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Therapeutic Approaches in Regenerative Medicine of Cardiovascular Diseases: From Bench to Bedside

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1. Introduction

Despite significant progress in medical research, which has led to lower infant mortality rates and increased life expectancy, cardiovascular diseases (CVDs) continue to be the largest contributors of morbidity and mortality in both developed and developing countries. In fact, they are predicted to be the leading cause of death by 2020 and responsible for 13.4% of total world-wide deaths by 2030. These diseases have a multifactorial origin, notably non-modifiable cardiovascular risk factors (CRFs) such as age, sex, race or family history, and modifiable CRFs, including smoking, hypertension, hypercholesterolemia, diabetes mellitus or hypertriglyceridemia (Picariello et al., 2011; Glynn & Rosner, 2005).

Currently, there are no effective treatments available for many degenerative diseases caused by the death or malfunction of specific cells. In this regard, the main physiopathology of CVDs is related to ischaemic heart disease, and approximately two-thirds of patients surviving an acute myocardial infarction (AMI) are left with debilitating congestive heart failure. AMI causes apoptosis and necrosis of cardiomyocytes, a specialized and differentiated cell population responsible for ventricular contraction, the main event of cardiac function. The low self- renewal rate of these cells (~0.06% of the total population of cardiomyocytes under normal conditions) produces, in cases of acute stress, the ventricular remodelling of non-ischaemic myocardium with scar formation, increasing the likelihood of a new crisis, progressive ventricular dilatation and heart failure, ending in death (Torella et al., 2007).

Organ transplantation is an appropriate therapeutic strategy for patients who have suffered end-stage heart failure and are unresponsive to conventional therapy but has drawbacks related to donor incompatibility, which can lead to rejection and eventually to Graft Versus

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Host Disease (GVHD), among other conditions. However, there are a number of preoperative and postoperative risks, besides the major challenge of finding an optimal donor heart and the elevated healthcare costs involved (currently ranging from 27,839 € and 51,575 € per patients), especially in view of the uncertain long-term outcomes of the procedure (Pérez -Romero et al., 2010).

1.1 Worldwide costs: The DALY factor

Besides the elevated mortality rates associated with CVDs, they contribute significantly to the burden of disability and disease in developing countries. In an update on the global burden of disease in 2004, the WHO provided a comprehensive assessment of the causes of loss of health in different regions of the world, demonstrating that the leading cost of the death in developed or high-income countries is heart disease, followed by stroke, lung cancer, pneumonia and asthma or bronchitis (WHO, 2004). The most widely-used measure of this burden is the life expectancy adjusted for disability (DALY), defined as the sum of life years lost due to premature death and years lost to disability. CVDs are considered to represent 86% of the total DALY-estimated disease burden among under 70-year-olds worldwide, indicating that global CVD-related deaths will rise from the 16.7 million estimated in 2002 to 23.3 million in 2030.

1.2 Regenerative biomedicine: Breaking down old paradigms?

Over the past decade, new therapeutic strategies have been proposed against diseases with an elevated prevalence world-wide for which standard treatments are not very effective, resulting in the emergence of a new medical science known as regenerative medicine. Scientific evidence has demonstrated that novel therapeutic solutions can be obtained for pathological situations with no current treatment and that existing therapies can be enhanced by the utilization of autologous or allogeneic stem cell transplantation. These derive from the discovery of adult stem cell populations in organs well known for their non-existent regeneration capacity, such as the heart and brain, which correlate with their early development in the embryo stage. Thus, some cardiomyocytes with proliferative capacity have been found in the heart after a myocardial infarction, although inadequate to meet requirements after an extensive AMI.

The discovery of multipotent cell populations in adult tissues has opened up new therapeutic possibilities for diseases that do not respond to conventional medical treatments. The ethical and legal issues involved in the use of embryonic stem cells (ESCs), the recently disclosed plasticity of adult-derived stem cells, their ability to be reprogrammed by defined factors into pluripotent stem cells and a greater understanding, thanks to a multidisciplinary approach, of the mechanisms underlying stem cell differentiation have led to the birth of the "Regenerative Medicine" concept. Unlike many existing products and therapies, this approach offers the potential for regenerative rather than merely palliative or symptomatic treatment. This novel specialty includes gene therapy, stem cell therapy, targeted drug therapy and cell reprogramming.

This emerging field seeks the maintenance, improvement or restoration of cell function, tissues and organs by applying methods mainly related to cell therapy and tissue engineering (Greenwood et al., 2006). The ultimate goal is to repair, replace or regenerate cells, tissues or organs that have lost their normal function. The essential elements for the success of regenerative medicine include cells, soluble molecules and three-dimensional

scaffolds that serve as anchor and stimulation for new tissue formation. In relation to CVD, the regeneration of vascular and myocardial tissues is considered a primary endpoint to limit the consequences of acute and chronic ischaemic heart disorders. Current clinical approaches include transdifferentiation techniques and the utilization of organic scaffolds (e.g., vessels or even the whole heart) by means of decellularization methods.

2. Stem cells and cardiovascular diseases

Stem cells are unspecialized cells that can renew themselves through cell division or can be differentiated to become tissue- or organ-specific cells with special functions (Wobus & Boheler, 2005). Up to the 8-cell morulla stage, cells in the embryo are totipotent and can produce the whole organism. Their utilisation is limited by ethical issues and current legislation in developed countries, besides inadequate knowledge of their behaviour and action mechanisms and of their impact on the body homeostasis.

Stem cells derived from the inner cell mass (ICM) of the blastocyst are no longer totipotent but they are pluripotent, having the ability to differentiate into all cell types of the body. These cells include human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). hESCs are capable of proliferating extensively in an undifferentiated state and in co-culture on fibroblast 'feeder' cells, and have the ability, once removed from feeders and/or grown in suspension as aggregates [called EBs (embryoid bodies)], to differentiate towards all three germ layers, and they can, in principle, give rise to all cell types of the body. hESCs contain factors that can induce reprogramming of the somatic cell nucleus (Allegrucci & Young, 2007; Cowan et al., 2005). Therefore, somatic cell fusion with ESCs regenerates pluripotent cells. However, pluripotent cells obtained by fusion contain both chromosomes from the ESCs and from the somatic cell, causing their post-implantation rejection (Takahashi & Yamanaka, 2006).

iPSC technology was developed to avoid these problems, generating a new type of pluripotent stem cell by directly reprogramming somatic cells rather than using embryos. It allows patient-specific stem cells to be obtained by using the same genome as that of the individual whose cells have been reprogrammed. Besides adult and foetal dermal fibroblasts, iPSCs have been created from multiple human tissues, including lung fibroblasts, keratinocytes (Aasen et al., 2008), fibroblast-like synoviocytes (Takahashi et al., 2007), cord blood (Giorgetti et al., 2009; Haase et al.,2009) , peripheral blood (Loh et al., 2009, Ye et al., 2009) mesenchymal stromal cells (Oda et al., 2010), oral mucosa fibroblasts (Miyoshi et al., 2010) and T-cells (Loh et al., 2010; Seki et al., 2010). These cells can be differentiated and can serve as a cell or disease model or may even lead to future stem cell therapies (Dimos et al., 2008, Park et al., 2008). However, there are a number of drawbacks in the use of retroviral vectors to create iPSCs, and considerable efforts have been made to find alternatives.

In addition, certain organs have multipotent stem cells with more limited differentiation ability (Wobus & Boheler, 2005), including foetal and adult stem cells obtained from bone marrow (BM) and postnatal organs and tissues. Stem cells of foetal origin express stem cell markers similar to hESCs, while their differentiation potential lies between that of hESCs and that of adult stem cells (Pappa & Anagnou, 2009). BM contains two types of multipotent stem cells: hematopoietic stem cells (HSCs), which give rise to all blood cell types (Orkin, 2000) and human mesenchymal stromal cells (hMSCs), which can be differentiated into multiple mesenchymal tissue cell types such as bone, cartilage, fat and muscle cells (Liu et al., 2009).

