# The use of stem cells in heart disease

# O uso das células-tronco nas doenças cardíacas

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Fernando P. COMPARSI[1], Sandrine C. WAGNER[2], Patricia PRANKE[1,3*]

*Endereço para correspondência: Laboratório de Hematologia e Células-tronco, Faculdade de Farmácia , Universidade Federal do Rio Grande do Sul (UFRGS). Av. Ipiranga n. 2752 Porto Alegre, RS, Brazil, Zip code: 90160-000 Tel. +55 51 3308-5257 or +55 51 3308 5275 Fax.+55 51 3308-5437. E-mail: patriciapranke@ufrgs.br
[1.] Faculdade de Farmácia, UFRGS, Porto Alegre, Brazil.
[2.] Curso de Biomedicina, Centro Universitário Feevale, Novo Hamburgo, Brazil.
[3.] Programa de Pós-graduação em Neurociências, UFRGS, Porto Alegre, Brazil.
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**ABSTRACT**
Stem cells are undifferentiated cells that are capable of both self-renewal and giving rise to several cell types. Cardiovascular disease is the most important cause of death in western society and, in spite of therapeutic advances in traditional medicine, it has not been possible to recover the original cardiac function. Similarly, Chagas´ disease is an important cause of heart failure in Latin America. The differentiation capacity of stem cells makes them an important source of cells for the regeneration of cardiac tissue. Research using animal models of myocardial infarction has shown promising results for the use of stem cells in heart disease therapy. Recent clinical trials have shown the safety and benefit of therapy with stem cells in cardiac regeneration. Research in this field is still in the early stages and needs to be consolidated to permit wider use of stem cells in clinical medicine. Nevertheless, cell therapy has been shown to have potential as a new approach in the treatment of heart disease, which takes the lives of millions people around the world.
**Key words.** stem cells, myocardial infarction, Chagas´ disease, cell therapy, cardiac regeneration.

**RESUMO**
As células-tronco são células indiferenciadas capazes de auto-renovação e de originar diversos tipos celulares. A doença cardiovascular é a causa mais importante de morte na sociedade ocidental e, apesar dos avanços terapêuticos da medicina tradicional, não tem sido possível recuperar a função cardíaca original. A doença de Chagas é também uma importante causa de insuficiência cardíaca na América Latina. A capacidade de diferenciação das células-tronco faz dessas uma importante fonte de células para a regeneração do tecido cardíaco. As pesquisas utilizando modelos animais com infarto do miocárdio têm mostrado resultados promissores através do uso das células-tronco para a terapia das doenças cardíacas. Recentes ensaios clínicos têm comprovado a segurança e os benefícios da terapia com células-tronco na regeneração cardíaca. No entanto, as pesquisas nesse campo ainda estão em estágios iniciais e necessitam ser consolidadas para permitir uma ampla utilização das células-tronco na medicina. Além disso, a terapia celular tem mostrado grande potencial como uma nova abordagem terapêutica para o tratamento das doenças cardíacas, as quais são responsáveis por tirar a vida de milhões de pessoas ao redor do mundo.
**Palavras-chave.** células-tronco, infarto do miocárdio, doença de Chagas, terapia celular, regeneração cardíaca.

## INTRODUCTION

Stem cells (SCs) are undifferentiated cells with a high proliferation capacity. SCs are capable of self-renewal, which is the ability to divide while remaining undifferentiated, thus maintaining a permanent pool of cells. They also have the ability to differentiate into a number of different cell types, given the appropriate stimuli[1].

Many researchers classify SCs according to their differentiation capacity, which can be totipotent, pluripotent and multipotent. In the fertilized egg, until about the third day, all the cells are considered totipotent SCs, which mean that these cells have the capacity to give rise to any one of the approximately 217 cells types of the human organism and the extraembryonic tissues. Pluripotent SCs give rise to all the more than 200 cell types, derived from the three embryonic germ layers (endoderm, mesoderm and ectoderm), but not the extraembryonic tissues. The capacity of multipotent SCs is limited to giving rise to the cells of a specific tissue or organ, such as those of the hematopoietic system, neurological system, liver, among others[1,2,3]. Plasticity is the capacity of SCs to give rise to cell types other than those that gave rise to them**.** This plasticity means that SCs have a promising therapeutic role.

Stem cells are divided into two large groups, according to their origin: Embryonic Stem Cells (ESCs) and Adult stem cells (ASCs). ESCs are totipotent or pluripotent. Generally, in human beings ESCs are obtained in the period until the blastocyst phase, which corresponds to approximately 150-200 cells, developed by the fifth or sixth day following fertilization[1,2]. In the blastocyst phase, the cells are considered pluripotent.

These cells also have a great capacity for self-renewal, proliferating in culture and remaining undifferentiated for an undetermined period. Moreover, they are clonable, that is, they have the capacity to give rise to genetically identical cell colonies. ASCs, found in the formed individual, are also capable of self-renewal and differentiation. Several studies have shown that some kinds of ASCs have more plasticity than previously believed[1,2]. Examples of these are Mesenchymal Stem Cells (MSCs) and Multipotent Adult Progenitor Cells (MAPCs) which, it has been suggested, have a similar degree of plasticity to pluripotent ESCs.

