

New Perspectives to Repair A Broken Heart

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Abstract: The aim of cardiac cell therapy is to restore at least in part the functionality of the diseased or injured myocardium by the use of stem/progenitor cells. Recent clinical trials have shown the safety of cardiac cell therapy and encouraging efficacy results. A surprisingly wide range of non-myogenic cell types improves ventricular function, suggesting that benefits may result in part from mechanisms that are distinct from true myocardial regeneration. While clinical trials explore cells derived from skeletal muscle and bone marrow, basic researchers are investigating sources of new cardiomyogenic cells, such as resident myocardial progenitors and embryonic stem cells. In this commentary we briefly review the evolution of cell-based cardiac repair, some progress that has been made toward this goal, and future perspectives in the regeneration of cardiac tissue.

Key Words: Cardiac cell therapy, heart failure, stem cells, cardiac regeneration, cardiac stem cells.

INTRODUCTION

Congestive heart failure and coronary artery disease are the leading causes of morbidity and mortality in western society, despite recent advances in medical and device-based therapies. Limited strategies are available to address and counteract the central pathophysiologic process underlying the development of heart failure, which is the loss of cardiomyocytes, permanently compromising myocardial contractile function [1-3]. The injured heart heals by scar formation rather than by cardiomyocyte regeneration. Heart regeneration seems to be too inefficient to repair the extensive myocardial injury that occurs in human disease. Heart transplantation is currently the last chance for end-stage heart failure, but it is hampered by severe shortage of donor organs and rejection. Cell-based therapies are a promising alternative, given the basic assumption that left ventricular dysfunction is largely due to the loss of a critical number of cardiomyocytes and therefore it could be partly reversed by implantation of new contractile cells into the post-infarction scar. During the last few years cellular therapy for the diseased heart, using a variety of different cells, has shown encouraging results on cardiac function in animal models of heart ischemia, even without clear cardiovascular differentiation of the transplanted cells and without clear corresponding results in the first clinical trials.

In fact, while in the field of skin [4], cartilage [5], bone [6], adipose tissue [7, 8], and cornea [9], regenerative medicine (including cell therapy) is already well established and

used clinically, therapeutic cardiac regeneration has to overcome some hurdles to be clinically feasible. We still need to develop efficient methods to selectively induce differentiation, to ensure cell yield, and to create transplantable tissue. The potential of cardiac cell therapy depends on the outcome of basic research and its application to the clinic.

In this review, a critical update will be presented on basic and clinical problems challenged by cardiac regenerative medicine, in particular by cell therapy. Furthermore, new perspectives on cardiac stem cells biology and their clinical deliverables will be discussed.

CELLS EMPLOYED FOR EXPERIMENTAL CARDIAC REGENERATION

The difficulty in regenerating damaged myocardial tissue has led researchers to explore the application of different stem cell types as possible sources for cell therapy, including embryonic stem cells (ESCs), cardiac stem cells (CSCs) that naturally reside within the heart, myoblasts (muscle stem cells), adult bone marrow (BM) derived cells, mesenchymal stem cells, endothelial progenitor cells, and umbilical cord blood cells. All have been tested in mouse or rat models, while some of them in large animal models as well, such as pigs, and in human clinical trials [10-14] (Table 1).

In the last five years the number of scientific publications concerning the experimental use of stem/progenitor cells for cardiac pathologies has grown up in a linear trend [15], with a slight decrease after 2006. This trend inversion reflects difficulties in finding adequate solutions to the many hurdles to be overcome for the clinical translation of a safe and effective cell therapy.

Many well-known problems exist and warrant further studies: the difficulty of inducing adult stem cells to cross

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Table 1. Stem Cell Populations Employed in Clinical Trials: Phenotype and *In Vivo* Results in Animal Models

Stem Cell Population	Phenotype	<i>In Vivo</i> Results	Controversial Issues	Ref.
Hematopoietic Stem Cells	Lin ⁻ / c-kit ⁺	+ CMs + SMCs + ECs ↑ LV function	Massive regeneration independent of cell fusion.	[99] [100]
	CD34 ⁺ / c-kit ⁺ / Sca1 ⁺	+ CMs ? SMCs + ECs ? LV function	Very low % of donor-derived cells.	[101]
	Lin ⁻ / c-kit ⁺ or Lin ⁻ / c-kit ⁻ / Sca1 ⁺	- CMs - SMCs - ECs ? LV function	Transplanted cells express hematopoietic markers.	[102] [103]
Mesenchymal Stem Cells	CD45 ⁻ / CD105 ⁺ / CD73 ⁺ / CD90 ⁺	+ CMs + SMCs + ECs ↑ LV function	Transdifferentiation.	[104] [105] [106] [107]
	CD34 ⁺ / CD45 ⁻ / CD29 ⁺ / CD44 ⁺	+/? CMs ? SMCs + ECs ↑ LV function	Immature CM phenotype.	[108] [109]
Endothelial Progenitor Cells	CD34 ⁺ (G-CSF-mobilized)	- CMs + SMCs + ECs ↑ LV function	Release of protective GFs.	[110] [111]
		+ CMs + SMCs + ECs ↑ LV function	Vasculogenesis and cardiomyogenesis.	[112]
Skeletal Myoblasts	No N-cadherin. No connexin43. Resistant to ischemia.	- CMs - SMCs - ECs ↑ LV function	Prevent LV remodelling (?) but induce arrhythmias.	[113] [114]

CMs: cardiomyocytes; SMCs: smooth muscle cells; ECs: endothelial cells; G-CSF: granulocyte-colony stimulating factor; LV: left ventricle; +: differentiate in; -: do not differentiate in; ↑: improvement.

their lineage boundaries (skeletal myoblasts and BM cells do not appear to transdifferentiate into cardiomyocytes); the commitment degree, the survival and the long-term retention of the injected cells and their properties (affecting adequate engraftment, proliferation, migration, differentiation and functional integration with the host); the timing and delivery strategies, also related to the specific heart pathology and to the patient to be treated.

