cell delivery conduit for injection into distal coronary bed. In order to prevent cell washout by antegrade blood flow and to increase the dwell time, the catheter balloon is inflated just before distal injection of the cells and continued occlusion for up to 5 minutes or the onset of significant ischemia (23). However, it is unknown whether this stop/flow technique is required to enhance cell retention within the infarcted area. This technique was effective and reproducible in other studies using other cell lines (28).

A number of clinical trials have examined the use of intra-coronary injections for cell delivery in different clinical settings and using different cell types (31, 33, 41). In the BOOST trial, 30 patients with acute ST-segment elevation MI who had successful percutaneous coronary intervention (PCI), were randomized to either the control group (n=30) that received maximum medical therapy or a treatment group (n=30) that received intra-coronary transfer of autologous BM cells in addition to the medical therapy. The procedure proved feasible and safe. There was no clinically significant myocardial ischemia, proarrhythmic effects or stent stenosis in the treatment group. At 6 months, those patients treated with BMSCs had clinically significant improvement in the mean global left ventricular ejection fraction (LVEF).

However, this safety profile was not proven in another clinical trial by Bartunek *et al.* (33). Using the same patient population as the BOOST trial, patients were randomized to either receive PCI with no additional treatment or PCI with CD 133^+ cells intracoronarily infused 12 days post intervention. Despite significant increase in ejection fraction in the intervention group, there was an increase in the number of coronary events including stent re-occlusion and in-stent restenosis.

The increase in the coronary events in this trial compared to the BOOST trial may be due to the different populations of BMSCs used. Bartunek *et al.* used enriched CD 133^+ compared to unselected BM aspirate in the BOOST trial. Bartunek also used higher cell concentration. Similar findings were observed in the MAGIC trial: despite improvement in cardiac function in the treatment group, there was an unexpectedly high rate of in-stent restenosis in patients who received G-CSF to mobilize BM stem cells. The difference was so significant that the study had to stop enrollment (34, 35).

In the TOPCARE-AMI trial (41), a total of 59 patients treated for Acute MI with PCI received intracoronary infusion of either circulating progenitor cells (CPC) or bone-marrow-derived progenitor cells (BMC). The 2 groups had a comparable safety profile with one coronary event occurring in each group. The functional improvement was statistically significant in the two groups with no difference between them with a 1-year follow up. However, a shortcoming of this study is that there was no control group that did not receive treatment with IC cell infusion. Therefore, the functional improvement observed in the 2 groups may be due to the PCI and not cell therapy. In addition, we cannot exclude the possibility that the IC cell infusion may have contributed to the two re-infarctions observed. There was, however, no evidence of myocardial damage observed by conventional histological methods.

Overall, IC delivery has proven to be feasible and reproducible. It is practical because it can be performed at the time of PCI or shortly after. However, its safety profile is still being questioned as some studies have shown increased risk of re-stenosis and infarction. Further studies are needed to investigate these risks and their clinical significance.

3.3. Retrograde coronary venous injection

This is another catheter delivery system but one that uses the venous system of the heart. The potential advantages of this delivery system include the limited invasiveness that allows the use of this technique in high-risk patients who are not candidates for surgical intervention. Thus, this system may allow the delivery of repeated doses of cells to the heart without the need of repeating a highly invasive technique. Unlike intra-coronary delivery methods, the venous system of the heart is free from obstructive diseases and thus provides a good platform for unobstructed cell delivery. In addition, manipulation of the catheter for coronary delivery in the venous system, as opposed to in the arterial one, eliminates the risk of potential procedural stroke.

The venous system can be used to deliver the cells to the heart by two techniques. The first involves accessing the venous system with a catheter by cannulating the coronary sinus through the right atrium. The angioplasty balloon is then advanced into the great cardiac vein. When in the selected cardiac vein, the balloon is inflated with consequent flow interruption. Under pressure, the cells are infused into the vein (42). In theory, this method delivers cells broadly and uniformly to the heart. However, given the tortuosity and variability of the coronary venous system, accessing certain veins could prove very difficult or even impossible.

The second technique also involves accessing the venous system by the same method described above. However, instead of delivering the cells as an infusate under pressure, a special catheter with a needle tip is used to deliver the cells into the myocardium. Unlike the endocardial approach, the transvenous approach delivers the cells parallel, rather than perpendicular, to the myocardium and thus is safer in cases of thinned myocardium, where there is risk for myocardial perforation with the perpendicular approach. This system may be limited with low therapeutic yield compared to the direct injection techniques but allows access to areas not reached by the latter method such as the septal area.

Initial studies conducted on a swine model have proven the feasibility of this delivery system (24). Using a composite catheter (TransAcess®) which incorporates a phased-array intravascular ultrasound tip for guidance (IVUS) and a sheathed extendable nitnol needle for transvascular myocardial access, Thompson *et al.* tested the feasibility of cell delivery in the swine. The coronary sinus is accessed through the venous system into the right atrium. Once the catheter is in the venous system, IVUS is used to confirm position in the coronary vein, with respect to the surrounding structures. A microinfusion (IntraLume) catheter is then advanced through the needle into the surrounding myocardium and the cells are delivered through the catheter. Through the anterior inter-ventricular coronary vein, it was possible to gain access to the anterior, lateral, septal, apical and inferior walls. This method proved safe with no procedure related complications including death, cardiac tamponade, and ventricular arrhythmia.

Newer second-generation catheters are more flexible. CrossPoint TransAccess catheter's flexibility is well suited for subselective vein access, such as the middle cardiac vein, which allows enhanced inferior wall access (24).

In a phase I clinical trial using a TransAcess® catheter described earlier (27), Siminiak *et al.* attempted the injection of skeletal myoblasts via the coronary sinus in 10 patients. This procedure was successful in 9 patients with no procedure related complications. Follow up at 6 months revealed improvement in NYHA class in all patients and improvement in EF in 6 patients. The limitation of this study was that the improvement was assumed to be due to the cell treatment despite the lack of objective evidence.

3.4. Systemic intravenous injection

There is mounting evidence that the body has a natural ability to mobilize stem cells from the bone marrow in order to repair the damaged heart in the event of acute ischemia (1, 3). Peripheral intravenous infusion of cells, as performed in BM transplant, will thus be an attractive route. This is a minimally invasive or no invasive route for myocardial regeneration. Animal experimental models have confirmed that, when systemically delivered, BM stem cells are capable of homing into the heart and engraft in the peri-infarct area (2, 12, 25, 43). The infused cells not only home into the heart but they have been proven to have therapeutic value. For example, IV administered endothelial progenitor cells (EPCs) in ischemic rats increased the capillary density and decreased the infarct size significantly in treated rats compared to control. Improvement was also evident in functional studies of the heart where the LVDd and LVDs were significantly lower and Fractional Shortening (FS) significantly improved in rats receiving EPCs (43).