2.1 The quest for an optimal adult stem cell type: Cardiac Stem Cells (CSC)

Although many different cell types have been considered for myocardial repair, the ideal candidate remains elusive. Adult cardiomyocytes are unable to survive, even when transplanted into normal myocardium, and the resident population of cardiac progenitors is insufficient after AMI. Therefore, researchers continue to search for the optimal cell type for myocardial repair, using various types of adult stem cells obtained from different sources, such as HSCs, endothelial progenitor cells (EPCs), cardiac stem cells (CSCs), muscle-derived stem cells (MdSTs), and MSCs. MSCs are frequently selected for their multiple advantages, such as their multiple anatomical localizations, easy isolation and cryopreservation, and paracrine potential.

The characteristics of CSCs make them an excellent candidate for cardiac regeneration. CSCs isolated from myocardial biopsies (Smith et al., 2009) express stem cell factor receptor (c-Kit) (Bearzi et al., 2007) and stem cell antigen-1 (Sca-1) on their cell surface (Iwakura et al., 2011). A direct relationship has been demonstrated between higher patient age and a smaller population of these cells, which represents a drawback for their therapeutic application, given that the main incidence of CVDs is among the middle-aged. However, intensive work is ongoing to activate these cells to proliferate and differentiate *in situ* using different techniques, such as induction with 5-azacytidine (5-aza), a potent inhibitor of DNA methyltransferase (DNMT1), in combination with TGF-β1 and vitamin C (Smits et al., 2009). Recent proposals include the use of hyperpolarization to differentiate CSCs into the cardiac phenotype, changing charges in the membrane potential (specific for each cell type) to enhance intracellular calcium levels and calcineurin signalling in CSCs and, more importantly, to upregulate the expression of cardiac-specific genes and proteins, leading to the formation of spontaneously beating cardiomyocytes (Van Vliet et al., 2010). Nonetheless, the specific capabilities of functional cardiac cells from these progenitors need to be more thoroughly studied (Gonzales & Pedrazzini, 2009).

2.2 Adult stem cell transplantation: Paracrine effects or change in phenotype?

Recently, cell-based therapeutic strategies have been aimed at replenishing the loss of myocytes by apoptosis and necrosis and at inhibiting the adverse effects of cardiac remodelling (Singla, 2009). Some studies have demonstrated improvement in ventricular function as an achievement of cardiac regeneration (Singla, 2009, Kumar et al., 2005). However, if a real clinical translation is to be achieved, researchers must follow strict criteria for the selection of an appropriate source of stem cells, taking full account of patient safety, production methods, and ethical and legal norms. It is necessary to establish an *in vitro* differentiation strategy to transform these cells into functional cardiomyocytes that can integrate in a damaged heart and exert therapeutic action.

The benefits of stem cell transplantation remain controversial and appear to depend on three mechanisms. First, the transplanted stem cells must be able to differentiate, even at a low rate, into heart or vascular cells. A second mechanism involves the immune and paracrine functions of stem cells and their interactions with an environment subject to cellular damage, with the transplanted cells having the capacity to induce the secretion of growth factors and cytokines that stimulate tissue repair after ischaemic injury, reducing the size of infarction. Finally, the pre-treatment of stem cells by transdifferentiation methods has been shown to produce a population of cells in pre-differentiated state, allowing their acquisition of the cardiac phenotype after transplantation. However, key issues have not yet

been elucidated, including identification of the most appropriate cell types or the factors released by stem cells and their role in cardiac repair and regeneration. The methods described below were conducted using hMCSs, which appear to be promising candidates for clinical translation, given their multipotency and ready availability, e.g., *via* liposuction.

2.2.1 Direct transplantation and cell tracking

Cell transplantation is emerging as a potential therapy to treat heart failure. Initial efforts have been focused on the cardiomyocytes and skeletal myoblasts. However, the mechanisms and the outcomes of transplanted cells during cardiac regenerative therapy remain unclear. In order to achieve the optimal concentration of stem cells for repairing the myocardial region of interest, cell delivery strategies need to consider different clinical settings and local milieus. Stem cells are believed to respond differently according to local signalling, and the success of transplantation depends to a large degree on damage signalling in the wounded area. In cardiovascular research stem cells have been delivered through coronary arteries or coronary veins or by means of peripheral-vein infusion. Alternatively, direct intramyocardial injections have been performed, utilizing a surgical, transendocardial, or transvenous approach. Delivery strategies also include the mobilization of stem cells from the bone marrow by means of cytokine therapy, with or without peripheral harvesting. In pre-clinical studies, when BM–derived hematopoietic cells were transplanted directly into the hearts of mice with AMI, no transdifferentiated BM–derived cardiomyocytes were found in the damaged myocardium. However, cell fusion has been found to occur at very low levels between BM–derived cells and host cardiomyocytes outside the infarction area (Nygren et al., 2004). A similar approach has been adopted using skeletal myoblast precursors. BM-stem cell (SC) transplantation has been the most widely used methodology in clinical studies, as shown in Table 1, although its association with arrhythmogenesis, among other adverse effects, has generated major controversy about its benefits.

The main problem with these methods is the lack of knowledge on the behaviour and integration of the transplanted stem cells. Many studies have relied simply on the presence of histological differences between hearts receiving cell transplants compared with non-transplanted patients (control group). However, the preferred approach is to utilize molecular analyses that are able to distinguish donor and host cells. For instance, treating donor cells with fluorescent cell-tracking dyes prior to transplantation has been used to monitor their survival *in vivo*. Because there is typically a high degree of donor cell death following transplantation, caution must be exercised to ensure that the observed signal arises from donor cells rather than from host cells, which have acquired dye released from dead or dying donor cells. Intrinsic genetic differences between donor and host cells as well as gene transfer have also been employed to monitor cell fate following transplantation (Lee & Segers, 2008). Hence, innovative non-invasive imaging techniques to effectively track the cells *in vivo* are vital for the adequate investigation of possible clinical applications, furnishing insights into the mechanisms underlying stem cell-based therapy and addressing some of the major concerns about translational cardiovascular stem cell therapy. Currently, there are three main approaches to stem cell tracking: (1) radioactive labelling for positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging, (2) iron particle labelling for magnetic resonance imaging (MRI), and (3) reporter gene labelling for bioluminescence, fluorescence, MRI, SPECT and PET imaging.

Clinical trial identifier number	Trial name	Number of patients	Cells	Primary end point	Route of cell delivery	Further Comments
NCT00669227	SCAMI	40	BM-SCs	LVEF	IC	Prior treatment with G-CSF
NCT00395811	CABG	50	BM-SCs	LVEF	IC	Stem Cell Therapy combined CABG
NCT00316381	REGENT	200	Subpopulation of CD34+/CXCR4+ BM-SCs	LVEF	IC	
NCT00587990	PROMETHEUS	45	MSCs	Safety	Transepicar-dial during CABG	Stem Cell Therapy combined CABG
NCT00548613	MESENDO	20	BM-SCs	New blood vessel formation	IC/IM	Combined stem cell therapy
NCT00939042	ALSTER	40	BM-SCs	LVEF	IC	
NCT00442806	APOLLO	48	ADRCs	Safety	Intravenous	
NCT00126100	REVIVAL-2	114	G-CSF-stimulated BMSCs/proge-nitor cells	Efficacy	Subcutaneous	Evaluation of the ↑ of circulating SC after stimulation
NCT00268307		41	BM-SCs	Safety	IC	
NCT00289822		75	BM-SCs	LVEF	IC	
NCT00626145		37	BM-SCs	LVEF	IC day 7 post PCI	Evaluation of reperfusion therapy for STEMI
NCT00810238	C-Cure	240	BM- derived cardiopoietic cells	LVEF	Transendocar dial	Efficiency of vascular regeneration in patients with CAD

ADRCs: Adipose-Derived Stem and Regenerative Cells; EPC: Endothelial progenitor cells; BM-SCs: Bone Marrow stem cells; MSCs: Mesenchymal stromal cells; G-CSF: Granulocyte Colony Stimulating Factor; STEMI: ST-Elevation Myocardial Infarction; PCI: Percutaneous Coronary Intervention; CAD: Chronic Coronary Artery Disease; CABG: Coronary Artery Bypass Graft; IC: Intracoronary; IM: Intramyocardial; LVEF: Left Ventricular Ejection Fraction.