In this contest, the paper of Zeisberg [16], showing that cardiac fibrosis is associated with the emergence of fibro-

blasts originating from endothelial cells, could be considered a milestone because it addresses a process important for the critical evaluation of the long-term fate of any injected cell. In this study, in fact, TGF- β release and endothelial-to-mesenchymal transition have been suggested to contribute to the persistence of fibrosis, while BMP-7 was proposed as an inhibitor of the process, and therefore as a valuable therapeutic tool. The same mechanism however could have a role in the temporary functional effect elicited by almost all kinds of injected cells, particularly those employed in clinical trials.

In other words, the cells to be used need to harbor a strong intrinsic and spontaneous potential of cardiac commitment, otherwise new scar will be added over the old one.

As previously suggested, the most appropriate cells for replacing dead cardiomyocytes appear to be cardiomyocytes of fetal or embryonic origin, since they can integrate functionally with resident myocardial cells [17]. This view is supported by experiments showing that transplanted fetal cardiac cells successfully engrafted into myocardial scars, connected with their host neighbors and improved function [18]. Moreover, in a recent study, Caspi [19] has shown that grafting of human ESC-derived cardiomyocytes into the normal and infarcted heart did not result in the generation of teratomas, as it occurs using undifferentiated ES cells. Instead the engrafted cardiomyocytes were shown to survive, proliferate, and integrate with host cardiac tissue [19]. However cardiomyocytes can not differentiate into coronary vessels, which obviously are also necessary for cardiac repair. Furthermore, their availability and scalability, in addition to immunological and ethical constraints, limit the use of fetal or embryonic tissue for any clinical application. Embryonic-like stem cells can be derived from neonatal and adult spermatogonial stem cells [20]. These cells have cardiac differentiation potential and could be used for autologous regenerative strategies without ethical and immunological problems [21]. Further studies are necessary to characterize these cells and to evaluate their behavior.

Another possible approach in cardiac repair concerns the use of mesothelial cells (MCs). MCs have an important role in the serosal homeostasis and repair after damage, and in preventing fibrous adhesion formation. Although MCs are of mesodermal origin, they show properties of both epithelial and mesenchymal phenotypes, displaying some degree of plasticity. MCs produce many cytokines and growth factors which can regulate inflammatory responses and stimulate tissue repair [22]. MCs also have the capacity to produce *in vitro* a variety of extracellular matrix macromolecules and to regulate their turnover [22].

Due to their multiple features and their endothelial properties, MCs are also used in tissue engineering and have been recently proposed as a cell source for cardiac repair. When transplanted into the damaged myocardium, MCs induced neoangiogenesis in the infarct scar and preserved heart function [23].

However, future studies warrant to fully understand their potential role and benefits for cardiac cell therapy.

Remarkable advances are also being made in generating pluripotent embryonic-like stem cells from somatic cells by viral expression of specific ESC-related genes and by somatic cell nuclear transfer [24].

The ideal cell to be transplanted for cardiac regeneration should probably be intermediate between a highly undifferentiated phenotype (ESCs) and a terminally differentiated cardiomyocyte. It should be characterized by a defined proliferative potential in the host without induction of immune reaction, by cardiac commitment and capacity to develop gap-junctions with the host cells, and should preferably be

resistant to ischemia, in order to avoid massive cell death and apoptosis, that are currently among the biggest hurdles for cell therapy translation.

With these premises, it seems obvious that the best cells to replace lost cardiomyocytes may be cells derived from the heart itself.

CARDIAC STEM CELLS AND CARDIAC REGENERATION

Emerging evidence suggests that several populations of stem/progenitor cells are present in the heart. A true cardiac stem/progenitor cell could be the ideal cell to repair a broken heart. However extensive basic research has yet to be performed to better understand the biology of these cells, that will foster the development of cell-based therapies for heart disease.

Evidence and Controversies on Mammalian Heart Regeneration

Typically, adult cardiac myocytes do not reenter the cell cycle when exposed to growth signals and further increase in cardiac mass during adulthood is achieved through hypertrophy. In contrast to most adult cardiomyocytes, fetal cardiomyocytes do proliferate. After birth, cardiomyocytes become binucleated, and withdraw from the cell cycle. Therefore adult mammalian cardiomyocytes have very limited potential for self renewal; the mammalian heart has historically been viewed as a terminally differentiated organ.

However the following observations lead to suggest that the mammalian heart has some potential intrinsic capacity to regenerate:

- The presence of myocytes undergoing mitosis and cytokinesis has been demonstrated in human hearts, especially under pathologic conditions (myocardial infarction, late cardiac failure) [25].
- Resident CSCs have been identified in the adult heart of humans and other mammal species (Table 2). These cells are self-renewing, clonogenic and multipotent, being able to differentiate into cardiomyocytes, smooth muscle and endothelial cells.
- From a very elegant genetic fate-mapping study [26], an indirect evidence has been obtained that an undefined population of stem/progenitor cells could replace adult mammalian cardiomyocytes after injury. In this study the authors used double transgenic mice, in which tamoxifen-inducible Cre-recombinase was under the control of the MHC promoter, and a reporter cassette under the actin promoter. The reporter gene was lacZ flanked by lox-sites, that would allow to switch to a GFP-reporter with induction of the Cre-recombinase. After a 4-OH-tamoxifen pulse, GFP was expressed through the inducible Cre-Lox system only in differentiated MHC-expressing cells, resulting in ~80% of GFP+ cardiomyocytes. After myocardial infarction (MI) or pressure overload, the percentage of GFP+ cardiomyocytes significantly decreased, indicating that a putative stem/precursor cells, had refreshed the cardiomyocyte pool.

