

## Adult Stem Cells for Myocardial Repair

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### Abstract

Cell transplantation for myocardial repair has emerged as a promising therapy for patients with heart failure. Recent *in vitro* and *in vivo* animal studies have suggested the potential of different cell types to differentiate into tissues required for cardiac repair namely endothelial cells and cardiomyocytes. In this review, we will focus on the potential of adult stem cells obtained from adult muscle and adult bone marrow to regenerate cardiac tissue. There are a number of studies suggesting that autologous myoblast or satellite cells can contribute *in vivo* to improve cardiac contractility. In fact, several clinical studies have been initiated using autologous *ex vivo* expanded myoblast in patients with myocardial infarction. We will review the rationale for this approach. Although attractive, the use of muscle derived progenitors has been questioned as these cells may not have the potential to differentiate into cardiac tissues. Besides, initial studies have been associated with a high incidence of cardiac arrhythmias. Bone marrow derived stem cells have also been explored *in vitro* and in animal models in the context of cardiac repair. The potential of adult bone marrow derived stem cells to differentiate into cardiomyocytes and endothelial cells will be summarized as well as the initial results of clinical trials using autologous bone marrow cells.

**Key words:** stem cells, cardiomyopathy

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Cardiovascular diseases are the leading cause of death in the developed countries. Loss and dysfunction of cardiomyocytes are characteristic of heart diseases and lead to heart failure as a consequence of irreversible cell loss. Unlike other tissues, the heart muscle has none or very small capacity of regeneration due to the lack of stem cells in the heart and the inability of the damaged heart cells to undergo repair or divide at least to a significant extent [2, 23]. The development of new techniques aim to repair the damaged heart with the introduction of stem cells with myogenic potential or the induction of resident myocardial cells to proliferate have great potential for treating heart failure and cardiomyopathy. The ultimate goal of cell therapy for cardiac diseases is to repair, replace or enhance the biological function of damaged cells in order to strengthen the heart muscle.

Studies of cell therapy for cardiac diseases have been an active field of research since the introduction of exogenous cells in a dog heart was first reported [29]. Subsequent studies have used immortalized myogenic cells [12, 26], embryonic stem cells [24], hematopoietic stem cells

[36, 37], endothelial progenitors [25], mesenchymal stem cells [60], fetal cardiomyocytes [68] or even induction of undamaged cardiomyocytes to replicate [51]. The goal of each of this cell population is to generate cells that can perform cardiac work, respond appropriately to adjacent cardiomyocytes and non-myocyte cells and exhibit a favorable response to physiological stimuli. Successful cardiac tissue engineering would provide a valuable alternative therapy for end-stage heart failure.

In this article we will focus on the potential of adult stem cells to differentiate into tissues amenable for cardiac repair meaning not only cardiomyocytes but also other tissues that may contribute to improve cardiac function. In particular we will examine the current evidence that supports a role for muscle and bone marrow derived adult stem cells in cardiac regeneration and will also described where we are from a clinical standpoint.

### Stem cells

Although not directly related to cardiac repair, but because the recent amount of information published regard-

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ing potential of stem cells we believe a few words about stem cells may help the reader to understand some of the concepts regarding stem cells and transdifferentiation. Stem cells have been defined as clonogenic cells that can undergo self-renewal as well as differentiation to committed progenitors and eventually to functional differentiated tissues. Stem cells can be subdivided according to their potential in totipotent stem cells capable of giving rise to both embryonic and extra-embryonic tissues, pluripotent stem cells, that may differentiate into tissues derived from any of the three germ layers, or multipotent stem cells, with a more limited differentiating capacity [1, 7, 65, 66].

It is generally accepted that ES cells are pluripotent. In contrast, it is generally accepted that adult stem cells are multi- but not pluripotent. However, recent studies describing adult stem cell plasticity have led to intense discussions whether some or all adult stem cells may have the same pluripotent capacity as ES cells. Although there is yet no "official" definition of stem cell plasticity it could be defined as the capacity of a given cell to acquire morphological and functional characteristics of a tissue different than the one from which the cell is originally derived [1, 41, 62]. True stem cell plasticity should include the following criteria: a single tissue-specific adult stem cell, for instance a hematopoietic stem cell (HSC), thought to be committed to a given cell lineage can under certain microenvironmental conditions acquire the ability to differentiate to cells of a different tissue. The differentiated cell types should be functional *in vitro* and *in vivo*, and engraftment robust and persistently in the presence (and absence) of tissue damage.