Table 1. Closed/Not recruiting autologous cell therapy trials in patients with coronary heart disease or AMI

2.2.2 Paracrine and immunology features

Two important features of MSCs are their immune-privileged and immunosuppressive functions. These qualities are very attractive for cell-based therapy because they may facilitate the use of allogeneic rather than autologous cells, which offers many potential advantages. Thanks to their immunosuppressive capacity, they may also suppress the immune response to cell injections or to the damaged myocardium itself during myocardial infarction. Findings by Aggarwal and Pittengerin on the lack of T-cell response to allogeneic hMSC transplantation indicated that these cells can modify the immune response in a dose-

dependent manner and can reduce the inflammatory response when present in appropriate amounts. Phase III clinical trials are under way on the optimal MSC regimen to prevent or treat GVHD during allogeneic HSC transplantation (Aggarwal & Pittenger 2005).
Secreted growth factors and cytokines are known to be important signalling molecules for modulating acute and chronic immune responses (Table 2). With regard to their immunomodulatory properties, MSCs inhibit T-cell proliferation and influence the maturation and expression profile of antigen-presenting cells such as dendritic cells. Research on the paracrine effect of MSCs in rat AMI models, using intramyocardial injection of culture medium with MSC-secreted growth factors and cytokines (conditioned medium), demonstrated their role in stromal network creation, neovascularization enhancement, angiogenesis promotion, and cardiac function improvement. Paracrine effects of MSCs may include the activation of different mechanisms against cell damage. Therapeutic models have shown that MSCs are recruited to infarction areas by the hepatocyte growth factor (HGF), which is released during necrosis and apoptosis and acts as a "homing signal". This migration of MSCs depends on interaction with c-Met, an HGF receptor (Parekkadan & Milwid, 2010).

Growth factors and cytokines	Functions	References
M-CSF, G-CSF, GM-CSF, SCF-1, LIF, SDF-1, Flt-3, IL 1, 6, 7, 8, 11, 14 and 15.	Signalling molecules and modulation of acute and chronic immune response.	Sumanasinghe et al., 2009 Kim et al., 2005.
VEGF, bFGF, PLGF and MCP-1.	Vasculogenesis and angiogenesis	Kinnaird et al., 2004.

M-CSF: Macrophage Colony-Stimulating Factor; G-CSF: Granulocyte Colony-Stimulating Factor; GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor; SCF-1: Stem Cell Factor; LIF: Leukemia Inhibitory Factor; SDF-1: Stromal Cell-Derived Factor-1; Flt-3: FMS-like tyrosine kinase 3; IL: Interleukin; VEGF: Vascular Endothelial Growth Factor; bFGF: basic Fibroblastic Growth Factor; PLGF: Placental Growth Factor; MCP-1: Monocyte Chemoattractant Protein-1

Table 2. Paracrine activity of MSCs

MSC-derived conditioned medium promotes the proliferation of CSCs and inhibits the apoptosis of CSCs induced by hypoxia and serum starvation. An upregulated expression of cardiomyocyte-related genes was also found in CSCs, including b-myosin heavy chain (b-MHC) and atrial natriuretic peptide (ANP), demonstrating the protective effects of this medium on CSCs and the enhancement of their migration and differentiation (Nakanishi et al., 2008). Preliminary results of *in vitro* assays with human adipose-derived mesenchymal stromal cells (hADSCs) by our group indicate that cytokine- and growth factor-rich conditioned media obtained from human cardiomyocytes in culture influence the acquisition of cardiac phenotype by hADSCs (unpublished data).

2.2.3 Cardiac regeneration: A transdifferentiation phenomenon

Regenerative biomedicine efforts have focused on attempts to reverse heart muscle failure by increasing the amount of human functional heart muscle through the transplantation of various adult stem cell types, notably CSCs, skeletal myoblasts, endothelial stem cells, and MSCs. BM stem cells have been reported to offer a wide range of benefits in blood cell diseases, but the action mechanisms underlying the change in phenotype have not been

clarified. *In vivo* transplantation studies have shown that MSCs can replenish the whole BM system in irradiated rodents and generate not only mesodermal cells but also cells with the characteristics of neural progenitors and neurons (Song & Tuan, 2004).

In this type of cross-lineage differentiation, also known as transdifferentiation, one cell type committed to and progressing along a specific developmental lineage is switched into another cell type from a different lineage by genetic reprogramming. This depends on the ability of adult stem cells to maintain their multidifferentiation potential even after exposure to specific inductive factors (Song & Tuan, 2004).

The conversion from adult differentiated cells must involve both the suppression and regulation of different genes in the cells, implying that genes from both cell types are co-expressed at some point. This phenomenon is currently under investigation by various research groups in an attempt to establish its true therapeutic value. Transdifferentiation studies have supported the notion that cell fate is controlled by master switch genes and that one or two factors can be sufficient to direct cells from one lineage to another (Burke & Tosh, 2005).

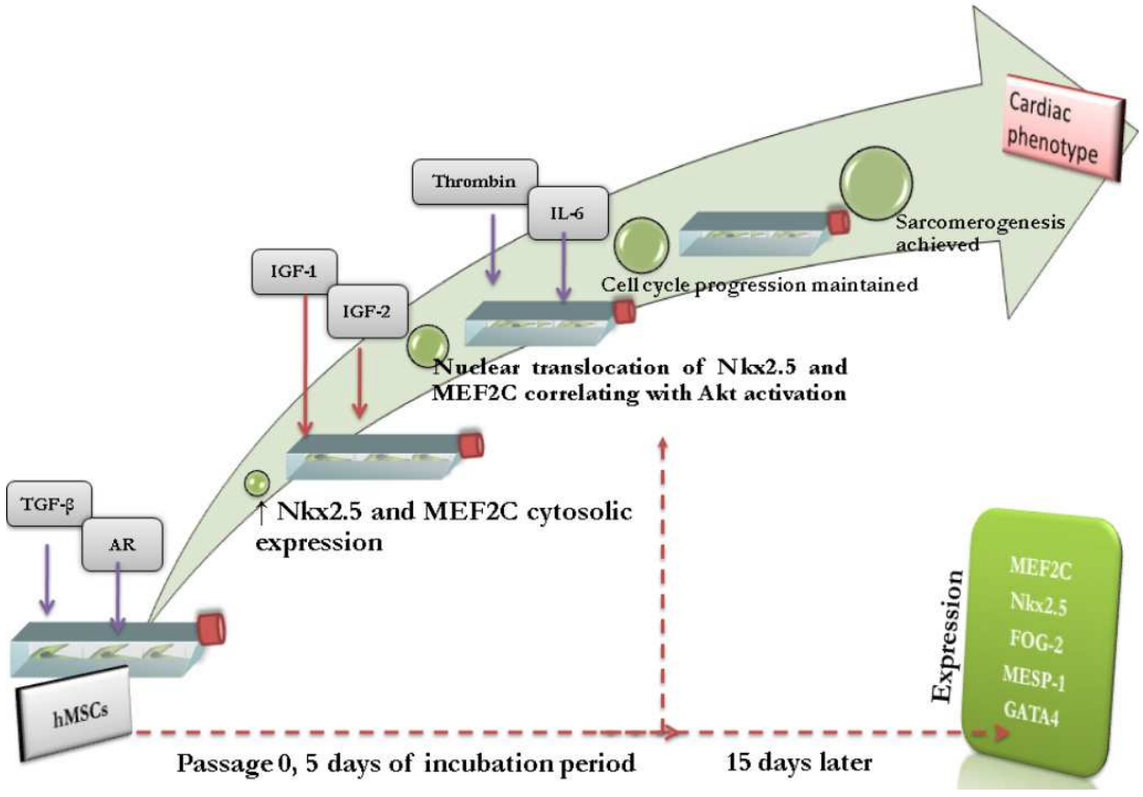

Fig. 1. Guided cardiopoiesis. This diagram depicts the effects of cytokines in MSCs cultures.

This process involves cardiopoiesis, defined as the final engagement of pluripotent or multipotent stem cells in the cardiac differentiation program. Cardiopoietic specification is based on emulation of the natural cardiac development in the embryo, which takes place in the mesoderm under the cardioinductive influence of the neighbouring endoderm (Figure 1). Cardiopoietic guidance emulates natural cardiac differentiation and can be achieved by stimulating stem cells with TGF-β1, BMP-2/4, FGF-2/4, IL-6, IGF-1/2, VEGF-A, EGF and activin-A. The identification of these factors has enhanced our understanding of the

mechanisms involved in differentiation and the signals that play a role in heart development (Behfar et al., 2010).