Several studies have reported cases of transdifferentiation, in which ASCs from one tissue type have given rise to mature cells of a different kind. ASCs from bone marrow (BM), which originates from the mesoderm, can give rise to skeletal muscle cells, derived from the mesoderm, neurons, derived from the ectoderm and hepatocytes, derived from the endoderm. Research has shown that SCs can acquire the phenotype of other cells by spontaneous cellular fusion[3]. When Mononuclear Cells (MNCs) from BM or neural stem cells were cultivated together with ESCs, they adopted the phenotype and plasticity of the latter through spontaneous cellular fusion. Recent clinical trials in cardiac therapy have demonstrated this. Among the different cell types that can be formed are cardiomyocytes, smooth muscle cells and vascular endothelial cells. The replacement of these cells permits a new approach to the treatment of cardiovascular disease, with marked advantages over conventional therapies[4].

The aim of this paper is to discuss cell therapy as a novel treatment for heart disease, to be considered as another option which can be combined with conventional therapies to achieve greater survival rates and better quality of life for patients with such morbid and debilitating diseases. We review recent research on the use of stem cells for cardiac regeneration, including *in vitro* studies, animal experimentation and recent clinical trials, which evaluate safety and feasibility of cell therapy in the treatment of heart disease. These have been carried out around the world and in Brazil, which has stood out in this pioneering field, particularly with reference to Chagas´ disease (ChD). These studies will support greater future efforts for research in this area and larger, more controlled clinical trials to more precisely determine the benefits of cell therapy in heart disease.

## CARDIOVASCULAR DISEASES

Congestive heart failure affects 5 million people in the United States of America with 400,000 new cases per year. Cardiac and circulatory problems represent the main cause of death and invalidity in the United States. According to data from the Brazilian Health Ministry, cardiovascular diseases constitute the most significant cause of death throughout Brazil[5]. In 2001, cardiovascular diseases were responsible for approximately 1.2 million admissions to the Public Heath System, and 260 thousand deaths[6].

Irreversible damage occurs early in the course of myocardial infarction (MI), and the number of cardiomyocytes lost is a major determinant in post-MI cardiac function and prognosis. The limited regenerative capacity of the heart results in the inability of the cardiomyocytes to re-enter in the cellular cycle and proliferate[4].

Infection by *Trypanosoma cruzi* causes American trypanosomiasis, or Chagas´ disease, which affects 16 to 18 million people, mostly in Latin America, 6 million of which are in Brazil[7]. Approximately 30% of these patients suffer a progressive destruction of the myocardium causing chronic chagasic cardiomyopathy[8] for which there is no cure. Thus, ChD remains a significant cause of morbidity and mortality in the most affected regions[9]. Chronic Chagasic Cardiomyopathy (CChC) may lead to heart enlargement and arrhythmia, causing cardiac dysfunction and death[8].

## CARDIAC REGENERATION

During embryogenesis and the beginning of post-natal life, cardiomyocytes proliferate extensively. However, some months later, the cardiomyocytes become terminally differentiated. Adult ventricular cardiomyocytes are refractory to cell cycle re-entry for reasons that include the lack of telomerase activity[10]. These observations have led to the widely held belief that the heart is incapable of regeneration.

Nevertheless, studies have shown that cardiomyocytes are not only capable of dividing, but also that this capacity is increased about ten-fold in severe heart failure. Cases where male patients receive a heart from a female donor offer a unique opportunity to investigate whether primitive cells migrate from receptor to the transplanted organ and whether such cells remain in the donated heart[12]. Quaini and colleagues[11] used the presence of the Y chromosome in cardiac cells of such patients to determine that the cells were originally from the receptor. They reported that 18% of the myocytes, 20% of the coronary arteries and 14% of the capillaries were of male origin, and were formed from the cells of the receptor in the post-transplant period.

Glaser and colleagues[12] employed the same kind of sex-mismatched heart transplants in order to investigate the migration of receptor cells to transplanted heart. They observed that, on average, 7.5% of the smooth muscle cells originated from the receptor, but no myocytes of male origin were found. At the same time, Müller and colleagues[13] reported an average of 0.16%, while Laflamme and colleagues[14] found an average of only 0.04% of cardiomyocytes from the receptor in transplanted hearts.

The great disparity between these research findings, and specially the non-confirmation, by other authors, of the notable results obtained by Quaini and colleagues[11], have generated intense debate on the methods used and, principally, on the criteria employed in interpreting the results.

Others factors, such as the shorter time interval between the heart transplant and tissue analysis and, consequently, the higher levels of infiltration by inflammatory cells into the cardiac tissue, may be responsible for the high levels of chimerism reported by Quaini and colleagues[11]. However, if confirmed, these results would lead to a paradigm shift from the accepted dogma that the heart is incapable of regeneration, to one in which stem cells from the host may be recruited to regenerate significant areas of the human heart[15].

Even if the potential of the heart to regenerate is not as great as that suggested by Quaini and colleagues[11], proving to be more limited, as indicated by the results reported by Müller and colleagues[13] and Laflamme and colleagues[14], precedents are opened for both the study and clinical use of the stimulation of this intrinsic regenerative capacity of the heart in the treatment of heart disease.

Recently, Oh and colleagues[16], using cardiac progenitor cells obtained from the myocardium of adult mice, have offered an explanation for myocyte regeneration. These cells, when injected into the jugular vein of infarcted mice, migrated to the heart, and after two weeks, acquired the phenotype of myocyte, by fusion and differentiation in similar proportions.

A recent interesting finding is that following acute MI, there was a naturally occurring significant increase in the levels of CD34+ progenitor cells in the peripheral blood, which peaked between 24 and 48 hours after the first symptoms appeared and gradually returned to normal values after 7 to 60 days[17] CD34 is well known as a hematopoietic stem cell (HSC) marker.