Adult stem cells have been found in most tissues including hematopoietic [10], neural [16], epidermal [64], gastrointestinal [30], skeletal muscle [48], cardiac muscle [18], liver [15], pancreas [9] or lung tissue [38] (for review of organ specific stem cells see special issue from J Pathol 2002, volume 197). Until recently it was accepted that tissue specific cells could only differentiate into cells present in the tissue of origin. However, this concept has been challenged by recent studies suggesting that cells originating from one germ layer (e.g. mesoderm) can generate tissues derived from a second germ layer (e.g. ectoderm or endoderm) [32, 41]. In addition, several recent studies have suggested that adult pluripotent stem cells may be obtained from the BM [20, 27] and from the brain [13, 21], capable of giving rise to tissues derived from all three germ layers. However, this unexpected plasticity of adult stem cells has been questioned by the observation of *in vitro* cell fusion between BM or neural stem cells (NSC) and ES cells [58, 67], and by the fact that some experiments have not been reproduced despite the intense efforts of different groups of investigators [6, 33].

### Skeletal Myoblast for Cardiac Repair

Satellite cells (SC) or skeletal myoblast are precursor cells of human skeletal cells. Under steady state conditions, satellite cells lie below the basal membrane of

muscle fibers but in response to injury, SC have the capacity to undergo proliferation and terminal differentiation into new functional muscle [47, 48]. Although there is some evidence suggesting that under special conditions, SC may adapt a different fate that muscle [35], it is generally accepted that SC are predetermined to differentiation into skeletal muscle fibers. The use of skeletal cells to repair cardiac defect is based on *in vitro* as well as *in vivo* experiments. Unlike cardiac muscle, skeletal muscle is characterized by fast-twitch fibers amenable of fatigue. However, it has been demonstrated that chronic electrical stimulation of skeletal muscle induces changes such as expression of slow-twitch fibers and can transform skeletal muscle into indefatigable muscle more akin to cardiac muscle [22]. The clinical experience with dynamic cardiomyoplasty has further demonstrated that autologous grafts of skeletal muscle can be adapted to perform cardiac work and enhance cardiac function [11].

Initial studies were performed with the goal of determining the fate of myogenic cells grafted into the hearts of animals and in general utilized myogenic cells lines as they were homogenous populations of well characterized cells [26]. The use of autologous graft of primary SC was facilitated by the development of culture protocols for the isolation and *ex vivo* expansion of SC and skeletal myoblast thus limiting the potential for tumor formation associated with cells lines [8]. One of the limitations of culturing primary myoblast is the contamination with other non-myogenic cells including fibroblast [34]. Transplantation of autologous skeletal myoblast has been successfully demonstrated in small (mice and rat) and large (sheep and dogs) animal models of myocardial infarction both by intramural implantation [5, 12, 17, 19, 34, 42, 54, 56] or by arterial delivery [53, 57]. Animal studies have suggested that myoblast delivered intracoronary are capable of transmigrate and integrate into the myocardial interstitium [46]. After introduction into the heart either directly or intracoronary, myoblast terminally differentiate into skeletal muscle and by day 7 they loss their proliferative capacity [34]. Implanted myoblast may survive for prolonged periods of time [17, 46]. These findings suggest that the cardiac environment is permissive for myogenic differentiation. There is more controversy regarding the potential of myoblast to adapt characteristics of cardiac muscle after implantation. Although some initial reports had suggested that after implantation, myoblast may transdifferentiate into cardiac muscle or at least acquire certain properties of cardiac muscle like expression of connexin 43, intercalated discs or slow-twitch MHC [12, 14, 34, 56] more recent studies indicate that myoblast do not transdifferentiate into cardiac muscle [45]. Differences between cell sources (neonatal or adult myoblast), species or the use of more rigorous techniques may explain some of these differences. In conclusion, the existing evidence does not support transdifferentiation of myoblast but only some conversion from fast to slow twitch phenotype. Another issue pertains to the capacity