Table 2. Resident Cardiac Stem Cells

Main Isolation Marker/Phenotype	SP Phenotype (Hoechst 33342 Efflux)			c-Kit		Sca-1	CSp Phenotype		SSEA-1	Isl-1	cKit/CD34		
	mouse	mouse	mouse CD31	rat	human (Clones)	mouse	mouse/human	human	rat	neonatal rat	mouse		
SSEA-1									+				
Oct-4		-							+				
Isl-1									+/-	+			
cKit (CD117)	-	low	-	-	+	+	-	+	+/-	+/-	-	-	+
Sca-1 (Ly 6)	-	high	+	+			+	+	-	+/-	-		+/-
MDR1(Abc1,Pgp)									-				+
Abcg2(MXR1,BCRP)		+							-	+/-			
CD90 (Thy-1)	-							+	+/-				+/-
CD133 (prominin)									-				
CD105 (endoglin)								+	+				+/-
CD34	-	low	-	-	-	-	-	+	+/-				+
CD45 (LCA)		low	-	-	-	-	-		-	-			-
CD31 (PECAM-1)		-	-	+			+	+/-	+/-	-			-
VEGFR2 (KDR,Flk-1)								+		+			
Blood lineage markers					-		-		-				
Cardiac transcription factors		+	+		+	-	+	+	+	+/-	+	+/-	+/-
<i>In vitro</i> differentiation method	-	Cc	Cc	-	DM	DM	5-Aza	Sp Cc	Cc	DM Cc	Cc	-	-
References	[34]	[35]	[36]	[37]	[115]		[39]	[44, 45]	[45]	[42]	[41]		[43]

+/-: subset; Cc: co-culture; DM: differentiating media; 5-Aza: 5-azacytidine; Sp: spontaneous. CSp: cardiosphere; SP: side population.

These studies indicate that, at least after injury, the heart has some limited capacity to regenerate and suggest that resident cardiac stem cells are involved in this process.

In the Hsieh study [26] it has been shown that in mice up to one year the percentage of GFP+ cardiomyocytes (that is the percentage of undifferentiated myocytes at the time of pulsing) remained unchanged, indicating no detectable replacement of cardiomyocytes by progenitor cells during normal aging. This observation supports the traditional view that cardiomyocytes are very long-living in the adult heart, and it is against the suggestion that the heart is a self-renewing organ in which myocyte regeneration occurs throughout the organism lifespan [27, 28].

However by following the ¹⁴C-dating of cardiac DNA, based on inadvertent pulse-labeling from atmospheric

nuclear tests, it was demonstrated both in pre- and post-atomic bomb born patients that, although the majority of cardiomyocytes are present from early development, their turnover is occurring during adulthood, at least until the third decade of life [29]. If cardiac regeneration occurs in the normal heart, the lack of detectable replacement of cardiomyocytes by progenitor cells during aging in the study by Hsieh could be explained (provided that these discrepancies could not be attributed to technical or methodological artifacts) assuming that cardiomyocytes are able to divide. Although it is generally accepted that cardiomyocytes lose their replicative capacity shortly after birth, it seems likely that the renewal of myocardial tissue during adult life might occur thanks to the ability of some cardiomyocytes to resume DNA synthesis and mitosis. This hypothesis is consistent with the observation that differentiated mononuclear cardio

myocytes have some proliferative potential and reenter the cell cycle [30]. Adult cardiomyocytes of lower vertebrates (Anuria, Urodela) proliferate considerably [31]. Zebrafish and newts hearts regenerate after injury with little or no scar formation. In newts regeneration occurs through dedifferentiation of specialized cells in blastema cells that redifferentiate afterwards into parenchymal cells [32]. In zebrafish, regeneration is supported by formation of blastema and by a subpopulation of epicardial cells [33]. Conversely the mammalian heart repairs itself poorly after infarction and regenerates poorly after injury.

CSCs Discovery

The discovery that the heart is not a terminally differentiated organ, but has a partial capacity to regenerate, gave a tremendous input in the last few years to the study of CSCs biology and increasing attention has been focused on the finding that the heart contains a reservoir of stem and progenitor cells [34-45]. These cells are positive for various stem/progenitor cell markers (c-Kit, Sca-1, Isl-1) and have Side Population (SP) properties. Although the relationship between the various CSCs and progenitor cells described awaits clarification (Table 2), nevertheless, they promise new therapeutic strategies for cardiac repair based on their regenerative potential. In fact, their presence into the heart, the frequent co-expression of early cardiac progenitor transcription factors, and the capability for *ex vivo* and *in vivo* differentiation towards the cardiac lineages offer the promise of enhanced cardiogenicity compared to other non-cardiac cell sources.

A well-known review by Garry and Olson [46] emphasizes a message shared by three recent studies [47-49], providing evidence for the existence during early development of cardiac progenitor cells that have the potential to differentiate in the three major cell types of the heart: myocytes, smooth muscle and endothelial cells. Beside the importance of each individual study, they raise still unresolved questions (relationship between the different progenitors described, variables in defining the differentiation pathway, markers and so on), particularly those related to the methods employed.

Due to the limited knowledge concerning the regulatory networks of proliferation, self renewal and lineage differentiation of cardiac progenitor cells, these studies tell us what is possible in our labs, but not necessarily what actually happens *in vivo* during development and disease. In other words, it is debatable to claim that one specific isolated cell is "the" only cardiac stem cell.

Types of Resident Cardiac Stem and Progenitor Cells

Different methods have been used to isolate CSCs from the heart, based on:

The Ability to Efflux Hoechst Dye (Side Population)

SP cells, which have the ability to efflux Hoechst dye, a process dependent on the expression of MDR1, Abcg2 or a comparable ABC transporter, have been identified in the developing, but also in the adult heart of mice [34, 35]. These cells are rare and their ability to differentiate into con-

tracting cardiac myocytes or to contribute to functional repair of damaged heart muscle has not been extensively evaluated yet. Among cardiac SP cells, the greatest potential for cardiomyogenic differentiation is restricted to cells negative for CD31 and positive for stem cell antigen 1 (Sca1) expression [36].