The mobilization of CD34+ and endothelial progenitor cells (EPCs) was observed in early stages of chronic heart failure, together with a reduction in circulation levels of these cells in advanced stages of the disease[18]. In patients with coronary arterial disease, the level of circulating endothelial progenitor cells is inversely related to the cardiovascular risk. Studies showed that the patients with lower levels of circulating $CD34^+$ and $KDR^+$ (kinase domain receptor) cells presented higher frequency of important cardiovascular events during the 12 month period in which they were followed[19].

Besides EPCs and HPCs, other subpopulations of cells from the bone marrow, such as cardiac progenitor cells, have been shown to be mobilized following damage to the myocardium[17,20]. Increased plasma and tissue levels of vascular endothelial growth factor (VEGF) have also been detected after infarction, as well as the tissue levels of stromal derived growth factor-1 (SDF-1), hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF), in the damaged region of the heart[17].

These results suggest the existence of cardiac progenitor cells in bone marrow that can be mobilized to the peripheral blood following acute MI and attracted to the heart by the release of chemotactic factors, where they repair the lesion.

It is important to highlight that the proliferation mechanisms of cardiac cells following a trauma such as MI are not capable of regenerating the heart and restoring original cardiac function[4,16].

Given this, the development of new therapeutic approaches that permit the regeneration of lost cardiac tissue is extremely important and stem cell therapy has a vital role to play[1].

## RESEARCH INVOLVING STEM CELLS IN THE REPAIR OF CARDIAC TISSUE

The use of autologous SC in the tissue engineering for cardiovascular diseases is promising due to the capacity of self-renewal, high proliferation and differentiation rate in specialized progenitor cells[21].

Several studies aimed at assessing the potential for the use of stem cells in the repair of cardiac tissue have been carried out. This approach has great advantages over heart transplant, especially given the scarcity of available heart donors to meet current transplant needs[1].

## ANIMAL EXPERIMENTATION

In order to verify the feasibility, safety and beneficial effects of stem cell transplantation for the treatment of heart disease, especially MI, experiments with animal models have been performed, inducing infarction in rats and mice. This is achieved by the occlusion of the coronary artery which leads to infarction of the cardiac region irrigated by that artery[1].

Orlic and colleagues[22] isolated lin$^-$ c-kit$^+$ cells from mouse BM, and injected them into the viable myocardium adjacent to the infarcted area, some hours after the arterial occlusion. Nine days after the transplant, 68% of the infarcted area had been replaced by newly formed myocardium. The developing tissue included vascular structures, proliferating myocytes, and expressed markers of cardiac cells in development. The expression of connexin 43, a protein responsible for intercellular connection and electrical coupling between myocytes, which is essential for the functional integration of heart muscle cells was also seen[23]. Of even greater significance was the improvement in the systolic and diastolic pressure when compared with mice that did not receive lin$^-$ c-kit$^+$ cells following infarction. These data show that the tissue formed from the transplanted cells had a positive impact on cardiac function[22]. However, direct injection into the heart, through thoracotomy, besides being technically difficult in mice, is highly invasive, resulting in high mortality and low grafting rates**.**

Therefore, Orlic and colleagues[24] checked the effects of the mobilization of BM cells through the injection of granulocyte colony stimulating factor (G-CSF) and Stem Cell Factor (SCF) in infarcted mice.

Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic growth factor which promotes proliferation and differentiation of neutrophil progenitors and also has immunomodulatory properties. G-CSF promotes the mobilization of stem and progenitor cells from bone marrow to the infarcted heart after acute MI. While there is controversy regarding the capacity of mobilized stem cells to differentiate into cardiomyocytes, the angiogenesis induced by G-CSF is a known effect in the infarcted heart[25].

Although some studies show that G-CSF can act directly on cardiomyocytes[25,26] promoting their survival after acute MI and inducing the regeneration of myocardium in mice, the mechanisms by which these benefits occur are not completely known yet. G-CSF may induce the release of anti-apoptotic proteins and inhibit the apoptosis of cardiomyocytes in the infarcted heart. Furthermore, it seems to reduce the apoptosis of endothelial cells, increasing the vascularization of the infarcted heart, protecting it against ischemia[26].

The results were encouraging, showing considerable increase in the survival of treated mice when compared to the control group: 85% to 35%, 6 days after the infarction, and 73% to only 17% after 27 days, respectively. It was seen that repair had only occurred in the treated group, with the reduction of the infarcted area from 64% to 39%, with the formation of new myocytes. These cells expressed desmin, nestin, α-sarcomeric actin, cardiac myosin and connexin 43, which are heart cell development and integration markers. The myocardium in formation occupied 76 ± 11% of the infarcted area and consisted of myocytes, endothelial and smooth muscle cells in proliferation. The ejection fraction was 48, 62, and 114% greater in treated than in untreated mice, at 9, 16, and 26 days following coronary occlusion, respectively. These results demonstrated that this non-invasive protocol was well tolerated by the recipient mice, and that autologous circulatory stem cells migrate to the damaged region of the ischemic heart and give rise to new cardiomyocytes and blood vessels, and so restore, at least partially, heart function[24].

Embryonic stem cells have the distinct potential for tissue regeneration, including cardiac repair[27] and they are promising for cardiac repair[28]. Human ESCs can give rise to cardiomyocytes in vitro[29] and ESC-derived cardiomyocytes are viable human heart cells that can functionally integrate with the recipient organ after transplantation[30]. Cai and colleagues[31] showed that transplantation of ESC-derived cardiomyocytes improved cardiac function in infarcted rat hearts.