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of myoblast for electromechanical coupling between implanted myoblast and cardiac cells. Although *in vitro* studies have suggested that electromechanical coupling can be established between cardiac and skeletal muscle [44] there is only minor *in vivo* evidence that this may be the case [34, 45, 46, 59]. Despite all these issues, the more relevant question resides on whether myoblast implantation improves cardiac function in damaged heart. Primary myoblast have been grafted into the injured myocardium of rats, rabbits, dogs, or sheeps [5, 14, 17, 34, 42, 43, 54, 56, 57]. Collectively, these studies clearly show long-term survival and differentiation of cells and importantly, functional improvement in left ventricular function after myocardial infarction.

Despite a number of open questions, the use of skeletal myoblast for cardiac repair has moved to clinical trials throughout the world. The use of SC for cardiac repair in patients with myocardial infarction has several advantages over other sources of stem cells. SC proliferation capacity is limited thus the potential of SC to undergo uncontrolled proliferation and tumor formation is minimal. Another advantage is that being autologous cells, it can overcome the shortage of organ donor as well as the requirement of immune-suppression after transplantation of allogeneic tissues. Myoblast are relatively resistant to ischemia so they may provide a more resistant tissue that cardiomyocytes. All these factors along with the fact that there is no ethical controversy regarding their use have prompted several groups to initiate clinical trials with autologous myoblast for cardiac repair (Table 1). The first case of a patient treated with autologous myoblast was reported by the group of Philippe Menasche [31]. A

patient with myocardial infarction successfully received direct intramyocardial injection of autologous skeletal myoblast simultaneously with coronary bypass surgery. Currently, more than 30 patients throughout the world, enrolled in different clinical trials have been treated with autologous myoblast administered intra-myocardium or percutaneously. No results have been reported so far except in abstract form. From some of these preliminary reports the mayor adverse event has been the incidence of cardiac arrhythmias. It is difficult at this time to make an assessment of the real incidence and potential mechanism of this adverse event as results have not been yet reported fully.

Our group has transplanted so far 10 patients with autologous myoblast by direct myocardium injection as adjunct to coronary bypass surgery. Although the follow up of our patients is very limited and the study has not been completed the procedure has been well tolerated with no adverse events including cardiac arrhythmias not having been detected post surgery. Unlike other studies, culture of autologous myoblast has been performed without the use of growth factor supplements or fetal bovine serum. From experiments performed in animal models, we have some preliminary evidence that culture of myoblast with xenogeneic serum may result in a severe inflammatory reaction within the heart (Rabago personal communication and unpublished observations).

How many cells do we need to inject?, what is the preferred way for injection?, are these cells capable of surviving long-term and if so, do they acquire characteristics of cardiac muscle?, can myoblast contribute to improved long-term cardiac function in patients with myo-

**Table 1: Clinical trials of cellular cardiomyoplasty with myoblast**

Country	Sponsor	Hospitals	Approach	Year <sup>1</sup>	N <sup>2</sup>
U.S.A	Diacrin	Temple Univ.	Surgery	2.000	
U.S.A	Diacrin	Cleveland Clinic; UCLA; Arizona Heart Inst.	Surgery	2.001	
U.S.A	Bioheart	Mount Sinai; DuKe Univ.	Surgery-Percut	2.003	
Argentina	Hosp. Avellaneda	Hosp. Avellaneda	Surgery	2.001	1
China	Nanjing Univ.	Nanjing Univ.	Surgery	2.002	3
China	Singapore Univ.	Singapore Univ. Hosp.	Surgery	2.002	1
Francia	Assistance Publique	Hôpital Bichat	Surgery	2.000	11
Holanda –Italia	Bioheart INC.	Thorax Center; Milano	Percutaneous	2.001	13
Polonia	Poznan Univ.	Poznan Univ. Hosp.	Surgery	2.002	10
España	Univ. Navarra	Universitaria Navarra; Clínico Salamanca; Juan Canalejo; Gregorio Marañón; M.Valdecilla	Surgery	2.002	10

<sup>1</sup>Year initiated, <sup>2</sup>Number of patients included as of September 2002

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cardial infarction or other cardiomyopathy?, what adverse effects can we expect from this therapy?, are some of the questions that need to be answered before this treatment can be translated into clinical practice.