The Presence of Cell-Surface Stem Cell Markers (Either c-kit or Sca-1)

In adult rat hearts a distinct population of CSCs has been isolated. These relatively small and primitive cells are negative for blood lineage markers and positive for c-kit, the receptor for the stem cell factor. These cells are self-renewing, clonogenic, and multipotent, giving rise to cardiomyocytes, smooth muscle and endothelial cells. When injected into the border zone of a 5-hour-old infarct in adult rats, these CSCs differentiated into newly formed myocardium, including cardiomyocytes, capillaries and arterioles in the infarcted area [37]. When delivered in a clinically relevant manner by intracoronary injection after a 90-minute coronary occlusion, followed by 4-hour reperfusion in rats, CSCs reduced the infarct, attenuated myocardial remodeling and ameliorated cardiac dysfunction 5 weeks after MI [38].

In the non-myocyte fraction of mice hearts, a resident population of CSCs, characterized by the expression of Sca-1, but lacking blood lineage markers or c-kit, has been reported [39, 40]. Even though these cells do not spontaneously differentiate *in vitro*, when exposed to 5-azacytidine [39] or oxytocin [40] a small fraction of them demonstrates biochemical evidence of cardiac myocyte differentiation. After intravenous injection in mice subjected to myocardial ischemia-reperfusion, Sca-1+ cells homed to the heart and differentiated into cardiomyocytes, in part because of fusion with host cells [39].

Expression of the Islet-1 Gene (isI1+ Cells)

Another population of CSCs, characterized by the expression of the LIM-homeodomain transcription factor islet-1 (isI1+ cells), has been recently described. These cells reside in the mature heart of newborn mice, rats and humans, and they are negative for c-kit or Sca-1, but express the early cardiac transcription factors Nkx2.5 and GATA4. When co-cultured with cardiomyocytes, isI1+ cells convert very efficiently to mature cardiomyocytes without cell fusion [41]. However, their low abundance and mere presence in very young animals and humans prevent their short-term application. A challenge regarding this cell population is whether isI1+ cells are present in the adult heart and whether they can be isolated, expanded, induced to differentiate or if they are able to regenerate damaged myocardium *in vivo*.

Expression of the Stage-Specific Embryonic Antigen (SSEA-1+ Cells)

Recently uncommitted cardiac precursor cells (UPCs) have been identified in the heart of adult rats through a typical embryonic antigen, SSEA-1, that is expressed early in heart development [42]. SSEA-1+ cells isolated from adult rats differ from neonatal cells because they do not express cardiac specific transcription factors (Nkx2.5, GATA4). This

suggests that only uncommitted stem cells persist in the adult heart. Adult UPCs in co-culture with cardiac-derived mesenchymal cells can differentiate in mature cardiomyocytes, endothelial cells, and smooth muscle cells through multiple stages, in which the cells co-express markers such as Oct4, Flk-1 or Sca-1 together with SSEA-1. Then, once committed, they finally express cardiac transcription factors such as Nkx2.5, GATA4 and Isl-1. Beating colonies are obtained by culturing UPCs in differentiating media or in co-culture with neonatal cardiomyocytes. UPCs improved ventricular function when injected in infarcted hearts, and SSEA-1+ cells are capable of forming new cardiomyocytes and endothelial cells in the infarct area [42].

Epicardially Derived Cells (EPDCs)

Limana [43] first identified cardiac progenitor cells outside of the previously described “niche” in human and mouse epicardium, which can migrate into the sub-epicardium where they generate a population of EPDCs. The authors described two distinct populations of myocardial and vascular precursor cells, expressing c-Kit or CD34 respectively [43]. Although c-Kit+ and CD34+ cells are negative for haematopoietic and endothelial markers (CD45-, CD31-), both populations have the ability to acquire the endothelial phenotype, as demonstrated by their capacity to uptake Ac-LDL-DiI, which is considered to be a specific marker for endothelial cells. A subset of c-Kit+ and CD34+ cells express cardiac transcription factors (Nkx2.5, GATA4). Their differentiation potential has been demonstrated only for c-Kit+ cells when a MI was induced in the mouse in the presence of an intact pericardial cavity.

Cardiospheres: A Novel Method to Isolate CSCs

Messina first debugged a methodology to isolate CSCs by primary culture of murine hearts or human heart tissue fragments derived from open heart surgery [44] (Fig. (1)). After few weeks of culture, a heterogeneous population of cells shed spontaneously from tissue fragments. Cells surrounding the “explants” express stem cell markers (c-Kit, Sca-1), endothelial progenitor cell antigens/markers (KDR, CD31) and the gap-junction protein Connexin43 (Cx43). They are clonogenic, and multipotent, giving rise spontane-

ously to cardiomyocytes, smooth muscle and endothelial cells. In suspension culture they form spherical multicellular clusters dubbed “cardiospheres” (CSps). CSps consist of proliferating c-Kit-positive cells primarily in their core, and of differentiating cells expressing cardiac (MHC, NKx2.5, TnI, ANP), vascular (SMA), endothelial (vWF) and mesenchymal (CD105, CD90) cell markers on their periphery [44, 45].

The 3D-structure of CSps probably resembles the actual architecture of cardiac tissue, in terms of intra- and extra-cellular interactions, which may have a fundamental role in regulating cellular functions such as proliferation, differentiation and angiogenesis. In fact, differently from CSCs isolated with other methods, CSps can lead to terminally differentiated cardiomyocytes, as demonstrated by their ability to beat, either spontaneously (embryonic and neonatal murine CSps) or after co-culture with rat neonatal cardiomyocytes (pig, human, adult mice CSps). Moreover it seems likely that the protective microenvironment of the CSps could enable stem cells (or cells with stem-cell function) to retain their potency, as in the “niche” [50].

It has not yet been determined which are the antigens that could unequivocally identify CSp-forming cells. Experiments carried out using transgenic mice suggest that c-kit positive cells have a major role in CSps formation and growth. Although the intracardiac origin of CSCs and adult myocytes is generally accepted, GFP+ CSps were obtained 3-4 weeks after heart injury in lethally irradiated mice, whose BM had been repopulated by haematopoietic cells from a syngeneic c-kit/GFP animal [51]. This suggests that, at least under these conditions, BM cells could replenish the cardiac c-kit+ stem cell pool, acquiring functional characteristics of specialized CSCs, such as the capability to form CSps.