The homing mechanism is dependent on signals from the heart, as studies on normal heart did not show any cells homing into them. Thus, this route would be applicable only after AMI because it relies on physiological signaling. As the heart signals are short-lived, this route would only be beneficial in a very narrow time window. It would thus be less useful for treating cases of chronic myocardial ischemia or cardiomyopathies where the signaling mechanisms are insufficient to cause homing of the cells. Attempts have been made to amplify these physiological signals by using different viral vectors that over express the cytokines that have been identified to play a major role in the homing mechanisms such as SDF-1, IL-8 and G-CSF (44-49).

Another major drawback of this method is that infused cells fail to reach their desired destination because of entrapment of the cells in the microvasculature of the lungs, liver and lymphoid tissue, especially the spleen. When stem cells are injected via the intravenous or intra-arterial routes in the absence of a MI, the cells were detected immediately, primarily in the lung and secondarily in the liver. At 48 hours, the cells were observed in the liver and considerable amounts were detected in the lungs and kidneys with very few cells in the heart (50). The primary reason why the stem cells are wedged in the lungs is probably due to cell diameter and cell attachment potential. The average size of the rat MSCs at the 1st passage of culture is between 20 and 24 microns in diameter, while the lung capillaries are about 10-15 microns in diameter. In addition, BM MSCs are attachment competent cells. They attach in fibronectin-facilitated manner *in vitro* and may attach to fibronectin-rich endothelia *in vivo*. Thus, when they pass the narrow capillaries in the lungs, they may attach to the endothelium in a receptor mediated fashion. In this regards, respiratory failure after BM transplantation is one of the most common complications in clinical practice and may be due to abundant cells lodging in the lung (51, 52).

Others studies have attempted to deliver the cells within the ventricular chambers in order to achieve a high concentration. In addition to its invasive nature, this approach has been only successful with techniques delivering the cells directly within the left ventricle. Animal studies that have tried to deliver cells in the right ventricle failed as the animals developed massive pulmonary emboli and died shortly after the infusion (53).

4. CELLULAR RETENTION AND ENGRAFTMENT

For effective cell therapy, cell retention within the targeted infarct area constitutes a critical point with regards to the benefit obtained. Cell retention is defined as the fraction of transplanted cells retained in the myocardium for a short period of time (minutes to hours) while engraftment is the amount of cells viable after a longer time period. The first prerequisite for the success of cell therapy is successful delivery and engraftment of transplanted cells. Despite the fact that early results with cellular cardiomyoplasty have been promising, initial results were received with skepticism because of lack of cellular retention and engraftment at target sites. Initial studies that have tried to explain this focused on cell apoptosis (54, 55). However, recent reports that have looked into the amount of cells retained showed that cells are lost too early for apoptosis to be the main cause.

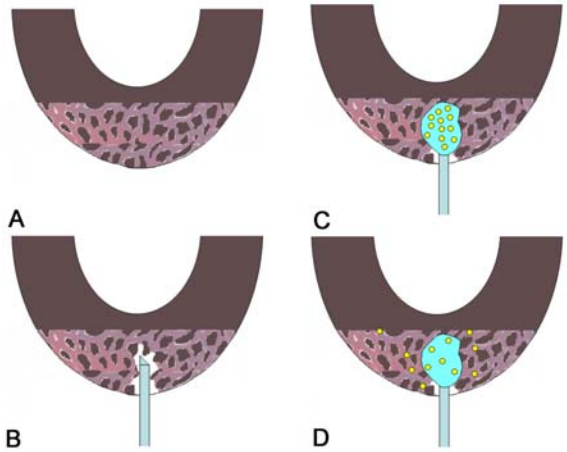

Figure 1. A. Diagram of cross section of the heart showing the blood vessels. B. As the needle pierces the epicardium and myocardium, vascular beds are disrupted. C. The pressure generated by the injection, coupled with that generated by the beating myocardium is able to D.force the cells through the disrupted blood vessels and into systemic circulation.

4.1. Cell loss due to "washout"

Our lab was one of the first to adopt the concept of "cellular washout" to explain the cell loss following intramyocardial cell delivery in cellular cardiomyoplasty. Teng *et al.* (56) studied the mechanical loss following direct intramyocardial injection in the beating heart. The study, which used fluorescent microspheres, similar in size to MSCs but immune to biological losses, looked into the amount retained in the heart after 10 minutes following direct intramyocardial injection. In a rat model, the microspheres retention after single injection into the epicardium was only 6.19% +/- 4.05% and this did not improve with the application of a purse-string occlusion of needle puncture site (5.44% +/- 5.66%). Thus, back-flow from the needle injection site does not account for the cell loss. The retention of the microspheres slightly improved to 8.83% +/- 3.29% if the same volume of microspheres were delivered via multiple injection sites and to 11.1% if a larger animal model was used (a piglet in this case).

The mechanism of "cellular washout" is illustrated in "Figure 1". When the cells are injected into the heart, the injectate forms a bleb within the myocardium. Since the cells were delivered via a needle injection, blood vessels within the myocardium are damaged as the needle pierces the muscles. Given the heart is a powerfully contractile organ, pressure within the injected bleb builds up and cells are forced into the damaged blood vessels since the diameter of the cells is smaller than that of the damaged blood vessels. These cells are washed out of the heart and into systemic circulation, eventually embolizing in capillary beds in the liver and lungs, etc.

Teng's study has many implications; the first is that cell loss resulting in a lower retention occurs too early for apoptosis to be the main culprit. Secondly, in direct intramyocardial injection, back flow is not the main port of mechanical loss, as application of purse-string to the injection site did not improve much the amount retained. Thirdly, the volume injected into the myocardium may have implications in cell retention. In a large animal model, Ishida *et al.* (15) looked into the adverse effect of different cell volumes injected directly into the myocardium. Large volumes compromised heart function. More than 10 ml cell suspension proved to compromise diastolic function of the heart and increased the wall pressure. This may translate into larger cellular washout as the pressure builds up on the bleb injected into the myocardium that contains the cells. Thus, by either decreasing the total volume injected or distributing the volumes into small aliquots with multiple injections, the amount retained maybe increased (17, 56).

One of the main limitations and variables for cell delivery to the heart is that the heart is a beating organ. Technically, it is more challenging to inject cells into a beating heart, especially in a small animal model, resulting in low retention with high standard deviation. In a study using mouse model, 25% of injected hearts did not have any traceable cells (12). This motion of the heart accounts for a significant loss as amount of cells retained in an arrested heart is significantly more than that in a beating heart (56). In addition, the heart is a very vascular organ with the myocardium extensively perfused and also has a very extensive venous drainage. Thus, cells injected in the myocardium are prone to cell washout as soon as they are delivered. Animal studies that looked into cell retention in the ischemic reperfused hearts have shown that they contain significantly lower cell retention than ischemic hearts that were non-reperfused. The cells injected were found in the blood vessels of the heart and at distant sites in the body, suggesting that the cardiac vasculature is the most likely route (10). The effect of vascular washout was also studied by comparing retention within scar tissue in 1-month-old MI and normal myocardium. The retention was significantly higher in the scarred myocardium than in normal myocardium (57). This has significant implications as areas of non-reperfused myocardium, despite having higher retention rate, may not be able to sustain the oxygen demand of the transplanted cells in order to improve the engraftment. Clinically, except in cases where reperfusion strategies are not an option, patients usually first receive reperfusion treatment before having cell therapy.