Min and colleagues[32] induced MI in rats and used ESCs from mice, cultivated for 7 to 8 days, permitting their differentiation. For the transplant, cells previously transfected with green fluorescent protein (GFP) were used. The cells were injected into two points adjacent to the ischemic region, and one point within the infarcted region, 30 minutes after infarction. Six weeks after the infarction, the animals that received the ESCs showed significant improvement in left ventricular function, when compared with rats that received cell-free culture medium (control). Considerable improvement was also seen in the ventricular contraction of the group transplanted with ESCs. The presence of GFP positive cells in the left ventricle shows that the implanted ESCs not only survived in the damaged myocardium, but also differentiated into mature cardiomyocytes 6 weeks after transplant.

The ability of cardiac-specified human ESCs to differentiate along the cardiomyogenic pathway following transplantation into infarcted myocardium raises the hope that these cells might become effective candidates for myocardial regeneration[29]. However, it is known that undifferentiated mouse ESCs can formed teratomas in infarcted hearts, indicating injury-related signals did not direct cardiac differentiation[28]. Their propensity for multilineage differentiation carries, however, the liability of neoplastic growth, impeding therapeutic application[27]. On the other hand, no teratoma was observed in hearts or any other organ of the body in studies from Tomescot and colleagues[29], when they studied the differentiation in vivo of cardiac committed human embryonic stem cells in postmyocardial infarcted rats[29]. Behfar and colleagues[27] showed that cardiopoietic programming established a strategy to hone stem cell pluripotency, offering a tumor-resistant approach for regeneration.

These controversial results show that the use of embryonic stem cells in patients needs to be more studied before its therapeutic application.

In a study using human peripheral blood MNCs, these cells were expanded in culture medium prepared for endothelial progenitor cells (EPCs) for 7 days. Infarction was induced in rats and after 3 hours $10^6$ EPCs were injected intravenously. After 28 days, the rats that received the cells exhibited lower left ventricle systolic and diastolic volumes, as well as greater cardiac contractility than rats from the control group. The increase in the neovascularization produced by EPCs resulted in a reduction in ventricular dilatation and maintenance of cardiac performance after myocardial ischemia in rats[33].

In a study carried out by Yeh and colleagues[34], infarction was induced in immunodeficient mice and 2.5 x $10^6$ $CD34^+$ cells from human peripheral blood were transplanted by infusion into the caudal vein. The infarcted area, but not the healthy portion of the heart, presented human cardiomyocyte-like cells that expressed HLA-ABC and cardiac protein (troponin T). Blood vessels in the infarcted region were also found to contain cells positive for HLA and smooth muscle α-actin or HLA and VE-cadherin, demonstrating that human CD34+ transdifferentiated into smooth muscle and endothelial cells in the damaged tissue. These events were not observed in mice that received only cells, in the absence of infarction.

In another study, BM cells were mobilized by the administration G-CSF in humans and then collected from the peripheral blood by means of leukapheresis. Forty-eight hours after infarction induced by occlusion of the left anterior descending coronary artery in athymic rats, 2 x $10^6$ human $CD34^+$ cells were injected into the caudal vein. After 2 weeks, a significant increase in microvascular density was seen in the infarcted area (with vessels constituted of human cells), and in the peri-infarcted region (with vessels containing rodent cells). Moreover, the rats that received human cells showed significant improvement in cardiac function, with relative increase of 22% and 34%, relative to post-infarction values, at 2 and 15 weeks, respectively. Furthermore, the frequency of apoptotic myocytes was 6 times lower in the treated group than in the control group[35].

The use of an alternative source of stem cells, from the mononuclear fraction of umbilical cord blood (UCB) has been studied in several diseases, including cardiac diseases. Recent studies in mice have shown that in 50% of animals with myocardial infarction the mononuclear cells (MNCs) from UCB migrate to the heart after the intravenous injection. Again in mice, when cells from UCB or BM enriched with CD133, a marker of EPCs, were directly injected in the infarcted area of myocardium, an improvement in the contractility of myocardium and in the neovascularization of ischemic myocardium was observed[36].

In an animal model of chronic ischemia in dogs, an increase in the vascularization and an improvement in the cardiac function were demonstrated after the implant of mesenchymal stem cells (MSCs). These cells differentiated into smooth muscle and endothelial cells, suggesting the formation of new vessels. Amado and colleagues[37] performed an allogeneic transplant of MSCs in pigs and achieved significant improvement in the cardiac function. On the other hand, Deten[38] and Balsam[39] and their colleagues, with the mobilization and intravenous injection of bone marrow cells, did not detect any sign of myocardium regeneration in the heart of infarcted mice.

These animal experiments have demonstrated the great potential of stem cells obtained from different sources, in the regeneration of cardiac tissue, through the revascularization of damaged tissue, and differentiation into cardiac cells, which leads to tissue restoration and the recovery of cardiac function. The cell types, animals, means of administration and main results of the presented studies are summarized in Table 1.

## CLINICAL TRIALS

A number of successful studies in the application of stem cell therapy in heart disease using animal models have stimulated ongoing clinical trials[1].