In order to increase cell yield, CSps can be expanded as adherent monolayers on fibronectin. Between 10 and 100 million cells can be obtained from a single biopsy in about 45 days [45]. Transplantation of human CSp-derived cells (CDCs) in the peri-infarct zone of SCID mice led to engraftment, migration, proliferation and multilineage differentiation, resulting in the replacement of dead tissue with new

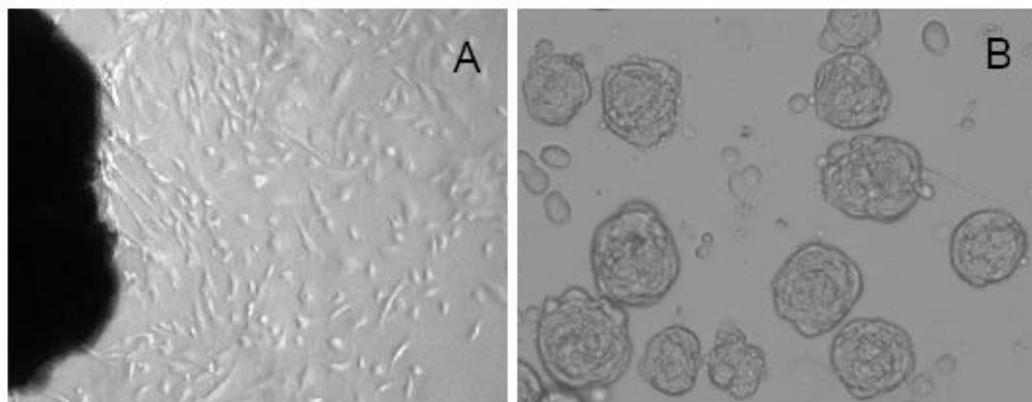


Fig. (1). Cardiosphere isolation steps. Explants are plated as primary cultures, and cells migrate from tissue fragments (A). CSp-forming cells are collected from the explant and then they spontaneously form floating CSps (B).

myocardium and in functional improvement after 3 weeks [45].

There are many characteristics that make CSps different from other kinds of CSCs populations which have been isolated so far, in particular from those isolated by selection of a single putative stem cell marker.

- The CSp isolation protocol is based on intrinsic and spontaneous functional properties of the cells, which are the ability to migrate from tissue explants and to grow as 3D-structures, resembling somehow a niche-like micro-tissue.
- CSps can lead to terminal differentiation: embryonic and neonatal murine CSps can start beating spontaneously, while other CSCs need the addition of differentiating agents and/or co-culture with cardiomyocytes in order to do so.
- CSps represent a heterogeneous population, including c-kit positive stem-like cells and other cell types expressing mesenchymal markers: these latter, beside endothelial and fibroblast phenotypes, might also have a feeder-layer role, allowing the activation of multiple mechanisms for cardiac repair beyond myocyte formation (paracrine pro-survival effects, angiogenesis, mobilization of endogenous CSCs).

TISSUE ENGINEERING

In the past few years, cardiac tissue engineering (CTE) has emerged as a new approach to regenerate hearts damaged by myocardial infarction. CTE combines the use of biomaterials with cell biology and medicine. Several tissue engineering approaches are being explored for cardiac repair. In this review we briefly present an overview on the most promising materials and interesting approaches employed for CTE (for a complete review see Zamaretti [52], Zimmerman [53] and Jawad [54]).

The ideal biomaterial for CTE, either synthetic or natural, should enhance cell attachment, growth and differentiation. Moreover it should promote revascularization and have electro-mechanical properties, allowing good integration with heart tissue. The most common approach for CTE is to seed cardiomyocytes onto pre-formed porous scaffolds made of polylactic acid, polyglycolic acid and their co-polymer polylactic-co-glycolic acid [55]. Other synthetic materials used in CTE are 1,3-trimethylene carbonate, D-L-lactide and their co-polymers [56], or the co-polymers ϵ -caprolactone-co-L-lactide [57, 58].

A very exciting approach is the cell-sheeting method that involves cell culturing on temperature-responsive culture dishes. These dishes are made of poly-(N-isopropylacrylamide) (PIPAAm), which at 37°C is hydrophobic and promotes cell attachment. When temperature decreases to 32°C the polymer becomes hydrophilic and the cell sheet detaches from the plate. Shimizu *et al.* demonstrated that the detaching cell layers can be collected and, when layered one on top of the another, the sheets fuse, beat synchronously and become vascularized *in vivo* [59-61].

Several groups are currently working with scaffold materials made of natural polymers such as collagen, gelatin and alginate. Zimmermann *et al.* used a combination of liquid collagen type I and matrigel with neonatal cardiac myocytes cultured in persistent mechanical load [62, 63]. Other approaches utilized a pre-formed, commercially available, tri-dimensional collagen matrix, obtaining promising results in terms of cardiac remodelling after implantation in an *in vivo* model of MI [64-70].

Chachques *et al.* reported the safety and feasibility of treatment with BMCs seeded onto a 3D collagen-type I-matrix in patients with left ventricular post-ischemic myocardial scar. The cell-seeded collagen matrix prevents cardiac remodeling and increases the thickness of the infarct scar with viable tissue, improving cardiac function [69, 71].

Another method that has emerged in the last years is an *in situ* CTE approach that involves injection of a mixture of biomaterials and cells. An example of this type of approach is the fibrin glue scaffold, consisting of two components: the first one consists of concentrated fibrinogen and aprotinin, and the second contains thrombin, CaCl₂ and cells. When delivered *in vivo* with a Duploject applicator, that provides simultaneous mixing and delivery, the two components form a solid gel matrix that supports neo-angiogenesis, cell survival and decrease in infarct size [72, 73]. Other natural biomaterials used with this kind of approach are collagen I [74-77] and matrigel [74, 77, 78]. Finally a similar approach was applied by Davis *et al.* that injected "self assembling" peptide nanofibers into the myocardium for recruitment of progenitor cells and promotion of vasculogenesis [79].