3. From the bench: Current status

Greater knowledge of heart development in the embryo and of the effects of the microenvironment on cardiac maturation has prompted researchers to reproduce these processes in the laboratory by means of different transdifferentiation techniques (Figure 2). Some of them have attempted to recapitulate the activation of cell signalling cascades involved in heart development (e.g., TGF-β and Akt) through the expression of GATA4, Mef2c and FGF 8/10 growth factors. A wide range of cell types and methods have been used to achieve the cardiac phenotype. In this regard, there is major interest in the novel technique developed by Takahashi & Yamanaka (2006), to induce pluripotency by reprogramming fibroblasts with key factors Klf4, Sox2, Oct4 and c-Myc. It is expected that the ability to derive patient-specific hiPSCs by somatic cell reprogramming may be useful for disease modelling and drug discovery as well as basic research.

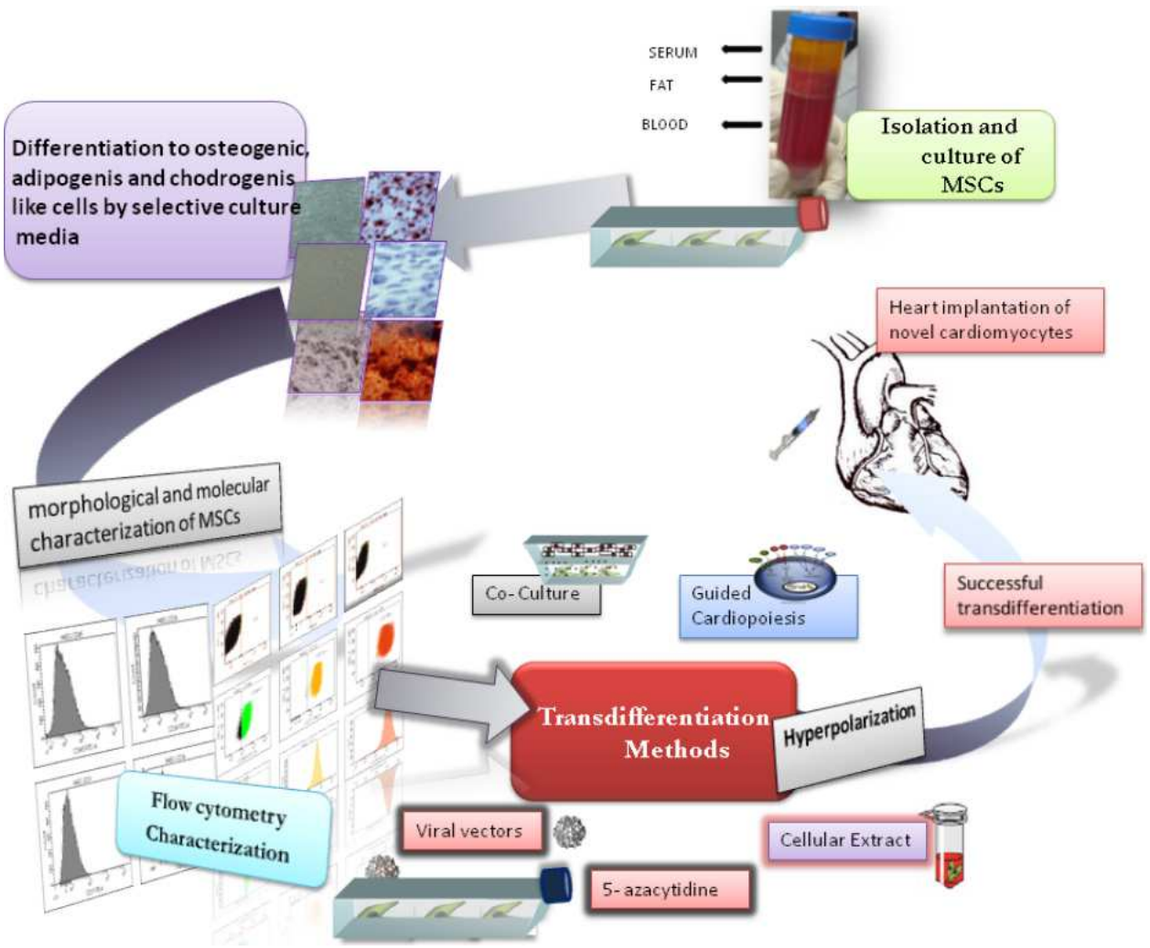

Fig. 2. Methods used to transdifferentiate MSCs to cardiomyocyte like-cells

3.1 Transdifferentiation methods using adult stem cells

3.1.1 Use of allograft cardiac tissue: The co-culture and cell extracts methods

The co-culturing of two different cell types, such as cardiomyocytes isolated from human biospies and hMSCs, which are grown together but separated by a membrane (with 0.4 – 0.3 µm pore size to avoid direct cell-to-cell interaction), has revealed the importance of the microenvironment in stem cell differentiation. This method is used to determine whether soluble chemical factors released from one cell type (which can diffuse though the membrane) are sufficient to induce transdifferentiation of the other cell type. Using this mechanism, Wang et al. (2006) showed *in vitro* the differentiation of BM stem cells into cardiomyocyte-like cells with expression of cardiac-specific genes. Similar results have been obtained by various authors (Antonitsis et al., 2007; Rangappa et al., 2003).

In the cell extract method, the intracellular components of one cell type are introduced into another cell type in order to switch from one cell pattern to another. Purified somatic nuclei or reversibly permeabilized somatic cells are incubated in a nuclear and cytoplasmic extract derived from a different cell type. The extract is believed to provide nuclear regulatory components that mediate alterations in the gene expression profile of the target genome. After exposure to the extract, the cells are resealed in a culture that contains calcium. By this means, hADSCs produced cardiomyocyte proteins after incubation with rat cardiomyocyte extracts. The success of these transdifferentiation strategies was demonstrated by observations of morphological changes, by findings on donor cell-specific genes, and by immunological evidence of alterations in protein expression (Gaustad et al., 2004).

Our group reported that adult cardiomyocytes obtained from human donors retain their capacity to induce cardiomyocyte differentiation of MSCs. Human adipose stem cells (hASCs) isolated from lipoaspirates were transiently permeabilized, exposed to human atrial extracts and then allowed to recover in culture. After 21 days, the cells acquired a cardiomyocyte phenotype, as demonstrated by morphologic changes (appearance of binucleate striated cells and branching fibbers), immunofluorescence detection of cardiac-specific markers (connexin-43, sarcomeric α-actinin, cardiac troponin I and T and desmin) and the presence of cardiomyocyte-related genes (cardiac myosin light chain 1, α-cardiac actin, cardiac troponin T and cardiac β-myosin). The relevance of these findings lies in the potential use of autologous extracts to induce stem cell reprogramming (Perán et al., 2010).

In conclusion, both techniques have an interesting potential in a therapeutic framework, due to their capacity to avoid immunologic responses from patients and produce a good clinical outcome.

3.1.2 Use of 5 –azacytidine and growth factors

The acquisition of a cardiac phenotype has been induced by the use of demethylation agents such as 5-aza, a cytosine analogue that can reduce DNA methyltransferase activity in the cells (Quian et al., 2011). However, *in vivo* studies with 5-aza showed that specific proteins were expressed in myocytes near the injection site in the myocardium, but these cells lacked the complete acquisition of cardiac phenotype and were not capable of symmetric contraction (Valiunas et al., 2004). Similar methods have been reported to produce cells with cardiomyocyte phenotype from human adipose-derived stem cells, and spontaneously beating cells were obtained after co-culture with neonatal rat cardiomyocytes (Choi et al., 2010).

3.1.3 Genetic modifications: Use of viral vectors

The genetic modification of MSCs is an interesting option for improving their therapeutic potential. The transfection of master genes or transcription factors that induce differentiation into the cardiac phenotype was recently developed as a transdifferentiation methodology. In cardiac research, efforts have been made to transfect BM-MSCs with viral constructions, using retroviral vectors that contain the master genes Csx/Nkx2.5 and GATA-4. Single-cell-derived MSCs overexpressing Csx/Nkx2.5 and GATA4 behaved as cardiac transient amplifying cells and retained their plasticity *in vivo*, but 5-aza treatment was required to achieve a complete cardiac phenotype. The frequency of cardiomyogenic differentiation was increased by co-culturing with foetal cardiomyocytes (Yamada et al., 2007).