Perin and colleagues[40] performed the autologous transplant of MNCs from BM in 14 patients with severe chronic ischemic heart failure. The control group consisted of 7 patients who received only conventional therapy. A NOGA catheter was used to administer 15 transendocardial injections delivering a total of 2 to 3 x $10^7$ cells per patient. The population of cells injected contained 2.4 ± 1.3% of $CD45^{dim}$ $CD34^+$ cells. The patients were evaluated two months after the transplant, and presented significant improvement when compared to the evaluation made prior to the procedure and the control group. The patients that received the transplant showed improvement in heart condition, with fewer symptoms of angina and greater exercise capacity. Improvement was observed in the ejection fraction measured by echocardiogram and the final systolic volume, as well as the cardiac tissue perfusion (a 73% reduction in the total reversible defect). The transplanted group was re-evaluated after 4 months, and angiography showed significant improvement in ejection fraction and end systolic volume. Moreover, improvement in mechanical function was seen in the cell injection sites. This research demonstrated the safety and efficacy of performing autologous transendocardial transplant of MNCs from BM.

Strauer and colleagues[41] were the first to perform the intracoronary autologous transplant MNCs from BM in patient with acute MI. Ten (10) patients received injections of around 2 x $10^7$ MNCs during angioplasty while a control group of 10 patients had only angioplasty. Three months after the procedure the patients that received cell therapy presented a significant reduction in the infarcted area (from 30 ± 13% to 12 ± 7%), a significant increase in motility in the affected region, as well as reduction in the end systolic volume.

In a study using $AC133^+$cells selected from the BM of patients with MI, a group of 6 patients received 1,5 X $10^6$ cells directly into the peri-infarcted area, together with coronary

**Table 1.** Experiments in animal models using SCs in cardiac regeneration.

| Cells | Animal/Route of Administration | Main Results | References |
|---|---|---|---|
| lin$^-$ c-kit$^+$ from BM from mice | Border of infarcted area in mice | 1) Regeneration of 68% of the infarcted region; 2) New My;3) cardiac function improvement | 22 |
| Cells mobilized from BM by G-CSF and SCF | Infarcted mice | 1)Increased survival; 2) New My;3)cardiac function improvement | 24 |
| differentiated ESCs in mouse | around and in the infarcted region in rats | 1) New My;2) cardiac function improvement | 32 |
| expanded EPCsfrom human PB | Intravenously in infarcted immunodeficient rats | 1) Increased vasculogenesis of ischemic tissue; 2) cardiac function improvement | 33 |
| CD34$^+$ from human PB | Intravenously in infarcted immunodeficient mice | 1) New My, SMCs and vascular endothelial cells | 34 |
| CD34$^+$ from human PB mobilized by G-CSF | Intravenously in infarcted immunodeficient rats | 1) Vasculogenesis in the infarcted area and angiogenesis in the periphery; 2) cardiac function improvement | 35 |
| MNCs fromUCB | Intravenously in infarcted mice | Migration of cells to heart in 50% of animals with AMI | 36 |
| Selected CD133 cells from UCB or BM | Injection in infarcted area in mice | Cardiac function improvement | |

SCs: stem cells; BM: bone marrow; G-CSF: granulocyte colony stimulating factor; SCF: Stem Cell Factor; ESCs: Embryonic Stem Cells; PB: Peripheral Blood; EPCs: endothelial progenitor cells; My: myocytes; AMI: acute myocardial infarction. SMCs: smooth muscle cells; MNCs: mononuclear cells; UCB: umbilical cord blood.

revascularization. This resulted in an increase in global ventricular function and perfusion of the infarcted region 3 to 9 months after the procedure. AC133$^+$ cells include a sub-population of CD34$^-$ cells, have great potential of inducing angiogenesis, and may be responsible for the increased perfusion in the cardiac tissue, though the affect of revascularization surgery cannot be ignored[42].

Tse and colleagues[43] reported a significant decrease in angina episodes and the use of nitrates in 8 patients with ischemic heart disease, 3 months after autologous transendocardial transplant of MNCs. NOGA system was used to electronically map the hearts of the patients and inject the cells into the ischemic area of the myocardium. A significant improvement in the cardiac contraction was noted in the patients.

Menasché group pioneered the use of skeletal myoblasts, with first implant of autologous myoblasts into a patient suffering from infarction. They followed up 10 patients submitted to the same treatment and noted improved cardiac function and increased ejection fraction. On the other hand, ventricular arrhythmia was seen in 4 patients, which has led to concern regarding the safety of skeletal myoblast transplant[44]. The presence of arrhythmia may be linked to the lack of a functional integration capacity in the newly formed tissue. This incapacity is due to the absence of electrical coupling between the new muscle cells and the cardiomyocytes, which was confirmed by the absence of connexin 43 and cadherin expression in the grafted cells[45].

Schächinger and colleagues[46] performed intracoronary autologous transplant of progenitor cells in 59 patients with acute MI. Twenty nine patients received MNCs from BM and the other 30 received peripheral blood cells expanded *in vitro* and with characteristics of endothelial progenitors cells. In the preliminary evaluation of the first 20 patients, the two groups (9 and patients, respectively) obtained comparable results in the follow-up four months after the procedure. Both showed significant improvement in ejection fraction, end systolic volume, cardiac contraction and perfusion, when compared to baseline values and the control group of 11 patients that received only conventional treatment[47]. After 12 months follow-up of all the patients an even greater improvement was noted in the ejection fraction, along with a decrease in end systolic volume and maintenance of end diastolic volume, demonstrating a favorable ventricular remodeling and no infarction spread[46]. Based on the observation that progenitor cells derived from both BM and peripheral blood increased the neovascularization in ischemic tissue[33,35], it has been suggested that improvement in ventricular function following experimental induction of a MI is due to the stimulation of angiogenesis. Thus, the

enhanced blood flow makes it possible to prevent myocardial remodeling and limits myocytic apoptosis, reduces collagen deposition and scar formation[33,35,47]. While arrhythmia has appeared to be an important limitation to the use of SCs derived from skeletal myoblasts[44], in this study none of the transplanted patients presented arrhythmia, demonstrating the safety of the procedure[46].