Clinical Trials

Although experimental studies with BM stem cells in animal models gave rise to controversial results in terms of cardiac trans-differentiation and regeneration, they have been employed in several human clinical trials that took place in the last few years, and most of them are undergoing clinical evaluation (Table 3). BM is, at present, the most frequently used source of cells for clinical cardiac repair. In most clinical trials BM-derived cells or peripheral blood progenitor cells were transplanted both in patients with acute MI and chronic ischemic cardiomyopathy. Cell number, administration route and time of delivery also differed among studies. BM stem cells have been delivered in three ways, depending on the disease and pathological status of the patients: intracoronary cell delivery, catheter-based endocardial injection or direct intramyocardial implantation during open heart surgery [80-82].

All studies showed an excellent safety profile that was associated with a modest-to-low improvement in ventricular function, and only in few cases the injection of BMCs resulted in a consistent functional benefit (Table 4). Generally the advantage of intracoronary infusion is that cells migrate, *via* the microcirculation, to the perfused infarct region in a favourable environment for their survival, and this is a prerequisite for stable engraftment. On the other side, it limits their delivery only to the perfused regions and requires an increased number of cells to be administered. Moreover the

Table 3. Protocol Details of Some of the Completed Clinical Trials for Cardiac Cell Therapy

Study Name	N° of Patients	Cell type/ Treatment	Number of Cells (*10 ⁶)	Delivery Route	Infusion Time d= Days; m= Months; y= Years	Rand.	Follow-up (Months)	Main Endpoints
Strauer <i>et al.</i> [116]	10	BMMNCs	28	Intracoronary	5-9 d. post MI	-	3	Safety
TOPCARE-AMI [95, 96]	59	CPCs/BMCs	18-213	Intracoronary	4.9+/-1.5 d. post MI	+/-	4-12	Safety and feasibility
TOPCARE-CHD [117, 118]	23+34+ 35	CPCs/BMCs	22+/-11 CPCs 205+/-110 BMCs	Intracoronary	MI> 3 m.	+	3-6	LVEF
REPAIR-AMI [119, 120]	101+103	BMCs	50 ml BM aspirate	Intracoronary	3-7 d. post MI	+	4-12	LVEF
BOOST [97, 98]	30+30	BMMNCs	2.46	Intracoronary	4.8+/-1.3 d. post MI	+	6-18	LVEF
Fernandez-Aviles <i>et al.</i> [121]	20	BMMNCs	78 +/- 41	Intracoronary	13.5+/-5.5 d. post MI	-	6	Safety and feasibility
IACT study [122]	18	BMMNCs	6*15-22	Intracoronary	5 m.-8.5 y. post MI	-	3	
Janssens <i>et al.</i> [123]	33+34	BMMNCs	304+/-128	Intracoronary	2-3 d. post MI	+	4	LVEF
ASTAMI [124, 125]	50+50	BMMNCs	54-130 (inter-quartile range)	Intracoronary	4-8 d. post M.I	+	6	LVEF, LVEDV and infarct size.
Chen <i>et al.</i> [126]	34+35	MNCs	6ml*(8000-10000cells/ml)	Intracoronary	18 d. from PCI	+	3-6	LVEF
Katritsis <i>et al.</i> [127]	11+11	MSCs+EPCs	1-2	Intracoronary	242d.<MI <3y.	-	4	Scar size
Tse <i>et al.</i> [128]	8	BMMNCs	15 injection; 10 ⁷ cells/mL	Endomyocardial	severe I.H.D.	-	3	Safety and feasibility
Perin <i>et al.</i> [129]	14+7	BMMNCs	25.5+/- 6.3	Endomyocardial	end-stage I.H.D.	-	2-4	Safety
Perin <i>et al.</i> [130]	11+9	BMMNC	15*2	Endomyocardial	end-stage I.H.D.	-	2-6-12	Safety and efficacy
Fuchs <i>et al.</i> [131]	10	unfractionated ABM	2,4ml*(32.6+/-27.5) cells/ml	Endomyocardial	angina III-IV rev ischemia	-	3	Safety and feasibility
Stamm <i>et al.</i> [132, 133]	12	BMCs; AC133+	1.5	Open heart surgery (CAGB)	10 days <MI< 3 months	-	3-10	Safety and feasibility
Li <i>et al.</i> [134]	6+30	BMMNCs	50-100	Open heart surgery (CAGB)	concomitant with CAGB		1-12	Safety and feasibility
MAGIC [135, 136]	10+10+ 7	G-CSF mobilized PBSCs	1500+/-500	Intracoronary	MI>48 h or clinically stable CMI	+	6	Safety and feasibility
Erbs <i>et al.</i> [137, 138]	14	G-CSF mobilized CPCs	69+/-14	Intracoronary	after CTO	+	3	Safety and feasibility
Boyle <i>et al.</i> [139]	5	G-CSF mobilized CD34+	66.9+/-17.6	Intracoronary	CIHD	-	12	Safety and feasibility
Ozbaran <i>et al.</i> [140]	6	G-CSF mobilized PBSCs	13-80	Open heart surgery (CAGB)	Congestive heart failure	-	4	Safety and feasibility

Table 3. Count....