### Bone marrow derived stem cells for cardiac repair

The bone marrow contains several populations of stem cells. In addition to blood-forming cells (hematopoietic stem cells), mesenchymal stem cells or stromal stem cells and endothelial progenitor cells can be isolated from the bone marrow. All these types of stem cells have been utilized in animal models for cardiac repair with different success. Very limited clinical experience has been reported with bone marrow mononuclear cell which includes different types of stem cells as well as a large proportion of terminally differentiated and committed progenitors, mesenchymal stem cells and hematopoietic cells [52].

The potential of BM or PB cells to differentiate into cardiac muscle has been suggested by several studies. Some investigators have used BM mononuclear cells either directly implanted into the heart or by percutaneous infusion [61] and even a small number of patients with myocardial infarction treated with coronary angioplasty have received autologous BM into their coronary arteries with some functional benefit [52]. Because there is no information regarding the nature of the cells that may contribute to cardiac muscle regeneration, the relevance of these studies is at best very limited. More compelling evidence for the capacity of BM cells to regenerate cardiac muscle stems from the group of Orlic and Anversa [36]. These authors demonstrated in a mouse model of myocardial infarction that direct injection into the heart of Lin<sup>-</sup> kit<sup>+</sup> BM cells results in more than half the infarct area being colonized by donor cells within 9 days. Donor cells acquired phenotypic characteristics of cardiomyocytes and contributed to improved cardiac function and improved survival of the animals. In a subsequent study this same group of investigators demonstrated that cardiac infarct size was significantly reduced and cardiac function significantly improved in animals in which SCF and G-CSF was used to mobilize HSC and progenitors from the BM. This was associated with increased proliferation of cardiac myoblasts, smooth muscle cells and endothelial cells in the infarct tissue, leading the authors to conclude that progenitor cells for these three tissues were mobilized from the BM. Although these studies suggest that cardiac defects due to ischemia may improve by local infusion of or mobilization of progenitors from the BM, the etiology of the cell(s) responsible for this effect is not clear. Despite the fact that these cells can be found in the HSC-rich Lin<sup>-</sup> kit<sup>+</sup> fraction of BM, proof that HSC trans-differentiate into myoblasts is still lacking and in fact very recent reports have seriously questioned the potential of hematopoietic stem cells to trans-differentiate [63].

Angioblasts or endothelial progenitor cells can also be found both in the PB and BM and identified based on ex-

pression of surface antigens such as AC133 and VEGFR-2 also found on HSC [3, 25, 39, 50]. In mouse models of limb ischemia, endothelial progenitors are mobilized from the BM and contribute to neo-angiogenesis in the ischemic limb [3, 4, 55]. Likewise, in a rat model of cardiac ischemia, mobilized CD34<sup>+</sup> CD117<sup>bright</sup> AC133<sup>+</sup> VEGFR2<sup>+</sup> cells contribute to repair of the rat heart after myocardial infarction by the remarkable capacity to generate new capillaries within the infarct zone [25]. Although neo-angiogenesis by mobilized PB stem cells could be demonstrated, no generation of new cardiac muscle cells (myocytes) was observed in this study.