Another study used SCs from peripheral blood mobilized by G-CSF for the autologous transplant during heart by-pass surgery. The stem cells were infused into the coronary artery or injected into non-fibrotic areas of the myocardium, adjacent to the infarcted region. There was improvement in the clinical conditions of the 5 patients, mainly in those that had recently suffered infarction. However, further research will be necessary to confirm the results[48].

Fernández-Avilés and colleagues transplanted autologous MNCs from BM in 5 patients with acute MI due to stenosis of the left anterior descending coronary artery. A catheter was used to infuse around 5 x $10^7$ cells into the affected artery, 10 –15 days after infarction. Six months after the transplant, an improvement was noted, though not significant, in the ejection fraction and final diastolic and systolic volumes. The same authors later published the results of a study containing 20 patients that showed significant improvement in ejection fraction and end systolic volume after 6 months, reflecting improved cardiac contraction, while in the control group (13 patients) no significant improvement was seen[49].

A randomized clinical trial was carried out with 60 patients that had suffered acute MI and percutaneous coronary intervention. A catheter was used to transplant 2.5 x $10^9$ nucleated cells from BM (containing approximately $10^7$ $CD34^+$ cells) into the coronary artery linked to the infarction. The patients were followed-up for 5 to 6 months after the transplant and magnetic resonance was used to determine the left-ventricular ejection fraction. The group of 30 patients that received cell therapy presented a significant elevation of 6.7 ± 6.5% in LVEF**,** while in the control group the elevation in LVEF was only 0.7 ± 8.1%. Moreover, the absence of the pro-arrhythmia effect in the transplanted patients demonstrates the safety of cell therapy in cardiac regeneration following MI[50].

Ince and colleagues[51] studied the effect of mobilization of $CD34^+$ cells from BM in patients suffering from acute MI. Fifteen patients received subcutaneous injection of G-CSF for 6 days and traditional treatment, while the control group received only the conventional treatment. During the treatment with G-CSF, the level of circulating $CD34^+$ cells increased 20 times, while they remained constant in the control group. In evaluations made 4 and 12 mounts after the treatment, the patients who received G-CSF showed significant improvement in cardiac motility, ejection fraction and cardiac geometry, when compared to the control group. Moreover, no adverse effects were observed, including electrical instability or accelerated restenosis.

In a randomized double blind study with 114 patients with acute MI, 56 patients received daily subcutaneous doses of G-CSF for five days, while 58 patients received placebo, during the same period. Increases in circulating $CD34^+$ cells, as well as in the level of granulocytes, monocytes and lymphocytes were observed in the patients who received G-CSF. However, the study showed that although the treatment produced a significant mobilization of stem cells, the use of G-CSF did not change the size of the infarction or the left ventricular function after the acute MI in patients. On the other hand, in contrast with other studies, the risk of coronary restenosis did not increase and other collateral effects were not observed[52].

In a study carried out by a group of Brazilian researchers, 9 patients received cell therapy for the treatment of heart failure due to ChD, hypertension and unknown origin (idiopathic dilated cardiopathy). Two patients received GM-CSF to mobilize SCs from BM that were then collected in the peripheral blood for leukapheresis and administered via intracoronary method using a catheter. In the other 7 patients, the cells were merely mobilized to the peripheral blood by the administration of GM-SCF, with the aim of observing whether the cells migrate to the damaged cardiac tissue to induce repair. The preliminary results showed there was improvement in the condition of the first patients that received the treatment, with improved ejection fraction and endurance during exercise. Three of these patients have now left the heart transplant waiting list[9].

Since October 2003, in Rio Grande do Sul, stem cell transplant has been performed on 7 patients with heart failure, and the patients are responding well to the treatment[53]. In another study, published in 2004, Sant'Anna and colleagues[53] showed the results of SCT in 7 patients with cardiac failure, who responded well to the treatment.

Tura and colleagues[54] have designed clinical trials to test for the efficacy of autologous bone marrow derived mononuclear cell therapies in four different cardiopathies: acute and chronic ischemic heart disease, and Chagasic and dilated cardiomyopathy. In each trial 300 patients will be enrolled and receive optimized therapy for their specific condition. Additionally, half of the patients will receive the autologous bone marrow cells while the other half will receive placebo. Many phase I clinical trials using cell therapy for cardiac diseases have already been performed. The few randomized studies have yielded conflicting results, rendering necessary larger well controlled trials to test for efficacy of cell therapies in cardiopathies.

All the published clinical trials have involved autologous transplants, that is, the patients own cells were used, so avoiding the risk of rejection and the need for immunosuppression, inherent in allogeneic transplants. The cell types used, the administration method, the number of patients and the types of heart disease are summarized in Table 2.