4.2. Factors affecting cell survival and engraftment

Direct epicardial injection of the cells allows for visualization of the ischemic area and in theory facilitates the delivery of a maximum number of cells in the intended area. However, when the distribution of the injected cells within the hearts was studied, only 59% of directly injected cells were detected in the previously identified underperfused MI segments whereas 41% were not. At the same time, 30% of segments that were identified as under perfused did not contain any detectable cells (11). At 4 days, only 0.44% of cells have survived (12). These findings were also supported by another study where rats treated with direct myocardial injection had only 18 of their 32 previously identified underperfused MI segments showing engraftment of injected cells. These results prove that despite direct visualization of the ischemic area,

methods of accurately delineating the underperfused segments need to be developed and utilized in order to optimize cell delivery during direct intramyocardial injection.

Distribution of engrafted cells within the heart is time gated. Surviving cells were found a the site of needle injection very early but at later times, the surviving cells were found far from the injection site and most likely entered the myocardium by crossing the endothelium (12). This probably involves adhesion molecules and is similar to the translocation of leukocytes into tissues during inflammation.

Different cell types used gave different cell retention and engraftment. This may be due to cell size as larger cells are less likely to be washed out into the circulation. Thus, when larger cells were used, the amount of immediate retention dramatically increased (7, 58). Furthermore, different cell types have different potentials to resist initial ischemia and multiply following the reduction of the acute inflammatory event (59, 60). For instance, in a study comparing different cardiomyocytes cell lines (60), adult cardiomyocytes did not survive under any conditions. In contrast, fetal and neonatal cardiomyocytes formed viable grafts under all conditions.

Studies on ischemia/reperfusion model in swine showed that, at 5 weeks, cells that are injected via the coronaries survived only in the infarct border zone (28). Two clinical studies have looked into the engraftment and bio-distribution of cells following intracoronary injection (38, 39). Hoffman *et al.* (38), using either radiolabelled unselected BM stem cells or CD34-positive (CD34+) cells, delivered them into the coronary arteries of the culprit vessels in patients with acute ST-segment elevation MI who had undergone stenting of the infarct related artery 5 to 9 days earlier. After about 75 minutes of intracoronary transfer, patients underwent 3D PET imaging. In the patients receiving unselected-BM cells, 1.3% to 2.6% of cells were detected in the infarcted myocardium while the remaining activity was primarily seen in the liver and spleen. In those receiving CD34-enriched cells, there was a significant increase in the heart retention to 14%-39%. Both types of cells were detected in the infarct border zone of the culprit blood vessel but the presence was more pronounced with CD34-positive cells. No significant activity was detected in any other areas of the myocardium. However, Blocklet *et al.*, also using enriched CD34+ cells was not able to achieve the same retention rates. After 1 hour of intracoronary infusion, PET images showed only 5.5% +/- 2.3% of the administered cells remained in the myocardium. As in the former study, PET activity in the heart was in the border zone and the rest was found in the liver and spleen. Lower retention in this study compared to Hofmann's study may be due to delayed intracoronary infusion following the MI. In Hoffman's study, cells were transfused 5 to 9 days after stenting. In this study, it was performed after 7 to 21 days. The expression of adhesion molecules, needed for trans-endothelial migration, seems lower the longer we wait post-MI.

Longer monitoring of the heart reveals cell engraftment only in patients with acute myocardial infarction but not in those with chronic heart failure (63). This may be due to lack of expression of adhesion molecules and/or signaling factors in the chronic state.

The feasibility of IV administration of cells for myocardial therapy had been documented earlier (3). However, this route of cell delivery produces very limited retention and engraftment in the heart. Endothelial progenitor cells that were radioactively labeled were injected intravenously in rats 24 hours after myocardial infarction or sham operation (control). At 24 to 96 hours after the injection, only 1.02% +/- 0.19% of radioactivity was traced in the hearts of sham-operated animals but the radioactivity was doubled to 2.03% +/- 0.37% in animals with myocardial infarction (61). Barbash *et al.* also obtained similar results, with less than 1% of cells migrating to the infarcted myocardium. The study points out the very low retention in the heart via homing mechanism in the normal heart that significantly increases in the presence of myocardial infarction.

The retention of cells is dramatically increased if the delivery of cells is more proximal to the heart. Cells that are injected within the left ventricle of sham operated animals had an increase in the retention to 2.69% +/- 1.54%. This retention was more than doubled at 24 hours in animals that had myocardial infarction (4.70% +/- 1.55%) (61). This again could be due to increased expression of adhesion and signaling molecules by the ischemic heart that result in better cell homing and migration to the target infarct area (36, 37, 62, 63). This finding was also supported by Barbash *et al.* (53) who also found that, after left ventricular cavity infusion of MSCs, there was a drastically better uptake in the heart and specifically in infarcted compared with sham-MI hearts. This result is probably due to the higher concentration of cells that enter the coronary circulation which migrate to the ischemic areas guided by signaling molecules (64-67). Thus, despite the fact that cells are being delivered systemically, those that reach the heart show preferential distribution to the ischemic areas. Clusters of donor cells were identified at the border zone of the infarct but not at remote intact myocardium or sham-MI hearts (53, 61). After IV administration in an ischemic model, EPCs were also found principally in the ischemic area and were rarely distributed to non-ischemic myocardium outside the risk area defined by LAD occlusion (43).

4.3. Comparative studies on cell delivery techniques

Several studies have compared the efficacy of the therapeutic potential of different delivery routes in the treatment of injured hearts. Hou *et al.* (14) evaluated the fate of peripheral mononuclear cells (PMNCs) after IM, IC and retrograde coronary venous (RCV) delivery in an ischemic/reperfusion swine model. Six days after the myocardial ischemia, animals were randomized into one of the three delivery groups. The distribution of injected cells was assessed by γ-emission counting of harvested hearts that received ^{111}Indium oxide labeled PMNCs after 1 hour of cell delivery. IM injection had significantly more

retention (11% +/- 3%) compared with IC (2.6% +/- 0.3%). RCV retention was lower (3.2% +/- 1%) than IM but that difference did not reach statistical significance. IC had the highest percentage of cells lost into the pulmonary circulation with 47% +/- 1% of cells found in the lungs compared to 43% +/- 3% for RCV. IM delivery had the lowest loss to the lungs: only 26% +/- 3% of the injected cells were traceable to the lungs. Significantly lower percentages of injected cells were traceable in other organs, including the liver and spleen. This study showed that despite the fact that the majority of delivered cells are not retained in the myocardium, the IM routes has proven to be the most efficient. However, it was also the least consistent (18-fold variability among the animals) with a wide standard deviation in the results. It is worth noting that in this study, right sided distribution i.e. in the lungs, of delivered cells is far in excess to that filtered by left sided organs such as the liver and spleen. This finding suggests that injected cells are largely drained into the myocardial venous or lymphatic system and pumped by the right ventricle to the lungs, rather than being lost through arterial conduits or inside the ventricular lumen. This study demonstrates that IC is highly reproducible but is associated with the lowest retention of cells.