In contrast to hematopoietic and endothelial cells, mesenchymal stem cells (MSCs) are derived from somatic mesoderm. MSCs have demonstrated their potential to differentiate into functional mesodermal derived tissues. MSCs differentiate into osteoblasts, chondrocytes, adipocytes, and skeletal myoblasts [28, 40]. Recent studies also suggested that MSCs differentiate into cardiac myoblasts *in vitro* as well as *in vivo* [28, 60, 61]. MSCs culture in the presence of 5-azacytidine acquire phenotypic characteristics of cardiomyocytes including electrophysiological activity and spontaneous beating in culture [28]. Injection of human MSC into an infarct mouse heart resulted in differentiation to cells staining positive for certain cardiac muscle markers after 1 week [60]. Using a swine model of myocardial infarction, 2 different groups have demonstrated that injection of MSCs resulted not only in engraftment of cells that acquire characteristic of cardiomyocytes but more importantly contribute to improvement of cardiac function [49, 61]. Although most animal studies have employed direct intramyocardial injection, the intracoronary approach is undoubtedly attractive from a clinical point of view. Patients not subsidiary of bypass surgery or patients with acute myocardial infarction could benefit from this approach without the risk of surgery. Intracoronary injection may pose other limitations for this type of treatment: cells may be trapped in small capillaries without reaching the myocardium, or may distribute and be lost in the systemic circulation. All these issues need to be addressed in animal models.

Several clinical trials have also been initiated using marrow stromal cells or MSCs for cardiac repair (Table 2). However, the results of these studies are still awaited and only some preliminary presentations have been done. The only clinical study of cardiac repair using bone marrow derived cells published so far involves the use of BM mononuclear unselected cells directly administered intracoronary [52]. Ten patients were treated with intracoronary transplantation of autologous, mononuclear bone marrow cells in addition to standard therapy of MI that included re-canalization of the infarct-related artery by percutaneous transluminal coronary angioplasty (PTCA) and subsequent stent implantation. Although the study was not randomized, the investigators compared 10 patients treated with the PTCA standard procedure with another 10 patients that were treated also with intracoronary

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**Table 2: Clinical Trials of Cellular Cardiomyoplasty with bone marrow cells**

Country	Sponsor	Hospitals	Approach	Year	Nº
<b>Brasil</b>	<b>Incor, Sao Paulo</b>	Incor, Sao Paulo	Surgery - HSC	2.002	1
<b>Japón</b>	<b>Yamaguchi Univ.</b>	Yamaguchi Univ. Ube	Surgery-MSc	2.001	10
<b>Alemania</b>	<b>Dusseldorf Clinic</b>	Heinrich Univ. Dusseldorf	ACTP-IC-HSC	2.001	10
<b>Alemania</b>	<b>Rostock Univ.</b>	Rostock Univ.	Surgery – MSC	2.001	3
<b>Italia</b>	<b>Padova-Milano Univ.</b>	Padova-Milano Univ.	Surgery	2001	
<b>España</b>	<b>Univ. Valladolid</b>	Universitario Valladolid	PTCA-IC-HSC	2.002	3
<b>España-Francia</b>	<b>Univ.Navarra-Miltenyi</b>	Universitaria Navarra Clínico Salamanca Juan Canalejo Gregorio Marañón M. Valdecilla Clínico Valencia Central Asturias Rangueil. Toulouse Georges Pompidou. Paris	Surgery-AC133	2.003	

**HSC: hematopoietic stem cells; EPC:endothelial progenitor cells; MSC:mesenchymal stem cells; PTCA:percutaneous transluminal coronary angioplasty; IC: Intracoronary**

infusion of  $5-20 \times 10^6$  MNC from BM at a median of 7 days after acute myocardial infarction. The functional results after 3 month follow-up suggested that cell therapy was associated with a reduction in the infarct region, increased infarction wall movement, improvement in stroke volume index, left ventricular end-systolic volume, contractility and myocardial perfusion of the infarct region. Although promising, these results are certainly questionable as most of the effects observed could be due to the standard treatment of the MI. Besides, the use of unselected cells prevents any conclusion regarding the potential effect of the cells.

### Conclusions

With the limited availability of organs for transplantation, cell transplant is a novel and promising treatment strategy for patients with heart failure. Although results in animal models and initial clinical trials are promising it is too early to establish any real conclusions as to the usefulness of such therapy. Besides, a number of fundamental questions remains: what is the best type of cells for cardiac repair and how many cells do we need to inject to be effective?, what is the best time (acute or chronic) for administration of cells and what is the best method for cell delivery? Or maybe, different situations may require different cell types, what cardiac diseases may benefit from this treatment? We are really at the beginning of a new and challenging field. New animal studies as well as phase I rigorously designed clinical trials are needed to try to answer some of this questions and many new ones that will arise.

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