**Table 2.** Clinical trials using cellular therapy in patients with heart disease

| Cells | Administration | Patient disease | N: | References |
|---|---|---|---|---|
| MNCs from BM | Transendocardial guided by NOGA | CHF | 14/7 | 40 |
| MNCs from BM | Transendocardial guided by NOGA | CHF | 8 | 43 |
| MNCs from BM | intracoronary (catheter) | AMI | 10/10 | 41 |
| MNCs from BM | intracoronary (catheter) | AMI | 5 | 49 |
| MNCs from BM | intracoronary (catheter) | AMI | 9/11 | 47 |
| PB Expanded *in vitro* | intracoronary (catheter) | AMI | 11/11 | 47 |
| $AC133^+$ Cells from BM | Direct injection into border of infarcted region, during by-pass surgery | AMI | 6 | 42 |
| Skeletal Myoblasts | Direct injection into infarcted Myc, during by-pass surgery | CHF | 10 | 44 |
| PB mobilized by G-CSF | Injection into coronary and Myc during by-pass surgery | CHF | 6 | 48 |
| Nucleated cells from BM | intracoronary (catheter) | AMI | 30/30 | 50 |
| PB mobilized by G-CSF | Injection of G-CSF | AMI | 15/15 | 51 |
| PB mobilized by G-CSF | Injection of G-CSF | AMI | 56/58 | 52 |

N: number of patients that received cell therapy / control group (when existent); MNCs: Mononuclear Cells; BM: Bone Marrow; PB: Peripheral Blood; G-CSF: granulocyte colony stimulating factor; Myc: myocardium; CHF: chronic heart failure; AMI: acute myocardial infarction.

## STEM CELLS IN CHAGAS´ DISEASE

The discovery of the pluripotential capacity of SCs from BM, and with it, the emergence of new perspectives for the treatment of, until then, incurable degenerative diseases, as well as the promising results of the use of these cells in cardiac regeneration[22,40,50], has led to the study of the use of cell therapy in the treatment of CChC.

A pioneering study in relation to ChD is being undertaken by a group of researchers from different Brazilian institutions, including Fiocruz from Bahia, a state with a high incidence of this disease. It has recently been demonstrated that BM cells injected intravenously into mice with chronic ChD migrated to the heart and provoked a significant reduction in the inflammatory infiltrates and in interstitial fibrosis, characteristic properties of CChC. Beneficial effects were seen within 6 months after transplant from BM. Massive apoptosis of inflammatory cells in the myocardium was noted after the therapy with BM cells. However, there was no increase in parasitism, indicating that the marked reduction in cardiac inflammation was not due to a generalized immunosuppression. It was also found that a fraction of the cells expressed cardiac myosin, but only in the hearts of chagasic mice, showing the need for the presence of tissue damage to provoke the migration of the cells and induce repair. Of particular importance is the finding that cells obtained from the BM of both normal and chagasic mice had comparable effects in inducing repair in the chagasic heart. The results show that the transplant of cells from BM is effective in the treatment of CChC and that the autologous transplant of BM can be used as an efficient therapy for patients afflicted by this pathology[55].

Based on the experience obtained in research with the transplant of SCs for ChD in mice and the encouraging results achieved in these studies[55], a surgical protocol has been applied, since July 2003, with the aim of improving the quality of life patients with ChD. Preliminary results have demonstrated the success of the procedure in 5 patients[56] that received an intracoronary autologous injection of SCs from BM via cardiac catheterism[57]. The aim is to extend the study to more 25 patients[56].

## DISCUSSION

Although experiments with animal models of infarction have shown the differentiation of SCs from BM into cardiomyocytes[22], the extent to which this is reproducible in humans is unknown. The confirmation of the presence of cardiac cells from the host in the transplanted hearts supports the differentiation of SCs into cardiomyocytes *in vivo* in the human heart. However, the inconsistency of the results from different research groups as hampered the attainment of more solid conclusions.

It is also important to try to discover the action mechanism of SCs. Transdifferentiation and cellular fusion have both been suggested as the action mechanisms involved in the tissue regeneration process induced by SCs. However, the low frequency with which some studies have demonstrated these events[3] has led to suggestions that other mechanisms may be involved. One such mechanism would be that the delivered SCs, release growth factors at the damaged tissue, and assist the "modulation" of the damaged environment. These factors may stimulate the proliferation of resident or circulating SCs and tissue regeneration. Much further research will be necessary to improve our understanding of these and other, as yet unknown action mechanisms of SCs.

Other studies with animals have indicated angiogenesis in the ischemic tissue, induced by transplanted cells, as an important mechanism in the recovery of cardiac function[33,35]. The evidence to support this includes the reduction in the occurrence of angina and increased myocardial perfusion, without alteration to heart contraction, as confirmed by Tse and colleagues[43]. Another important finding is the increase in perfusion and cardiac contraction in viable but non-functional regions of the myocardium that received BM derived SCs, reported by Perin and colleagues[40]. The findings of Stamm and colleagues[42] also suggest that angiogenesis plays an important role in the infarcted tissue leading to cardiac regeneration. The group noted the increase in ventricular function and myocardial perfusion in patients that received transplanted cells with high angiogenic potential. Furthermore, Assmuss and colleagues[47] demonstrated significant improvement in cardiac contraction and perfusion in patients that received *in vitro* expanded EPCs, derived from peripheral blood cells.

Although the results of clinical trials point to angiogenesis as the main process responsible for cardiac regeneration, the proliferation of cardiomyocytes and the differentiation of progenitor cells, probably also play important roles. However, the complete mechanism is far from being understood. Perhaps, the increase in perfusion induced by angiogenesis in the ischemic region facilitates improved intrinsic regenerative capacity of the heart, through the increase availability of oxygen and nutrients for cellular proliferation and tissue regeneration.

Other questions, besides the mechanisms responsible for the cardiac regeneration induced by SCs, regarding the use of cell therapy in heart disease remain unanswered. An example is the necessity or possibility of cell manipulation for the purification of a specific population or *in vitro* differentiation prior to transplant.