For human clinical proposes, Ad- vectors are safer than RV- vectors because they do not integrate into the cellular DNA, and therapy with Ad-transduced MSCs is likely to be less immunogenic than the direct administration of Ad-vectors to affected tissues, due to the anti-inflammatory properties of MSCs. Moreover, transgenic expression in proliferating stem cells is only transient, due to the non-integrative properties of Ad-vectors. Nonetheless, short-term therapeutic gene expression may be sufficient or even desirable for the treatment of diseases such as myocardial infarction, in which the transient paracrine effects of MSCs enhance tissue healing and repair responses (Reiser et al., 2005).

Two studies recently reported the direct reprogramming of murine fibroblasts into functional cardiomyocytes. Srivastrava´s group developed a method to recruit and transdifferentiate resident cardiac fibroblasts near the infarction area. This group enforced the expression of developmental cardiac transcription factors Gata4, Mef2c, and Tbx5 and produced cardiomyocytes that matched a normal cardiomyocyte gene expression profile (Ieda et al., 2010). The efficiency of this direct reprogramming was higher in comparison to reprogramming to iPSCs (20% vs. <0.1%) and it was more rapid, but the emergent cardiomyocytes could not be expanded in culture, which is a shortcoming.

In another study, Ding´s group obtained cardiomyocytes by virally transducing mouse embryonic fibroblasts with genes encoding for the transcription factors Oct4, Sox2 and Klf4, followed by modified standard reprogramming medium, in which leukaemia inhibitor factor was removed and foetal bovine serum was added at 1-15% (Efe et al., 2011). This new development may help eliminate some of the obstacles to the use of traditional ESCs or iPSCS, including teratoma formation due to residual undifferentiated cells, and it may lower the cost and delivery time to patients thanks to higher yields and faster production.

3.2 Transdifferentiation methods using iPSCs

Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. The reprogramming of adult cells into ESCs enables the generation of patient-specific stem cells and therefore has enormous potential for the analysis and treatment of degenerative diseases. iPSCs are typically derived by transfection of certain stem cell-associated genes into non-pluripotent cells, such as adult fibroblasts. Transfection is typically achieved through viral vectors, such as retroviruses. Transfected genes include the master transcriptional regulators Oct-3/4 (Pouf51) and Sox2, although some other genes (e.g., Klf4, c-Myc) have been used to enhance the efficiency of induction.

Among various methods used to induce differentiation in pluripotent cell lines, the most widespread is to grow undifferentiated cells as aggregates in suspension, which causes them to form EBs in which cardiac cells spontaneously develop (Son et al., 2011). Cardiomyogenic mesodermal progenitors are normally formed in the embryo during gastrulation as cells of the epiblast pass through the primitive streak (Nury et al., 2009).

Many protocols for directed differentiation to cardiomyocytes are based on these developmental principles. In general, two directed differentiation strategies have been applied. First, the co-culture of hESCs with cardio-inductive cell types, such as endodermal cell line END-2. Cardiac induction by END-2-conditioned medium can be (partly) mimicked by insulin depletion (Freund et al., 2008), inhibition of p38 MAPK (Graichen et al., 2008) and addition of prostaglandin E.

A second method is to induce gastrulation by the use of specific growth factors. Several studies have shown that various combinations of BMP4, WNT3a and Activin A induce gastrulation-like events and meso-/endoderm development in hESCs (Sumi et al., 2008; Vijayagavan et al., 2009). A similar approach uses an efficient cardiac differentiation protocol based on transient stimulation with BMP4, bFGF and activin A followed by VEGF and WNT inhibition through DKK. Both approaches have allowed considerable progress to be made over the past few years with respect to the efficiency of cardiomyocyte differentiation, but its use in clinical therapies remains plagued with numerous problems, including inadequate purity. The same methods are currently used for the generation of iPSCs-derived cardiac cells.

3.2.1 Future applications of iPSCs technology in heart disease

The possibility of creating patient-specific embryonic stem cell-like cells may complement the rather slow development of heart disease models based on hESC. However, their value in elucidating disease mechanisms and as models for drug discovery remains to be demonstrated. The uniformity and reliability of the differentiation of several well-studied hESC lines may make these the most useful near-term research tool; therefore, research on the targeting and genetic modification of hESCs is still vital. In this regard, the use of iPSCs models has opened a new alternative to study illness, especially in countries were legal issues do not allow the use of hESC.

The generation of heart disease models is a highly complicated process, due to the variety of aetiologies and subtypes of CVDs and their complexity. Initial approaches have avoided multi-gene diseases and focussed on heart diseases associated with mutations in single genes, which are most likely to have an effect at single cell level. This is the case of channelopathies, which are related to mutations in a small set of genes that encode cardiac ion channels and sarcomeric proteins. In this way, the phenotypes of these diseases are characterized by disturbed ion channel function (cardiac channelopathies) or impaired contractility (cardiomyopathies). Heritable cardiac channelopathies include long-QT syndrome (LQTS), Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia, cardiac conduction disease and sinus node dysfunction (Priori & Napolitano, 2006.

Recent successes have been reported using human iPSCS as models for human heart disease. Two groups generated human iPSCS lines from heart disease patients that showed a disease phenotype in culture. Carvajal-Vergara et al. (2010) produced cardiomyocytes from two human iPSCs lines derived from two patients with a heterozygous T468M

substitution in *PTPN11* (protein tyrosine phosphate, non-receptor type 11), which leads to Leopard syndrome, offering novel insights into signalling pathways related to this disease.
Another study by Moretti et al. (2010) investigated an ion channel mutation in patients with LQT1. LQTS is a life-threatening congenital condition characterized by cardiac arrhythmias and sudden cardiac death. These patients have a *KCNQ1* gene mutation that results in an elongated QT-interval. The *KCNQ1* gene encodes *I*Ks, the ion channel controlling the slow component of the delayed rectifier K+ current, which is partially responsible for the repolarising phase of the action potential. The authors were able to recapitulate this electrophysiological phenotype in culture by using a whole-cell patch-clamp electrophysiology method after creating human iPSCs lines from two patients in a single family with an R190Q missense mutation in *KCNQ1*. Using a similar method, Itzhaki et al. *(*2011) developed a patient/disease-specific human iPSCs line from a patient with type-2 LQTS, which is due to the A614V missense mutation in the *KCNH2* gene, coaxing these iPSCs to differentiate into the cardiac lineage. The LQTS human iPSC-derived cardiac-tissue was used as a model to evaluate the potency of existing and novel pharmacological agents that may either aggravate (potassium-channel blockers) or ameliorate (calcium-channel blockers, K_{ATP}-channel openers and late sodium-channel blockers) the disease phenotype.

4. Pre-clinical studies: A step to the bedside

Initial animal experiments found that the transplantation of BM-SCs after AMI and ischaemic cardiomyopathy is associated with a reduction in scar size and improvements in the ejection fraction of the left ventricle (LVEF) and tissue perfusion (Dawn & Bolli, 2005). Other pre-clinical studies demonstrated an improvement in myocardial function after repair of the infarcted area through implantation of EPCs derived from peripheral blood, BM or umbilical cord blood (UCB) (Leor & Marbel, 2006; Perin & López, 2006).
MSCs have also been used in a model of chronic myocardial ischaemia in large animals. Twelve dogs that received intramyocardial injections of 100 million cells showed improved systolic function (by echocardiography) in comparison to controls, both at rest and under stress situations; in addition, the MSCs were able to transdifferentiate into endothelial cells and smooth muscle cells, enhancing vascularization (Perin et al., 2003). Skeletal myoblasts were also studied in the setting of chronic myocardial ischaemia, specifically to treat myocardial scarring, and were found to improve the LVEF and the adverse effects of ventricular remodelling, due to their high capacity to engraft in scarred myocardial segments (Leor et al., 1996).