Study Name	N° of Patients	Cell type/ Treatment	Number of Cells (*10 ⁶)	Delivery Route	Infusion Time d= Days; m= Months; y= Years	Rand.	Follow-up (Months)	Main Endpoints
Pompilio <i>et al.</i> [141]	4	G-CSF mobilized AC133+	13-200	Open heart surgery (CAGB)	10 days <MI< 3 months	-	3-6	Safety and feasibility
Losordo <i>et al.</i> [142]	24	G-CSF mobilized CD34+	0.05 - 0.1 - 0.5 cells/kg b.w.	Endomyocardial	Angina III-IV	+	3-6	Safety and feasibility
Briguori <i>et al.</i> [143]	10	CD34+	90-140	Intramyocardial	Angina III-IV	-	1-3-6-12	Safety and feasibility
FIRSTLINE-AMI [83, 84]	25+25	G-CSF 10µg/kg b.w. 6 days	None	Subcutaneous	85+/- 30 min. after PCI	+	4-12	Safety and efficacy
G-CSF STEMI [85-87]	39+39	G-CSF 10µg/kg b.w. 6 days	None	Subcutaneous	1-2 d. after acute ST elevation	+	6	change in systolic wall thickening
REVIVAL-2 [88]	56-58	G-CSF 10µg/kg b.w. 5 days	None	Subcutaneous	5 d. after MI	+	4 to 6	LV size and LVEF
Pagani <i>et al.</i> [90]	5	myoblasts	300	Open heart surgery	concomitant with LVAD implantation.	-		Immunohistochemical staining
Menaschè <i>et al.</i> [89]	10	myoblasts	871	Open heart surgery	concomitant with CAGB	-	10.9 +/-4.5	Safety and feasibility
Herreros <i>et al.</i> [144, 145]	12+14	myoblasts	200	Open heart surgery	MI>4 w. concomitant with CAGB.	-	3-12	Safety and feasibility
Siminiak <i>et al.</i> [146]	10	myoblasts	0.4-50	Open heart surgery	MI>3 m. concomitant with CAGB.	-	12	Safety, feasibility and efficacy
Dib <i>et al.</i> [147]	30	myoblasts	10-30-100-300	Open heart surgery	concomitant with LVAD or CAGB	-	4 years	Safety and feasibility
Smits <i>et al.</i> [148]	5	myoblasts	196+/-105	Endomyocardial	MI> 4 w.	-	3-6	Safety and feasibility
Ince <i>et al.</i> [149]	6+6	myoblasts	210±150	Endomyocardial	MI> 4 w.	-	12	Safety and feasibility
POZNAN [150]	10	myoblasts	<100	Percutaneous transcatheter venous	MI> 3 m.	-	12	Safety and feasibility

BMNC: bone marrow mononuclear cell; CPC: circulating progenitor cell; EPC: endothelial progenitor cell; MSC: mesenchymal stem cell; G-CSF: granulocyte colony stimulating factor; PBSC: peripheral blood stem cell; IHD: ischemic heart disease; CIHD: chronic ischemic heart disease; LVAD: left ventricular assist device; CAGB: coronary artery bypass grafting; CTO: recanalization of chronic coronary total occlusion; PCI: percutaneous coronary intervention; Rand: randomization. CMI: chronic myocardial infarction.

outcome after intracoronary route may be negatively affected by cell uptake from the circulation or by embolic risk. Another approach is to deliver cells directly into the scar tissue by catheter-based injection or during open heart surgery by multiple direct injections. However these methodologies are limited by the risk of ventricular perforation and they may induce arrhythmias.

A different approach is that of enhancing CSCs and progenitors activation and inducing their migration toward the infarct region by treatment with G-CSF. The rationale of this methodology derives from multiple studies in animal models (Table 1) and requires, for successful results, the reperfusion of the ischemic site. Briefly if we analyze different random-

ized trials with G-CSF treatment after acute myocardial infarction, discrepancies arise again about the real beneficial effect on cardiac function [83-88]. Unfortunately, despite many relevant clinical trials have used BM stem cells or their mobilization, once again relevant doubts still remain about the therapeutic efficacy of these cells for cardiac regeneration (Table 4).

Autologous skeletal myoblasts are another potential source for cardiac repair. Skeletal myoblasts (SMs), or satellite cells, are the reservoir of regenerative cells for the skeletal muscle. SMs feature several attractive characteristics as therapeutic cells, including the autologous origin, which overcomes immunologic and ethical concerns. They can be

Table 4. Improvement in Ventricular Function in Some Clinical Trials with Control Group

Study	Treatment/ Follow-up	LVEF%			LVESV			LVEDV		
		Baseline	4	12	baseline	4	12	baseline	4	12
REPAIR-AMI [119, 120]	months	Baseline	4	12	baseline	4	12	baseline	4	12
	control	46.9± 10.4	49.9± 13.0	Clinical outcome	75± 32	80± 45	Clinical outcome	139± 46ml	153± 57ml	Clinical outcome
	BMCs	48.3± 9.2	53.8± 10.2		67± 26	67± 30		128± 38ml	141± 43ml	
BOOST [97, 98]	months	Baseline	6	18	baseline	6	18	baseline	6	18
	control	51.3± 9.3	52.0± 12.4	54.4±13.0	40.6±16.9 ml/m ²	42.6± 23.5 ml/m ²	41.0±24.7 ml/m ²	81.4± 16.9 ml/m ²	84.9± 21.9 ml/m ²	85.0± 24.2 ml/m ²
	BMCs	50.0± 10.0	56.7± 12.5	55.9±14.7	43.0±14.7 ml/m ²	42.4± 23.9 ml/m ²	42.5±25.0 ml/m ²	84.2± 17.2 ml/m ²	91.7± 26.0 ml/m ²	90.3± 26.5 ml/m ²
Janssens et al. [123]	months	Baseline	4		baseline	4		baseline	4	
	control	46.9± 8.2	49.1± 10.7		44.4± 12.3 ml/m ²	45.0±17.9 ml/m ²		83.1± 14.7 ml/m ²	85.9± 19.5 ml/m ²	
	BMCs	48.5± 7.2	51.8± 8.8		42.2± 10.5 ml/m ²	41.0 ±15.5 ml/m ²		81.2 ± 14.0 ml/m ²	84.1± 20.8 ml/m ²	
TOPCARE-CHD [117, 118] * = crossover to another group CTR→ BMC/CPC CPCs→ BMCs BMCs→ CPCs	months	Baseline	3	6 *	baseline	3	6	baseline	3	6
	control	43± 13	42± 13	Improve- ment mainly in BMCs treated.	55± 36 ml/m ²	55± 32 ml/m ²	-	90± 38 ml/m ²	87± 33 ml/m ²	-
	CPCs	39± 10	39± 10		62± 31 ml/m ²	60± 26 ml/m ²	-	96± 34 ml/m ²	93± 30 ml/m ²	-
	BMCs	41± 11	43± 10		49± 26 ml/m ²	47± 26 ml/m ²	-	79± 29 ml/m ²	79± 29 ml/m ²	-
ASTAMI [124, 125]	months	Baseline	6		Baseline	6		Baseline	6	
	control	46.9± 9.6	48.8± 10.7		-	-		132.0± 34.6	142.7± 45.2	
	BMCs	45± 9.4	49.0± 9.5		-	-		136.1± 30.5	145.0± 42.0	
Chen et al. [126]	months	Baseline	3	6	baseline	3	6	baseline	3	6
	control	48± 10	53± 18	54± 5	No difference between groups	88± 19ml	-	No difference between groups	162± 27ml	-
	MSCs	49± 9	67± 11	67± 3		63± 20ml			136± 31ml	
Katritsis et al. [127]	months	Baseline	4		baseline	4		baseline	4	
	control	46.18± 7.37	47.72± 6.46		62.3 ± 29.4	59.23± 21.35		121.2± 35.2	115.32± 26.75	
	MSCs+ EPCs	41.5± 7.7	43.45± 6.65		69.1 ± 24.0	65.27± 26.49		128.0± 36.0	119.45 ± 36.98	
Perin et al.[129]	months	baseline	2		baseline	2		baseline	2	
	control	36± 11.73	31.85± 7.55		89.42± 26.23 cc	98.85± 20.52 cc		135.71± 26.08 cc	145± 27.62 cc	
	BMCs	30± 5.56	35.5± 7.85		146.78± 53.46 cc	123.21± 47.88 cc		211.35± 76.89 cc	189.14± 67.54 cc	
FIRSTLINE-AMI [83, 84]	month	baseline	4	12	-	-	-	LVEDD		
								baseline	4	12
	control	47± 5	42± 4	45± 8	-	-	-	55± 4mm	59± 4mm	58± 4mm
G-CSF	48± 4	54± 8	56± 9	-	-	-	55± 3mm	54± 5mm	54± 5mm	