Freyman *et al.* (29) compared IC with EC and IV instead of IM and RCV routes. In his study using a swine model of myocardial ischemia/reperfusion, female swine were randomized to one of the 3 delivery methods. 14 +/- 3 days post delivery, hearts were harvested and utilizing both Dil and fluorescence *in situ* hybridization (FISH) labeling techniques, engrafted cells were quantified. For IC and EC injection, 14-day retention represented 6% and 3% of the administered dose. For the IV infusion group, none of the infarcts contained a measurable number of cells. In none of the delivery groups were cells detected in remote, non-infarcted myocardial samples. Washout to the lung was lowest for EC when compared to IC and IV.

In a study comparing direct epicardial injection via an open chest operation against percutaneous endomyocardial catheter cell delivery the latter had a significantly better retention than the former (43% +/- 15% vs. 15% +/- 21%) (17). This finding was supported in another study that used "Cell-Fix" catheter for endocardial injections vs. direct epicardial injections. The catheter uses electrophysiological guidance to identify the ischemic areas. It has proven to provide a better coverage of the ischemic area compared to the epicardial technique, which relies on surgical visualization. Percutaneous endocardial injections allowed a 63.7% +/- 8.25% ischemic tissue coverage compared to 41.2% +/- 8.1% for the epicardial route (9).

5. ON ENHANCING CELL RETENTION AND SURVIVAL

Currently, little is known regarding the optimal delivery parameters as discussed above. As the mechanisms of cardiac functional improvement are still not clear, it is assumed that more cell retention and engraftment should translate into better functional improvement. Therefore, it is important to understand the factors that may affect cell survival and thus we could try to optimize the conditions for cell delivery.

In order to overcome the huge loss of cells noted, we assume the injected solution should deliver the maximum amount of cells to the heart. This is certainly true with the IV route, where a high concentration is needed to ensure that enough cells are able to migrate across the endothelium into the myocardium and produce a meaningful therapeutic effect (66). However, increasing the cell load has many limitations and side effects. For instance, because a large percentage of cells delivered through the IV route become lodged in the lung (53), it may translate into a higher incidence of pulmonary emboli, acute lung injury or respiratory distress syndrome (68). The lodging of cells has also been reported in other organs, such as the liver and spleen with yet unknown consequences (22, 53). However, there is concern that, given the multipotency of these cells, there may be an increased risk for malignant transformation in these organs (29).

Direct cell delivery methods have also proven to have limitations concerning the increase in cell load. Direct IM cell implantation involves injecting cells to areas that are limited in their blood supply. Therefore, increasing the cell load would expose the cells to a lower perfusion and augment the likelihood of cell death due to ischemia. A large bolus of cells will create a necrotic core that will secrete pro-inflammatory cytokines, killing healthy cells and thus counteracting the main aim of increasing the cell load (58). On the other hand, with the IC route, cells are delivered through coronaries that have been unblocked. Although the areas are better perfused, increasing the cell load would mean that the injectate has increased cellular density, which may result in decreased coronary blood flow (29) and increase in the incidence of infarction and myocardial damage (29, 30).

Studies have proven that cell signaling and cytokines play an important role in cell homing and engraftment (44-49). Thus, it is of utmost importance that the signaling mechanisms are optimal for the mode of cell delivery. For instance, SDF-1 is an important cytokine that has been proven to play a pivotal role in cell chemotaxis and transendothelial migration (44, 45, 65). Studies that have looked into increasing the expression of SDF-1 at the time of cell delivery have demonstrated increased cell retention in the heart (44). Other signaling factors also play important roles. Thus, methods of amplifying molecular signaling using viral vectors may help in cell homing, retention and engraftment in the ischemic heart, which may translate into better functional improvement.

Various factors have to be optimized in order to achieve the best retention and engraftment. Temporal delivery of cells following an ischemic event is very important. Although IV routes of cell delivery may depend on the released signaling molecules and inflammatory cytokines after MI to achieve maximum homing and engraftment -thus the cells need to be delivered early in order to obtain the maximum benefit, it seems that this may

not hold true for other modes of direct cell delivery. As much as some of these signals may be important in the differentiation of implanted cells, they may have adverse effects because of the excessive oxidative load. Hu *et al.* (69) studied the optimal temporal delivery of BM MSCs following myocardial infarction. Rats received IM injections of MSCs at 1hr, 1 week and 2 weeks after MI. It was found that the greatest benefit in terms of reduction of left ventricular dilation, reduction in infarct size and improvement in cardiac function was observed in rats that received cells 1 week after MI. This corresponded with increased survival of the engrafted MSCs. This optimal delivery time could be correlated with the inflammatory response. Following massive myocardial necrosis, leukocytes and inflammatory cells rapidly infiltrate into the ischemic myocardium and peak between 24 and 72 hours. By 1 week, the majority of the infarct zone is composed of granulation tissue and the acute inflammatory reaction is almost resolved. By 2 weeks, scar tissue begins to form and ventricular remodeling starts. As the inflammatory response could be the culprit in reducing the survival of cells immediately delivered following MI, some studies have tried to reduce this inflammatory response (8, 59). One of the methods described in myoblasts transplantation is control of inflammation by inhibiting IL-1. This has proven to improve myoblasts survival (59). Suzuki *et al.* (8), administered IL-1beta antibody and improved the survival of male skeletal muscle precursor cells, thus obtaining a 2-fold increase in the number of surviving cells at 72 hours. Others have looked into factors that are known to increase the survivability of MSCs in harsh conditions. Fibroblast Growth Factor-2 (FGF-2) has mitogenic activity for various cells of mesenchymal, neuronal, and epithelial origin. It activates several signaling components related to cell survival including mitogen-activated protein kinase (MAPK) (70) and protein kinase C (PKC) (71). FGF-2 also stimulates the growth and development of new blood vessels (72). Knowing this, Song *et al.* (73), introduced FGF-2 gene into MSCs *ex-vivo* before transplantation. In hypoxic conditions, the transfected cells displayed a threefold increase in viability as well as increased expression of the anti-apoptotic gene, *Bcl2*. They also showed increased expression of troponin T (Tn-T) and voltage gated Ca^{2+}channel (Ca V2.1). There was associated increase in new blood vessel formation in the FGF-2-MSC group compared to the control MSCs group, as judged by the levels of alpha smooth muscle actin and von Willibrand Factor (vWF). This finding suggests that the FGF-2 indeed promoted angiogenesis (73).

Several studies have implicated the role of apoptosis in cell death and decrease in the cellular engraftment (54, 60). However, in a study by Muller-Ehmsen, the caspase inhibitor AcYVADemk failed to improve transplanted cell survival at 24 hrs. This suggested that apoptosis did not play a major role in cell loss (58).