5. To the bedside: Cardiac cell therapy

The rapid development of stem cell technology has raised hopes for novel and even revolutionary treatments for cardiac and other disorders with tissue damage, awakening the keen interest of the pharmaceutical industry. These expectations are largely based on the 30-year history of successful BM-HSC transplantations in patients with blood diseases and cancer (Thomas et al., 1959; Shizuru et al., 2005). However, few stem cell therapies have been widely accepted by the medical community, and all of these use tissue-specific stem cells. These include BM or UCB stem cell transplantation to treat blood diseases or restore the blood system after cancer treatments, skin stem cell therapies for burns and limbal stem cells for corneal replacement, among others. In each case, stem cells repair the same tissue as that from which they are derived.

Cardiac cell therapy has been attempted in more than 1000 patients worldwide, and the results of the first meta-analysis recently became available. The interest in the use of adult stem cells in cardiac regeneration began after the demonstration by Orlic et al. (2001a) that murine BM-derived cells regenerated heart muscle after direct injection at the site of the previously induced myocardial infarction. These promising results generated significant interest in its clinical application, with the launch of numerous randomized clinical trials on the efficacy of cell therapy in patients with coronary disease, including 37 trials in Europe alone. The interpretation of their results should consider the effectiveness of the cell transplantation method, the delivery time and the cell type used. In this section, the NIH-supported ClinicalTrials website (ClinicalTrials.gov) was searched for closed and ongoing clinical trials in the field. Keywords included "heart", "myocardial infarction", "heart failure" and "cell therapy" (Table 3).

5.1 Bone marrow-derived stem cells

The TOPCARE-AMI study provided the first major clinical report on the transplantation of BM stem cells after myocardial infarction (Leistner et al., 2011). Patients were randomized to receive BM-derived mononuclear cells (29 patients) or EPCs (30 patients) by intracoronary infusion. Both groups showed improved LVEF at 4 months and reduced infarct size at 12 months, but there was no randomized control group with which to compare results. More recently, in a sub-group of the same patients, the authors observed a significant increase in LVEF (by cardiac MRI) and a reduction in infarct size (by delayed enhancement MRI). Interestingly, the migratory capacity of the infused cells proved to be the most important predictor of myocardial remodelling (Britten et al., 2003).

Some trials failed to show a significant increase in cardiac function, whereas others evidenced improvements comparable with those obtained by established treatment regimens (Reffelmann & Kloner, 2009). A range of results has been reported, from a low to a 14% improvement in LVEF (Fernandez-Aviles et al., 2004; Perin & López, 2006; Stamm et al., 2007; Tatsumi et al., 2007).

Many clinical studies have evaluated the therapeutic potential of human BM-derived stem cells (e.g. MSCs or mononuclear cells) to improve cardiac function after myocardial infarction (Gonzales & Pedrazzini, 2009; Mathiasen et al., 2009; Wei et al., 2009). However, no evidence has been reported of cardiac regeneration through differentiation of implanted stem cells into cardiomyocytes and other cardiac cell lineages. Some studies, but not all, have reported beneficial effects on heart function and on symptoms (Janssens et al., 2006; Lunde et al., 2006; Schachinger et al., 2006; Meyert et al., 2006). However, it has been suggested that these benefits are short-term, and a five-year follow-up study found that treatment with BM cells did not achieve sustained improvements in heart function (Meyer et al., 2009). Nonetheless, according to the results of all of these studies, the therapeutic use of human BM stem cells appears to be safe.

A meta-analysis of 18 randomized and non-randomized clinical trials, including 999 patients with AMI and chronic ischaemia, reported encouraging results after the transplantation of BM-MSCs. In comparison to controls, the transplantation improved the LVEF by 5.40% ($P < 0.001$), reduced the post-AMI scar area by 5.49% ($P = 0.003$) and reduced the left ventricular end-systolic volume by 4.80 ml ($P = 0.006$). According to the results of this meta-analysis, BM-MSC therapy appears to be safe and can be used in large-scale randomized trials to assess its impact on cell therapies (Abdel-Latif et al., 2007).

Clinical trial identifier number	Trial name/ Sponsor	Expected enrolment	Acquisition Method/ treatment	Primary end point	Route of cell delivery	Further Comments
Cardiac Stem Cells (CSCs)						
NCT 00474461	SCIPIO	40	Harvested from RAA	LVEF	IC	Evaluation of the regeneration capacity of CSCs in non-viable myocardial segments in patients with ICM
NCT 00981006	ALCADIA	6	Clonally Cell isolation	Safety	IM	Stem Cell Therapy combined with bFGF release and using a gelatin Hydrogel Sheet during CABG
NCT 00893360	CADUCEUS	30	Harvested from biopsies	LVEF	IC	Evaluation of the regeneration capacity of CSCs
Skeletal Myoblasts (SMs)						
NCT 00526253	MARVEL	170	Unknown	LVEF	Catheter delivery system	Evaluation of Myocell™ effects
NCT 00102128	Genzyme	30	Harvested from the thigh muscle	LVEF	Unknown	Evaluation of the regeneration capacity of SM
NCT 00908622	PERCUTANEO	50	Harvested from biopsies	LVEF	Percutaneous	Cardiomyoplasty benefits with SMs in patients with old AMI.
Endothelial Progenitor Cells (EPCs)						
NCT 00936819	ENACT-AMI	100	Harvested from apheresis, One group transfected with eNOS	LVEF	IC into the IRA	Combined Cell and gene therapy

RAA: Right atrial appendage; IRA: Infarct Related Artery; ICM: Ischaemic Cardiomyopathy; bFGF: Basic Fibroblastic grow factor; CABG: Coronary Artery Bypass Graft; eNOS: human endothelial nitric oxide synthase

Table 3. Ongoing clinical trials using different autologous stem cells.

5.2 Mesenchymal stromal cells

Animal studies are ongoing to determine whether MSCs can be used to treat arthritis, non-healing bone fractures or spinal cord injuries, among other diseases. Although it is also possible that these or similar cells modulate the immune system in response to injury, their use as cardiomyocyte-like cells *via* transdifferentiation methods remains controversial. A major advance in this field was achieved by Behfar et al. (2010), whose results with MSCs using guided cardiopoiesis methods allowed this approach to be evaluated in preclinical studies. They obtained a complete cardiac phenotype by using an endoderm-like protein cocktail with hMSCs harvested from patients with coronary artery disease. The hMSCs were injected into the myocardium of nude infarcted mice and followed up for a year to assess functional and structural endpoints. The recombinant cardiogenic cocktail guidance secured the cardiopoietic phenotype across the patient cohort, achieving a superior functional and structural benefit in comparison to unguided counterparts, with no adverse side effects (Behfar et al., 2010).

However, there is evidence of a low retention of the infused cells in the area of interest, which would reduce the effectiveness of this approach. Current strategies include the use of hMSCs modified with viral vectors carrying genes that increase the affinity of these cells to the sites of cell damage, e.g., through activation of HIF-α factor, which is expressed in states of hypoxia (Xue et al., 2010).

5.3 Skeletal myoblasts

The promising experimental results achieved by Leor et al. (1996) with skeletal myoblasts in a chronic myocardial ischaemia model, described above in section 4, generated significant interest in their possible clinical application. The first clinical trial was conducted at Paris-Descartes University using autologous skeletal myoblasts isolated from muscle biopsies from patients with severe heart failure, expanded *ex vivo* and then transplanted. The clinical status and ejection fraction of the treated patients improved over time, with a strikingly low incidence of hospitalizations for heart failure (0.13/patient-years). Moreover, the risk of arrhythmia could be controlled by medical therapy and/or on-request automatic cardiac defibrillator implantation (Menasche et al., 2001).

Therapy with skeletal myoblast transplantation was tested in the MAGIC trial (Myoblast Autologous Grafting in Ischaemic Cardiomyopathy). An injection of stem cells or culture medium was randomly administered to patients and, although initial fears of serious arrhythmias did not materialize, the results showed no significant benefit from the stem cell treatment.

The ambiguous results of clinical trials reflect differences due to the diversity of cell types and administration routes used. The timing of the assessment of ventricular function post-transplantation may also explain some discrepancies in the results. Several of the benefits achieved by this therapy appear to be transient (Meyer et al, 2009), consistent with the findings of similar studies conducted in animals (van Laake et al., 2008).