While different studies have used cells derived from different sources, no conclusion has been reached on the cell type best indicated for implant. A further point to be considered is that of the best cell administration route. NOGA appeared to be one of the more promising methods, as the transendocardial injection can be guided by electromechanical mapping, allowing the administration of cells into viable but non-functional regions of the myocardium. Intracoronary infusion via catheter into the artery responsible for the infarction, following its desobstruction, is another strategy and permits the use of larger volumes of cellular suspension and the contact of the cells with all the affected tissue. Though these strategies are less invasive than direct injection into the myocardium through thoracotomy during by-pass surgery, and have been shown to be safer in the clinical trials in which they have been employed, the need to use NOGA has been debated. Attempts have been made to substitute NOGA by other procedures, because of its invasive nature and high cost.

Less invasive methods of SCs implant have shown promising results. Nevertheless, perhaps cell implant itself may not be necessary. Maybe the administration of cytokines, such as G-SCF or SCF, which mobilize BM derived SCs to the peripheral blood, is sufficient to ensure stem cell migration to the damaged cardiac tissue. Alternatively these cells may be collected from the peripheral blood after mobilization with cytokines, and then implanted into the heart to increase their concentration in the target tissue. Though such approaches remove the need for cell aspiration from BM, they have been less tested in animal experiments and clinical trials, and their efficacy and safety have not yet been established.

Kang and colleagues[58] found a high incidence of restenosis in the infarcted artery in patients that received G-CSF, despite cardiac function improvement. However, there were few patients in the study sample, and the result has not been confirmed by other research[51].

Controversy remains regarding the results and procedures employed in recent studies. Silva and colleagues[59] demonstrated increased vascularity and improved cardiac function in a canine chronic ischemia model, using mesenchymal stem cells differentiated into smooth muscle cells and endothelial cells. On the other hand, Deten and colleagues[37] found no detectable signs of myocardial regeneration in infarcted mouse heart with the mobilization or intravenous injection of BM cells.

An important problem to be solved is that the mobilization of BM derived cells to the peripheral blood takes several days, and hence increases the time delay between infarction and treatment. The early induction of angiogenesis in the infarcted tissue and the consequent increase in perfusion appear to inhibit late ventricular remodeling and the

deterioration of the myocardium, thus preserving cardiac function[33,35,42,47]. While tissue damage is an important factor for the migration of SCs and their incorporation into the affected tissue, the ideal time period for treatment initiation following infarction remains to be established.

It is known that delivered factors from myocardium during the inflammatory process play an important role in increasing homing, migration and implantation of systematically infused stem cells. Despite the controversy surrounding the capacity of mobilized SC to differentiate into cardiomyocytes and the fact that the effects of G-CSF on the advance of atherosclerosis are not fully understood, the use of this growth factor seems to represent a promising therapy for ischemic events[25].

The CXCR4 molecule is a recently discovered marker involved in the process of attraction of stem cells to the sites of damaged tissue. When tissue is damaged, $CD34^+CXCR4^+$ cells migrate to the site of the lesion, attracted by SDF-1 secretion, the ligand of the CXCR4 receptor. Niches of SDF-1 are found in the damaged organs and they are delivered during the tissue damage. The organization of cellular niches is known to have a key role in regulating normal stem cell differentiation and in the regeneration of tissue. Thus, $CXCR4^+$ cells are important in the regeneration of damaged organs, showing the regenerative capacity of stem cells[60], such as HSC and MSC, which express this marker on their cellular surface. Bone marrow and skeletal muscle also contain a population of CXCR4 cells that express specific genes for muscle progenitor cells and that can be mobilized to peripheral blood[20]. These cells are attracted by the SDF-1 delivered in the damaged area of the organ and they can help in the repair of the infarcted heart[17,20].

Clinical trials have included both patients suffering from acute MI, and patients with chronic heart failure. Though it is not yet possible to compare the results due to the low number of studies and of patients, in both cases improvement has been seen in patients. An important factor is that there are more patients with chronic heart failure and their situation is more severe, as frequently there is no option available other than heart transplant. Clinical trials until now have included patient follow-up of at most 12 months, and as yet it is not known whether transplanted cells survive in the heart for longer periods, nor for how long the beneficial effects will remain.

There is a need for continued research with animal models as well as clinical trials to enhance our understanding and facilitate the wider use of SCs in the treatment of heart disease. Though, advances in stem cell therapy have broken paradigms and shown it to be a promising treatment for the regeneration of tissue, especially in the heart.

## CONCLUSION

Stem cells have great potential in the regeneration of cardiac tissue, through the formation of new myocytes, endothelial and smooth muscle cells. The partial repair of the infarcted heart, seen in several studies, suggests that transplanted cells respond to signals from the damaged myocardium, that provoke their migration, proliferation and differentiation within and around the infarcted area. Of greater importance, is the significant improvement observed in the clinical condition of the patients submitted to SCs treatment. In the studies so far performed, improvement has been reported in hemodynamics, cardiac geometry, myocardium perfusion and contraction, and endurance during exercises, as well as reduction in the occurrence of angina. Moreover, the relative safety observed in these studies encourages the performance of larger randomized controlled studies, to better evaluate the potential of cell therapy and disseminate its therapeutic use.

The treatment of the sick heart in patients with ChD by cell therapy is particularly important in Brazil and Latin America. The results obtained with cell therapy in animal models of CChC and the recent implementation of a surgical protocol for the autologous transplant of SCs in chagasic patients give new hope to millions of people that suffer from ChD[57].

Stem cell therapy therefore points to a promising future for regenerative medicine, and has become a great hope in the treatment of heart disease, adding to the therapeutic arsenal of the clinician in the combat of diseases that represent such a heavy burden for society.

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