Another method that could result in improved cell retention is to reduce the volume of the injected cells (17). When the volume injected in a swine model was reduced from 100 microliters to 10 microliters the retention increased from 20% +/- 25% to 98% +/- 85% in endocardial injection and from 9% +/- 9% to 36% +/- 50% in epicardial injections. Similar findings were demonstrated by Teng *et al.* who showed that for same volume of the injectate, multiple smaller volume injections produced a better retention compared to a single larger volume injection (56).

One of the methods described to improve survival is pre-treatment with vascular endothelial growth factor before cell delivery (74, 75). One of the hypotheses of cell loss we discussed earlier is ischemic cell death secondary to cells being delivered into poorly perfused areas. Stimulation of the same areas with Vascular Endothelial Growth Factor (VEGF) to induce angiogenesis before cell delivery may aid cell survival. Retuerto *et al.* pretreated ischemic myocardium with an adenovirus encoding VEGF 121 3 weeks before delivering fetal cardiomyocytes to the same area. 2 weeks later, rats pretreated with VEGF before cell transplant had nearly double the exercise tolerance compared to controls. However, Chachques *et al.* did not demonstrate this functional improvement in their study. Animals treated with a combination of VEGF and myoblasts did not show any functional or angiogenic advantage over those treated with myoblasts alone. Those treated with myoblasts either alone or in combination with VEGF showed significant limitation of left ventricular dilation and regional functional area change. This is despite the fact that animals treated with VEGF alone showed a higher capillary density in the per-infarct area compared to the other groups. The different results seen in this study compared to studies that did find an effect may be due to the fact of simultaneously administering VEGF and cells. This may have resulted in an inflammatory effect due to cell injection that reduced or neutralized the VEGF effect. It may also be due to the relatively delayed delivery of VEGF after 3 weeks. (76). This study has two important implications; the first is that angiogenesis alone may not result in improvement of cardiac function in the absence of cell therapy. Second, the temporal relationship between delivery of angiogenic growth factor and delivery of cells is not very clear yet. More studies are needed in this topic, in order to optimize the effects of angiogenic agents in relation to stem cell therapy.

Cell size, as described earlier, could play an important role in retention and subsequent engraftment. Thus the larger the cell used, the more of it will be retained (60) probably as a result of decreased " wash-out" into the cardiac vasculature (56).

Different cell types exhibit different robustness in surviving in the myocardium once delivered. It appears that the more primitive the cell line used the more likely it will survive. Using fetal, neonatal and adult cardiomyocytes, Reinecke *et al.* studied how the developmental stage of cardiomyocytes affected their survival once implanted into ischemic myocardium. They found that adult cardiomyocytes did not survive under any conditions at day 6 post-transplant. Even when studied at day 1, the vast majority of adult cardiomyocytes had features typical of coagulation necrosis, including loss of nuclei and more intensely eosinophillic cytoplasm. In contrast, fetal and

neonatal cardiomyocytes formed viable grafts under all conditions (60).

Proximity to the ischemic heart may also play a role in increasing retention. Barbash *et al.* (53) demonstrated increased cellular retention when the cells are delivered within the left ventricle compared to those injected distal intravenously.

Some investigators tried to innovate with materials used in other medical and surgical fields in order to increase cell retention. Christman *et al.* (77), when injecting the cells in the myocardium, used fibrin glue mixed with skeletal myoblasts. Skeletal myoblasts mixed with fibrin glue covered a greater area of the myocardium and contained a significantly greater myoblast density than control. This resulted in a smaller scar area and a higher arteriolar density. The fibrin glue is a biopolymeric scaffold that becomes semi-rigid upon injection. The cells contained within this biological glue are prevented from escaping and are thus retained in the myocardium. Others have studied using various biodegradable scaffolds to increase cell retention and survival.

The composition of the extracellular matrix (ECM) of the heart is known to be important in maintaining the normal geometry of the heart. Following MI, the ECM is deeply altered and collagen type I, which is important in maintaining this matrix, is decreased from 80% to 40%. Thus, Cortes-Morichetti *et al.* (78) have engineered a collagen matrix onto which Umbilical Cord Stem Cells (UCSCs) were seeded and grafted onto the epicardium of infarcted ventricle. Compared to both control (medium injection) and cell injection, the cell plus matrix group had both higher wall thickness and lower end-diastolic volume (EDV) at 45 days. However, the cell plus matrix group did not show significant improvement in EF over the cell therapy group. Many mechanisms are believed to be responsible for this improvement, but increased cell retention and survival may be the main one. By increasing the cell retention, the cells are allowed to exert their paracrine effects more effectively. The increased engraftment may be due to the possible anti-apoptotic effect that the collagen matrix exerted by lowering the LV parietal stress.

6. SUMMARY AND CONCLUSIONS

The first step in stem cell therapy for cardiac diseases is to deliver appropriate cells effectively to the desired site. There are two major challenges for cell delivery. First, the myocardium is a unique tissue that continuously and forcefully contracts, such that any injectate containing the stem cells could easily be squeezed out mechanically. Second, there is a continuing controversy regarding the mechanism of action how the implanted cells exert their therapeutic effects. Currently, the proposed mechanisms include transdifferentiation of stem cells into cardiomyocytes, cell fusion, angiogenesis and various paracrine effects that range from cytokines to ECM enzymes. Such ambiguity contributes to our incomplete understanding of the dose-response relationship, which then compromises our choice for optimal cell delivery strategy. Here, we reviewed the current state of art in this field, and explored the future challenges for our knowledge and skills in order to realize the goal of advancing successful regenerative therapy for heart diseases.

7. REFERENCES

1. Y. Wang, H. Haider, N. Ahmad, D. Zhang and M. Ashraf: Evidence for ischemia induced host-derived bone marrow cell mobilization into cardiac allografts. *J Mol Cell Cardiol*, 41, 478-87 (2006)
2. W. Jiang, A. Ma, T. Wang, K. Han, Y. Liu, Y. Zhang, A. Dong, Y. Du, X. Huang, J. Wang, X. Lei and X. Zheng: Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium in vivo: a time-series study. *Pflugers Arch*, 453, 43-52 (2006)
3. J. D. Abbott, Y. Huang, D. Liu, R. Hickey, D. S. Krause and F. J. Giordano: Stromal cell-derived factor-1alpha plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation*, 110, 3300-5 (2004)
4. L. C. Amado, A. P. Saliaris, K. H. Schuleri, M. St John, J. S. Xie, S. Cattaneo, D. J. Durand, T. Fitton, J. Q. Kuang, G. Stewart, S. Lehrke, W. W. Baumgartner, B. J. Martin, A. W. Heldman and J. M. Hare: Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci U S A*, 102, 11474-9 (2005)
5. S. Fuchs, L. F. Satler, R. Kornowski, P. Okubagzi, G. Weisz, R. Baffour, R. Waksman, N. J. Weissman, M. Cerqueira, M. B. Leon and S. E. Epstein: Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. *J Am Coll Cardiol*, 41, 1721-4 (2003)
6. H. F. Tse, Y. L. Kwong, J. K. Chan, G. Lo, C. L. Ho and C. P. Lau: Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet*, 361, 47-9 (2003)
7. P. C. Smits, G. van Langenhove, M. Schaar, A. Reijs, W. H. Bakker, W. J. van der Giessen, P. D. Verdouw, E. P. Krenning and P. W. Serruys: Efficacy of percutaneous intramyocardial injections using a nonfluoroscopic 3-D mapping based catheter system. *Cardiovasc Drugs Ther*, 16, 527-33 (2002)
8. K. Suzuki, B. Murtuza, J. R. Beauchamp, N. J. Brand, P. J. Barton, A. Varela-Carver, S. Fukushima, S. R. Coppen, T. A. Partridge and M. H. Yacoub: Role of interleukin-1beta in acute inflammation and graft death after cell transplantation to the heart. *Circulation*, 110, II219-24 (2004)
9. J. C. Chachques, A. Azarine, E. Mousseaux, M. El Serafi, M. Cortes-Morichetti and A. F. Carpentier: MRI evaluation of local myocardial treatments: epicardial versus endocardial (Cell-Fix catheter) injections. *J Interv Cardiol*, 20, 188-96 (2007)
10. J. Dow, B. Z. Simkhovich, L. Kedes and R. A. Kloner: Washout of transplanted cells from the heart: a potential new hurdle for cell transplantation therapy. *Cardiovasc Res*, 67, 301-7 (2005)