5.4 iPSCs technology

The need for deeper knowledge of iPSCs is acknowledged by the scientific community, but only one clinical trial is using this cell type, in an attempt to induce pluripotent stem cells from cell cultures of skin biopsies (Royan Institute, Iran, CI. NCT00953693). This trial is related to hepatic regeneration. The strategy is basically the same for all research purposes:

after isolation of fibroblasts from the patient`s skin biopsy, the cells are transfected after 3-4 weeks with the four factors used by Yamanaka. Transfected cells are isolated by morphological selection in order to generate allogeneic hiPSCs (Seifinejad et al., 2010). In the field of heart disease, basic research includes aspects such as the generation of cardiomyocytes derived from human ES/iPS cells from post-AMI heart failure patients (Itzhaki et al, 2011). These results will be important for drug discovery screening in relation to QT prolongation and cardiac cell transplantation therapy.

6. Funding research through industry: Development of patents in cell therapy

Improved understanding of the development of the human heart has led to proposals for strategies that emulate the cardiogenic programme, which are currently being tested in pre-clinical and clinical studies. There are an increasing number of patents in this area as well as on the establishment of cell lines. A patent operates as a *quid pro quo*: the patent owner obtains the exclusive right to make, use and sell an invention in exchange for its public disclosure. Some scientists believe that patents harm the research environment by increasing secrecy and costs. Others consider that the regenerative medicine sector requires access to funding and resources for the protracted procedures required, including not only the research and clinical trials but also the legal issues that must be addressed with respect to patent protection for regenerative medicine-related intellectual property. Interestingly, regenerative medicine may change the conventional reliance on patents and their finite exclusivity, which is a truly major issue for the pharmacy industry. Patent registers in the USA show a growing number of patents for the treatment of CVDs using stem cells, representing a validation of the therapeutic potential of this approach. In Europe, there is a slight trend towards the creation of patents related to methods for isolating cell populations with cardiopoietic potential and for preparing enrichment culture media, which are in pre-clinical stages of application.

A search of the sources "Patent Lens" of the organization "Initiative for Open Innovation (IOI)", "Patentscope" of the World Intellectual Property Organization (WIPO), and the "Invenes", "Esp@cenet", "Free patents online" and "Latipat" databases of the *Spanish Patents Office*, shows that patent applications to commercialise cell therapies for CVDs centre on three main topics: i) methods to deliver stem cells to the damaged myocardium, ii) methods to isolate and differentiate autologous cells harvested from patients, and, finally, iii) viral vectors for gene delivery.

6.1 Achieving the cardiac cell phenotype: Producing stem cell- derived cardiomyocytes

Given the difficulty of isolating and culturing heart cells, many studies have focused on developing methods to induce the differentiation of stem cells into the myocardial phenotype. For this purpose, MSCs are the most widely used cells in the patents and clinical trials reviewed, followed by HSCs, but knowledge of the existence of cardiac progenitor populations has made these the object of the most recent studies. One example is the development by Chien et al. (2011) (Patent application US 2011/0003327) of methods to identify and isolate atrial progenitors and, in some cases, atrial progenitors that are positive for Islet 1 (Isl1) and sarcolipin, which are growth factors responsible for the negative regulation of second heart field progenitor cell differentiation (Buckingham et al., 2005).

Another invention of this team relates to methods for isolating cardiovascular stem cells, placing cell populations in contact with agents reactive to Isl 1, Nkx2.5 and Flk-l and separating reactive from non-reactive cells (Chien & Chang 2008; Patent application WO 2008/054819 A2).
The use of MSCs from different sources is reflected in the patent of Büscher et al. (2010) (US patent application 20100304477), who characterized a novel population of human cardiac adipose tissue-derived adult stem cells from the epicardial area. These cells are isolated by their surface marker profile and their gene and protein expression of different cardiac muscle cell components, including the cardiac factors GATA-4 and Nkx2.5, the sarcomere components beta-myosin heavy chain and α-actinin, and the regulators of electrochemical connection and intracellular calcium distribution, connexin-43 (Cx43) and SERCA-2, respectively.

6.2 How can stem cells be guided to the damaged myocardium?

Numerous techniques have been developed for this purpose, including the use of growth factors and molecules that deliver and mobilize stem cells to the injured site. This paracrine effect has been described in MSCs and also includes the activation of different mechanisms against cell damage. In this respect, granulocyte colony-stimulating factor (G-CSF) has proven useful alone or in combination with the stem cell factor (SCF) in the mobilization of BM stem cells. The pre-AMI mobilization of primitive BMCs by the administration of G-CSF and SCF (US Patent 7220407) results in a significant degree of tissue regeneration at the ischaemic site (Orlic et al., 2001b). It is also contemplated that human G-CSF therapy may be used in a similar manner along with other agents commonly used for the treatment of AMI in conjunction with reperfusion.
In the field of gene therapy, several methods have been developed for treating patients with CVDs, including heart disease and peripheral vascular disease. The preferred methods involve the *in vivo* delivery to the myocardium or peripheral ischaemic tissue of genes encoding angiogenic proteins or peptides by the introduction of a vector containing the gene into a blood vessel supplying the heart or into peripheral ischaemic tissue. The patent "Techniques and compositions for treating cardiovascular disease by in vivo gene delivery" (Hammond et al., 2003; US Patent Application 20030148968), describes a method that employs a replication-deficient adenovirus as vector. By injecting the viral vector stock with angiogenic protein- or peptide-encoding genes deeply into the lumen of one or both coronary arteries or grafts, it is possible to locally transfect an adequate number of cells, especially cardiac myocytes, into the affected myocardium. This methodology maximizes the therapeutic efficacy of gene transfer and minimizes undesirable angiogenesis at extracardiac sites and the possibility of an inflammatory response to viral proteins.

7. Clinical translation

The translation of research findings to the clinical setting can be a complex process. This discipline, based on interventional epidemiology, aims to increase the throughput of findings from the basic or early phase of clinical research in ageing and to use them to drive innovation in healthcare settings. The success of clinical translation depends on the capacity of scientists, clinicians, regulators and other experts to share their expertise with one other and on their ability to respond adequately to negative or positive clinical outcomes.

In the field of CVD, considerable efforts have been made to establish feedback between research groups and clinical practice. Universities and hospitals have developed clinical translation units in which stem cell-related issues are focused on the utility of plasma biomarkers of acute brain injury to improve the early diagnosis of acute stroke and on the prevalence and specific causes of ventricular repolarisation abnormalities in older patients undergoing psychoactive drug treatment, among others.

7.1 Cell therapy

As reported above, human cell-based medicine is proving increasingly attractive to the pharmaceutical and medical equipment industries, opening up the way for the self-funding needed for scientific development and the implementation of clinical trials. This is of particular importance at a time of intense pressure to reduce public health costs.

Progress has been made in developing protocols for obtaining and isolating stem cells, in establishing legislation that specifies the parameters of good manufacturing practice for large-scale production, and in accumulating pre-clinical data that validating the effects of this therapy. As a result, several types of stem/progenitor cells have been proposed for use in regenerative medicine, leading to the consideration of stem cells as pharmaceuticals.

Large pharmaceutical companies apply stem cell-based technologies and other proprietary methods in the area of regenerative medicine to bring patient-specific therapies from the laboratory to the bedside. This is the case of products being developed by Advanced Cell Technology, Bioheart Inc. and Cardio3 Bioscience companies (Figure 3).