11. N. S. Tran, Poussier, P. R. Franken, F. Maskali, F. Groubatch, C. Vanhove, L. Antunes, G. Karcher, J. P. Villemot and P. Y. Marie: Feasibility of in vivo dual-energy myocardial SPECT for monitoring the distribution of transplanted cells in relation to the infarction site. *Eur J Nucl Med Mol Imaging*, 33, 709-15 (2006)
12. C. Toma, M. F. Pittenger, K. S. Cahill, B. J. Byrne and P. D. Kessler: Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*, 105, 93-8 (2002)
13. N. Tran, P. R. Franken, F. Maskali, J. Nloga, P. Maureira, S. Poussier, F. Groubatch, C. Vanhove, J. P. Villemot and P. Y. Marie: Intramyocardial Implantation of bone marrow-derived stem cells enhances perfusion in chronic myocardial infarction: dependency on initial perfusion depth and follow-up assessed by gated pinhole SPECT. *J Nucl Med*, 48, 405-12 (2007)
14. D. Hou, E. A. Youssef, T. J. Brinton, P. Zhang, P. Rogers, E. T. Price, A. C. Yeung, B. H. Johnstone, P. G. Yock and K. L. March: Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation*, 112, I150-6 (2005)
15. M. Ishida, S. Tomita, T. Nakatani, K. Kagawa, T. Yamaguchi, M. Suga, Y. Ohtsu, H. Yazawa, T. Yagihara and S. Kitamura: Acute effects of direct cell implantation into the heart: a pressure-volume study to analyze cardiac function. *J Heart Lung Transplant*, 23, 881-8 (2004)
16. B. Pouzet, J. T. Vilquin, A. A. Hagege, M. Scorsin, E. Messas, M. Fiszman, K. Schwartz and P. Menasche: Factors affecting functional outcome after autologous skeletal myoblast transplantation. *Ann Thorac Surg*, 71, 844-50; discussion 850-1 (2001)
17. P. M. Grossman, Z. Han, M. Palasis, J. J. Barry and R. J. Lederman: Incomplete retention after direct myocardial injection. *Catheter Cardiovasc Interv*, 55, 392-7 (2002)
18. K. Hamano, M. Nishida, K. Hirata, A. Mikamo, T. S. Li, M. Harada, T. Miura, M. Matsuzaki and K. Esato: Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J*, 65, 845-7 (2001)
19. M. Hendrikx, K. Hensen, C. Clijsters, H. Jongen, R. Koninckx, E. Bijnens, M. Ingels, A. Jacobs, R. Geukens, P. Dendale, J. Vijgen, D. Dilling, P. Steels, U. Mees and J. L. Rummens: Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: results from a randomized controlled clinical trial. *Circulation*, 114, I101-7 (2006)
20. C. Stamm, B. Westphal, H. D. Kleine, M. Petzsch, C. Kittner, H. Klinge, C. Schumichen, C. A. Nienaber, M. Freund and G. Steinhoff: Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet*, 361, 45-6 (2003)
21. C. Stamm, H. D. Kleine, Y. H. Choi, S. Dunkelmann, J. A. Lauffs, B. Lorenzen, A. David, A. Liebold, C. Nienaber, D. Zurakowski, M. Freund and G. Steinhoff: Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg*, 133, 717-25 (2007)
22. D. L. Kraitchman, A. W. Heldman, E. Atalar, L. C. Amado, B. J. Martin, M. F. Pittenger, J. M. Hare and J. W. Bulte: In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation*, 107, 2290-3 (2003)
23. W. Sherman, T. P. Martens, J. F. Viles-Gonzalez and T. Siminiak: Catheter-based delivery of cells to the heart. *Nat Clin Pract Cardiovasc Med*, 3 Suppl 1, S57-64 (2006)
24. C. A. Thompson, B. A. Nasseri, J. Makower, S. Houser, M. McGarry, T. Lamson, I. Pomerantseva, J. Y. Chang, H. K. Gold, J. P. Vacanti and S. N. Oesterle: Percutaneous transvenous cellular cardiomyoplasty. A novel nonsurgical approach for myocardial cell transplantation. *J Am Coll Cardiol*, 41, 1964-71 (2003)
25. B. Bittira, D. Shum-Tim, A. Al-Khaldi and R. C. Chiu: Mobilization and homing of bone marrow stromal cells in myocardial infarction. *Eur J Cardiothorac Surg*, 24, 393-8 (2003)
26. S. M. Devine, A. M. Bartholomew, N. Mahmud, M. Nelson, S. Patil, W. Hardy, C. Sturgeon, T. Hewett, T. Chung, W. Stock, D. Sher, S. Weissman, K. Ferrer, J. Mosca, R. Deans, A. Moseley and R. Hoffman: Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol*, 29, 244-55 (2001)
27. T. Siminiak, D. Fiszer, O. Jerzykowska, B. Grygielska, N. Rozwadowska, P. Kalmucki and M. Kurpisz: Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. *Eur Heart J*, 26, 1188-95 (2005)
28. A. D. Moelker, T. Baks, K. M. Wever, D. Spitskovsky, P. A. Wielopolski, H. M. van Beusekom, R. J. van Geuns, S. Wnendt, D. J. Duncker and W. J. van der Giessen: Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. *J Mol Cell Cardiol*, 42, 735-45 (2007)
29. T. Freyman, G. Polin, H. Osman, J. Crary, M. Lu, L. Cheng, M. Palasis and R. L. Wilensky: A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J*, 27, 1114-22(2006)
30. P. R. Vulliet, M. Greeley, S. M. Halloran, K. A. MacDonald and M. D. Kittleson: Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet*, 363, 783-4 (2004)
31. K. C. Wollert, G. P. Meyer, J. Lotz, S. Ringes-Lichtenberg, P. Lippolt, C. Breidenbach, S. Fichtner, T. Korte, B. Hornig, D. Messinger, L. Arseniev, B. Hertenstein, A. Ganser and H. Drexler: Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*, 364, 141-8 (2004)
32. B. Liu, S. M. Buckley, I. D. Lewis, A. I. Goldman, J. E. Wagner and J. C. van der Loo: Homing defect of cultured human hematopoietic cells in the NOD/SCID mouse is mediated by Fas/CD95. *Exp Hematol*, 31, 824-32 (2003)
33. J. Bartunek, M. Vanderheyden, B. Vandekerckhove, S. Mansour, B. De Bruyne, P. De Bondt, I. Van Haute, N. Lootens, G. Heyndrickx and W. Wijns: Intracoronary injection of CD133-positive enriched bone marrow

progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. *Circulation*, 112, I178-83 (2005)
34. J. W. Bulte, S. Zhang, P. van Gelderen, V. Herynek, E. K. Jordan, I. D. Duncan and J. A. Frank: Neurotransplantation of magnetically labeled oligodendrocyte progenitors: magnetic resonance tracking of cell migration and myelination. *Proc Natl Acad Sci U S A*, 96, 15256-61 (1999)
35. H. J. Kang, H. S. Kim, S. Y. Zhang, K. W. Park, H. J. Cho, B. K. Koo, Y. J. Kim, D. Soo Lee, D. W. Sohn, K. S. Han, B. H. Oh, M. M. Lee and Y. B. Park: Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet*, 363, 751-6 (2004)
36. K. Jaakkola, S. Jalkanen, K. Kaunismaki, E. Vanttinen, P. Saukko, K. Alanen, M. Kallajoki, L. M. Voipio-Pulkki and M. Salmi: Vascular adhesion protein-1, intercellular adhesion molecule-1 and P-selectin mediate leukocyte binding to ischemic heart in humans. *J Am Coll Cardiol*, 36, 122-9 (2000)
37. A. M. Lefer: Role of selectins in myocardial ischemia-reperfusion injury. *Ann Thorac Surg*, 60, 773-7 (1995)
38. M. Hofmann, K. C. Wollert, G. P. Meyer, A. Menke, L. Arseniev, B. Hertenstein, A. Ganser, W. H. Knapp and H. Drexler: Monitoring of bone marrow cell homing into the infarcted human myocardium. *Circulation*, 111, 2198-202 (2005)
39. D. Blocklet, M. Toungouz, G. Berkenboom, M. Lambermont, P. Unger, N. Preumont, E. Stoupel, D. Egrise, J. P. Degaute, M. Goldman and S. Goldman: Myocardial homing of nonmobilized peripheral-blood CD34+ cells after intracoronary injection. *Stem Cells*, 24, 333-6 (2006)
40. C. T. Mesquita, P. L. Correa, R. C. Felix, J. C. Azevedo, S. Alves, C. C. Oliveira, A. L. Sousa, R. Borojevic and H. F. Dohmann: Autologous bone marrow mononuclear cells labeled with Tc-99m hexamethylpropylene amine oxime scintigraphy after intracoronary stem cell therapy in acute myocardial infarction. *J Nucl Cardiol*, 12, 610-2 (2005)
41. V. Schachinger, B. Assmus, M. B. Britten, J. Honold, R. Lehmann, C. Teupe, N. D. Abolmaali, T. J. Vogl, W. K. Hofmann, H. Martin, S. Dimmeler and A. M. Zeiher: Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol*, 44, 1690-9 (2004)
42. E. C. Perin and J. Lopez: Methods of stem cell delivery in cardiac diseases. *Nat Clin Pract Cardiovasc Med*, 3 Suppl 1, S110-3 (2006)
43. A. Kawamoto, H. C. Gwon, H. Iwaguro, J. I. Yamaguchi, S. Uchida, H. Masuda, M. Silver, H. Ma, M. Kearney, J. M. Isner and T. Asahara: Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation*, 103, 634-7 (2001)
44. A. T. Askari, S. Unzek, Z. B. Popovic, C. K. Goldman, F. Forudi, M. Kiedrowski, A. Rovner, S. G. Ellis, J. D. Thomas, P. E. DiCorleto, E. J. Topol and M. S. Penn: Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*, 362, 697-703 (2003)
45. J. Yamaguchi, K. F. Kusano, O. Masuo, A. Kawamoto, M. Silver, S. Murasawa, M. Bosch-Marce, H. Masuda, D. W. Losordo, J. M. Isner and T. Asahara: Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation*, 107, 1322-8 (2003)
46. K. Schomig, G. Busch, B. Steppich, D. Sepp, J. Kaufmann, A. Stein, A. Schomig and I. Ott: Interleukin-8 is associated with circulating CD133+ progenitor cells in acute myocardial infarction. *Eur Heart J*, 27, 1032-7 (2006)
47. A. A. Kocher, M. D. Schuster, N. Bonaros, K. Lietz, G. Xiang, T. P. Martens, P. A. Kurlansky, H. Sondermeijer, P. Witkowski, A. Boyle, S. Homma, S. F. Wang and S. Itescu: Myocardial homing and neovascularization by human bone marrow angioblasts is regulated by IL-8/Gro CXC chemokines. *J Mol Cell Cardiol*, 40, 455-64 (2006)
48. E. Chavakis: IL-8: a new player in the homing of endothelial progenitor cells to ischemic myocardium. *J Mol Cell Cardiol*, 40, 442-5 (2006)
49. S. Minatoguchi, G. Takemura, X. H. Chen, N. Wang, Y. Uno, M. Koda, M. Arai, Y. Misao, C. Lu, K. Suzuki, K. Goto, A. Komada, T. Takahashi, K. Kosai, T. Fujiwara and H. Fujiwara: Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. *Circulation*, 109, 2572-80 (2004)
50. J. Gao, J. E. Dennis, R. F. Muzic, M. Lundberg and A. I. Caplan: The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs*, 169, 12-20 (2001)
51. S. W. Crawford and F. B. Petersen: Long-term survival from respiratory failure after marrow transplantation for malignancy. *Am Rev Respir Dis*, 145, 510-4 (1992)
52. T. Bojko and D. A. Notterman: Reversal of fortune? Respiratory failure after bone marrow transplantation. *Crit Care Med*, 27, 1061-2 (1999)
53. I. M. Barbash, P. Chouraqui, J. Baron, M. S. Feinberg, S. Etzion, A. Tessone, L. Miller, E. Guetta, D. Zipori, L. H. Kedes, R. A. Kloner and J. Leor: Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*, 108, 863-8 (2003)
54. M. Zhang, D. Methot, V. Poppa, Y. Fujio, K. Walsh and C. E. Murry: Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol*, 33, 907-21 (2001)
55. L. Ye, H. Haider, C. Guo and E. K. Sim: Cell-based VEGF delivery prevents donor cell apoptosis after transplantation. *Ann Thorac Surg*, 83, 1233-4; author reply 1234 (2007)
56. C. J. Teng, J. Luo, R. C. Chiu and D. Shum-Tim: Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: implications for cellular cardiomyoplasty. *J Thorac Cardiovasc Surg*, 132, 628-32 (2006)
57. N. Tran, Y. Li, F. Maskali, L. Antunes, P. Maureira, M. H. Laurens, P. Y. Marie, G. Karcher, F. Groubatch, J. F. Stoltz and J. P. Villemot: Short-term heart retention and