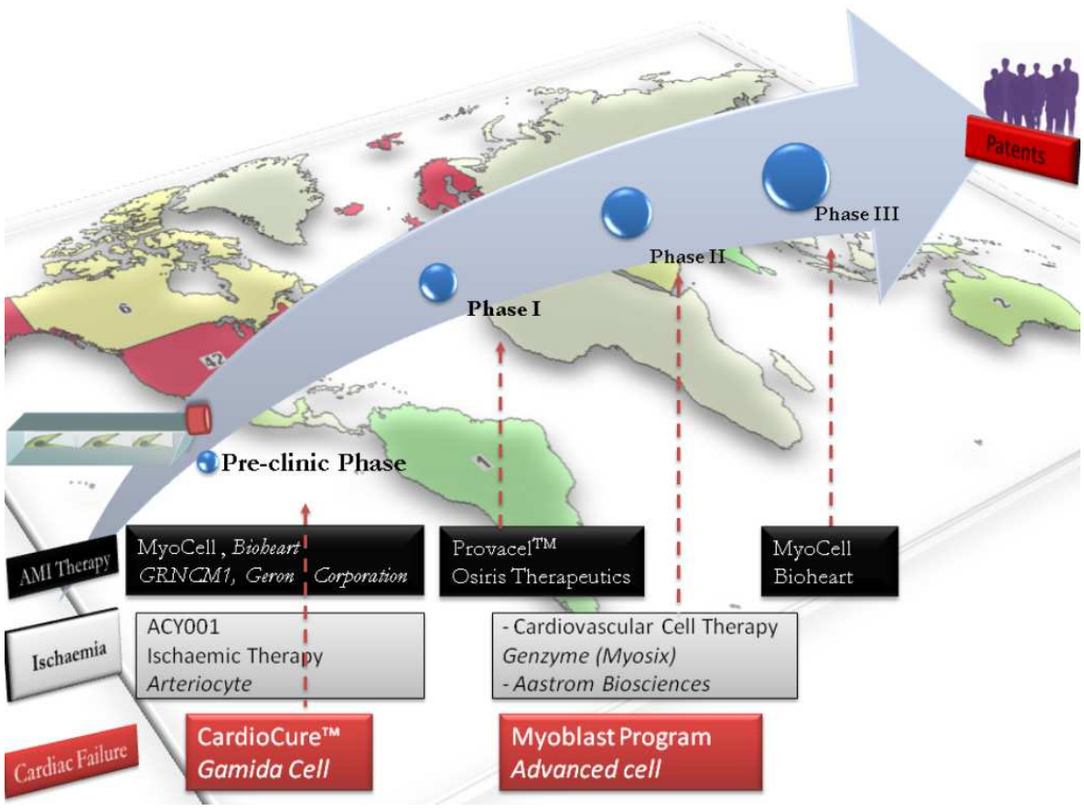

Fig. 3. Examples of products in development for cell therapy in diseases related to the cardiovascular system.

We highlight the initiatives taken by some U.S. drug companies, who have begun to market cell therapies after successful clinical trials. One example is Athersys Inc., whose MultiStem cell therapy has a broad potential to treat diseases such as AMI, stroke, and inflammatory bowel disease. MultiStem promotes tissue repair through the induction of therapeutic factors produced in response to signs of inflammation and tissue damage. Likewise, the Bioheart company is now marketing MyoCell as a result of the MARVEL trial, a randomized, double-blind, placebo-controlled, multicenter, phase II/III trial involving 330 patients in North America and Europe. MyoCell uses muscle tissue samples from the patient, isolating and expanding stem cells and then transplanting them by endoventricular injection into the patient's scar tissue (MyoStar ™).

7.2 Scaffolding: The development and translation of the tissue-engineered vascular graft

The post-mitotic and myogenic nature of myocardial tissue hampers the *in vitro* isolation and maintenance of cardiomyocytes. *In vitro* dedifferentiation is evidenced by the loss of sarcomeric distribution, observed in the distribution pattern of protein alpha actinin, troponin and connexin 43. These proteins are essential for muscle contraction. The same difficulty arises with the generation of cardiomyocytes *in vitro*, demonstrating that an effective approach for cardiac regeneration must include both specialized cells in a three-dimensional support or scaffold that provides the physical properties necessary for contraction. However, because of the heterogeneous population residing in the myocardium, attempts have focused on the generation of vascular replacements for larger vessels such as arteries. The utilisation by Dr. Taylor's group of a decellularisation method in mouse heart yielded encouraging findings in relation to the generation of bio-artificial hearts. They decellularised the hearts by coronary perfusion with detergents, preserving the underlying extracellular matrix and producing an acellular, perfusable vascular architecture with competent acellular valves and intact chamber geometry. They mimicked the composition of cardiac cells by reseeding these constructs with cardiac or endothelial cells. The group maintained eight constructs for up to 28 days by coronary perfusion in a bioreactor that simulated cardiac physiology, observing macroscopic contractions by day 4. By day 8, under physiological load and electrical stimulation, the constructs generate a pump function equivalent to around 2% of adult or 25% of 16-week foetal heart function in a modified working heart preparation (Ott et al., 2008).

Research by Dr. Christopher Breuer and Dr. Toshiharu Shinoka on tissue engineered vascular grafts (TEVG) is in the process of clinical translation for congenital cardiovascular diseases. In the United States, around 1 in 100 infants is expected to have heart defects, i.e., around 36,000 infants per year. Ten percent of these cases—over 3,600 infants—result in death. TVEGs are biodegradable synthetic scaffolds made of the same material as absorbable sutures and seeded with the individual's own cells. The scaffold degrades by hydrolysis, eventually leaving only the living vessel in the patient. Bone marrow aspiration is used to harvest the cells, inserting the needle through the cortex of the spinal bone and marrow. The marrow is drawn up and separated by density centrifugation, yielding BMCs that are directly seeded onto the scaffold by pipetting. The seeded scaffold is then incubated in the patient's plasma for two hours to attach cells to the scaffold. The graft is then ready for implantation. The whole process takes only a few hours, allowing a viable TEVG to be obtained on the same day as the cell harvesting (Nuti, 2010).

8. Conclusion

There have been numerous clinical trials of cell therapies for CVDs, and several problems have been identified. Questions that remain to be addressed in translational research from animals to humans concern the cell type, preparation time, delivery, cell number and optimization. The measurement of results must also be resolved, including the efficacy assessment method and length of follow-up. Nevertheless, the potential therapeutic contribution of adult stem cells is enormous. hESCs have been widely studied in relation to heart disease, but their use in cell therapy has been limited, in part due to ethically-based restrictions on research with this cell type in some countries and the lack of adequate funding.

With regard to iPSCs, the most important and immediate advances have been in relation to drug discovery, in the context of an urgent need for new approaches to meet the increasing incidence of myocardial infarction and heart failure in ageing populations. The number of novel drugs entering the market for heart disease is decreasing, whereas investment by the pharmaceutical industry in this area continues to increase, underlining the need for patents to safeguard the intellectual property of research groups and provide them with sufficient financial support to run clinical trials.

Criteria established by international scientific organizations such as the European Society of Cardiology and the American Society of Cardiology must be followed by researchers who address these issues. The correct design of future clinical trials is of particular importance before clinical stem cell therapy can be widely applied. In this regard, the International Society of Stem Cell Research (ISSCR, 2011) is taking the exceptional step of developing guidelines for patients, researchers and physicians, establishing safety procedures and providing patients worldwide with information to meet their concerns and describe their legal rights.

9. Acknowledgment

This work was supported in part by grants from the Instituto de Salud Carlos III (Fondo de Investigación Sanitaria, grant number PI10/02295), the Consejería de Salud (Junta de Andalucía, grant number PI-0384/2008), and the Consejería de Economía, Innovación y Ciencia (Junta de Andalucía, excellence project number CTS-6568).

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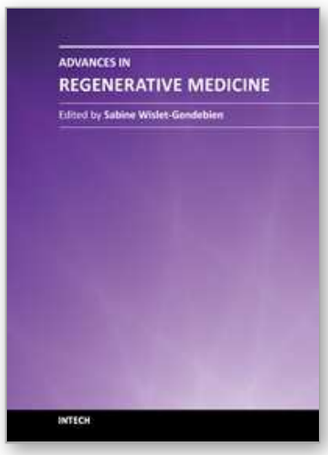

Advances in Regenerative Medicine
Edited by Dr Sabine Wislet-Gendebien

ISBN 978-953-307-732-1
Hard cover, 404 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Antonia Aránega, Milán Bustamante, Juan Antonio Marchal, Macarena Perán, Elena López, Pablo Álvarez, Fernando Rodríguez-Serrano and Esmeralda Carrillo (2011). Therapeutic Approaches in Regenerative Medicine of Cardiovascular Diseases: From Bench to Bedside, Advances in Regenerative Medicine, Dr Sabine Wislet-Gendebien (Ed.), ISBN: 978-953-307-732-1, InTech, Available from: http://www.intechopen.com/books/advances-in-regenerative-medicine/therapeutic-approaches-in-regenerative-medicine-of-cardiovascular-diseases-from-bench-to-bedside

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