distribution of intramyocardial delivered mesenchymal cells within necrotic or intact myocardium. *Cell Transplant*, 15, 351-8 (2006)
58. J. Muller-Ehmsen, P. Whittaker, R. A. Kloner, J. S. Dow, T. Sakoda, T. I. Long, P. W. Laird and L. Kedes: Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J Mol Cell Cardiol*, 34, 107-16 (2002)
59. Z. Qu, L. Balkir, J. C. van Deutekom, P. D. Robbins, R. Pruchnic and J. Huard: Development of approaches to improve cell survival in myoblast transfer therapy. *J Cell Biol*, 142, 1257-67 (1998)
60. H. Reinecke, M. Zhang, T. Bartosek and C. E. Murry: Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. *Circulation*, 100, 193-202 (1999)
61. A. Aicher, W. Brenner, M. Zuhayra, C. Badorff, S. Massoudi, B. Assmus, T. Eckey, E. Henze, A. M. Zeiher and S. Dimmeler: Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. *Circulation*, 107, 2134-9 (2003)
62. T. Takahashi, C. Kalka, H. Masuda, D. Chen, M. Silver, M. Kearney, M. Magner, J. M. Isner and T. Asahara: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med*, 5, 434-8 (1999)
63. S. Shintani, T. Murohara, H. Ikeda, T. Ueno, T. Honma, A. Katoh, K. Sasaki, T. Shimada, Y. Oike and T. Imaizumi: Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*, 103, 2776-9 (2001)
64. V. F. Segers, I. Van Riet, L. J. Andries, K. Lemmens, M. J. Demolder, A. J. De Becker, M. M. Kockx and G. W. De Keulenaer: Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. *Am J Physiol Heart Circ Physiol*, 290, H1370-7 (2006)
65. A. Peled, O. Kollet, T. Ponomaryov, I. Petit, S. Franitza, V. Grabovsky, M. M. Slav, A. Nagler, O. Lider, R. Alon, D. Zipori and T. Lapidot: The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood*, 95, 3289-96 (2000)
66. K. Imai, M. Kobayashi, J. Wang, Y. Ohiro, J. Hamada, Y. Cho, M. Imamura, M. Musashi, T. Kondo, M. Hosokawa and M. Asaka: Selective transendothelial migration of hematopoietic progenitor cells: a role in homing of progenitor cells. *Blood*, 93, 149-56 (1999)
67. B. Ruster, S. Gottig, R. J. Ludwig, R. Bistrian, S. Muller, E. Seifried, J. Gille and R. Henschler: Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood*, 108, 3938-44 (2006)
68. S. Schrepfer, T. Deuse, H. Reichenspurner, M. P. Fischbein, R. C. Robbins and M. P. Pelletier: Stem cell transplantation: the lung barrier. *Transplant Proc*, 39, 573-6 (2007)
69. X. Hu, J. Wang, J. Chen, R. Luo, A. He, X. Xie and J. Li: Optimal temporal delivery of bone marrow mesenchymal stem cells in rats with myocardial infarction. *Eur J Cardiothorac Surg*, 31, 438-43 (2007)
70. H. Tokuda, O. Kozawa and T. Uematsu: Basic fibroblast growth factor stimulates vascular endothelial growth factor release in osteoblasts: divergent regulation by p42/p44 mitogen-activated protein kinase and p38 mitogen-activated protein kinase. *J Bone Miner Res*, 15, 2371-9 (2000)
71. M. A. Nugent and R. V. Iozzo: Fibroblast growth factor-2. *Int J Biochem Cell Biol*, 32, 115-20 (2000)
72. F. Debiais, G. Lefevre, J. Lemonnier, S. Le Mee, F. Lasmoles, F. Mascarelli and P. J. Marie: Fibroblast growth factor-2 induces osteoblast survival through a phosphatidylinositol 3-kinase-dependent, -beta-catenin-independent signaling pathway. *Exp Cell Res*, 297, 235-46 (2004)
73. H. Song, K. Kwon, S. Lim, S. M. Kang, Y. G. Ko, Z. Xu, J. H. Chung, B. S. Kim, H. Lee, B. Joung, S. Park, D. Choi, Y. Jang, N. S. Chung, K. J. Yoo and K. C. Hwang: Transfection of mesenchymal stem cells with the FGF-2 gene improves their survival under hypoxic conditions. *Mol Cells*, 19, 402-7 (2005)
74. M. A. Retuerto, P. Schalch, G. Patejunas, J. Carbray, N. Liu, K. Esser, R. G. Crystal and T. K. Rosengart: Angiogenic pretreatment improves the efficacy of cellular cardiomyoplasty performed with fetal cardiomyocyte implantation. *J Thorac Cardiovasc Surg*, 127, 1041-9; discussion 1049-51 (2004)
75. T. K. Rosengart, E. G. Chedrawy, G. Patejunas and M. Retuarto: Vascular endothelial growth factor before cells. *J Thorac Cardiovasc Surg*, 129, 696 (2005)
76. J. C. Chachques, F. Duarte, B. Cattadori, A. Shafy, N. Lila, G. Chatellier, J. N. Fabiani and A. F. Carpentier: Angiogenic growth factors and/or cellular therapy for myocardial regeneration: a comparative study. *J Thorac Cardiovasc Surg*, 128, 245-53 (2004)
77. K. L. Christman, A. J. Vardanian, Q. Fang, R. E. Sievers, H. H. Fok and R. J. Lee: Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol*, 44, 654-60 (2004)
78. M. Cortes-Morichetti, G. Frati, O. Schussler, J.-P. Huyen, E. Lauret, J. Genovese, A. Carpentier and J. C. Chachques: Association between a cell-seeded collagen matrix and cardiomyoplasty for myocardial support and regeneration. Tissue Eng, in-press

Abbreviations: BMSC: bone marrow stem cell; UCSC: umbilical cord blood stem cell; PMNC: peripheral mononuclear cell; EPC: endothelial progenitor cell; AMI: acute myocardial infarction; IM: intramyocardial; IC: intracoronary; RCV: retrograde coronary venous; IVUS: intravascular ultrasound tip for guidance; PCI: percutaneous coronary intervention; LVEF: left ventricular ejection fraction; FS: fractional shortening; IL-1: interleukin-1; MAPK: mitogen-activated protein kinase; PKC: protein kinase C; FGF-2: fibroblast growth factor 2, EF: ejection fraction

Key Words: Cellular cardiomyoplasty, Cell delivery techniques, Intramyocardial injection, Intracoronary injection, Retrograde coronary venous injection, Systemic intravenous injection, Cellular retention, Washout, Cellular

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