Table 4. Count....

Study	Treatment/ Follow-up	LVEF%		LVESV		LVEDV	
		Baseline	3	baseline	3	baseline	3
G-CSF STEMI [85-87]	months	Baseline	3	baseline	3	baseline	3
	control	44± 9	49.5± 12	87± 21ml	85± 44 ml	149± 32 ml	162± 52 ml
	G-CSF	4± 12	47± 12	83± 38 ml	79± 46 ml	140± 44 ml	145± 61 ml
REVIVAL-2 [88]	months	Baseline	4 to 6	Baseline	4 to 6	Baseline	4 to 6
	control	49.2± 8.7	51.2± 9.0	46.1± 13.7 ml/m ²	43.7± 17.5 ml/m ²	89.6± 17.5 ml/m ²	87.8± 21.9 ml/m ²
	G-CSF	51.3± 8.2	51.8± 7.7	46.1± 15.2 ml/m ²	45.5± 15.5 ml/m ²	93.3± 18.7 ml/m ²	92.4± 20.5 ml/m ²
Herreros <i>et al.</i> [144, 145]	months	Baseline	12	baseline	12	baseline	12
	control	33.4± 9.1%	38.6± 11	141± 68.3ml	125± 55.8ml	221± 101ml	223± 64.1ml
	myoblasts	35.6± 2.3%	55.1± 8.2%	99.1± 26.8ml	69.1± 15.8ml	175± 39.3ml	166.1± 30ml
Ince <i>et al.</i> [149]	months	Baseline	12	-	-	LVEDD	
						baseline	12
	control	24.7± 4.6	21.0± 4.0	-	-	68.3± 4.9 mm	72.3± 5.0 mm
	myoblast	24.3± 6.7	32.2± 10.2	-	-	67.5± 6.0 mm	65.2± 6.0 mm

BMCs: bone marrow cells; CPCs: circulating progenitor cells; MSCs: mesenchymal stem cells; LVEF: left ventricle ejection fraction; LVESV: left ventricle end-systolic volume; LVEDV: left ventricle end-diastolic volume; LVEDD: left ventricle end-diastolic diameter.

readily isolated from relatively small skeletal muscle biopsies and amplified in an undifferentiated state *in vitro*. Promising experimental data in animal models of heart failure have led to several clinical trials, since June 2000 [89]. Although clinical trials have shown cell survival, differentiation toward a skeletal phenotype [90, 91] and improvement of global cardiac function following myoblasts injection (Table 3), they have also raised the issue of potential arrhythmogenicity [92]. Myoblast grafts represent arrhythmogenic foci because their electromechanical integration is limited by their inability to form gap-junctions [93], and also by their dihydropyridine phenotype, which markedly differs from that of cardiomyocytes. Myoblasts transplantation results in isolated islands of skeletal muscle tissue, mostly surrounded by scar tissue, so re-entry circuits can be formed and cause arrhythmias. It has also been proposed that some transplanted myoblasts might fuse with cardiomyocytes, thereby generating heterogeneity in calcium-signalling at the graft-host interface [94].

Generally the safety of cardiac cell therapy is well established, as well as its beneficial effects in terms of neovascularization and ventricular remodelling. However, long-term follow-up in most trials has shown that conventional pharmacological therapy has often the same outcome of cellular therapy [95-98] which probably works through indirect mechanisms. For example, it might decrease the death rate of the existing myocytes, improve neoangiogenesis or positively affect ventricular remodelling. The details of the possible mechanisms are unknown, but they could involve growth factors and cytokines secreted by the injected cells.

The small improvement in ventricular function though, especially in the long-term follow-up, is probably due to the inability of the cells to actually form new cardiomyocytes. It is also important to notice that all the differences in the trials protocols (number of injected cells, reperfusion time between ischemia and therapy, etc.) make it difficult to directly compare them one to the other.

CONCLUDING REMARKS

Extensive basic and clinical research performed during the last few years on cardiac regeneration and on the use of cell therapy to repair a broken heart led to two new important emerging concepts.

First, a large number of animal studies and clinical trials with different adult stem cells suggest that improved physiological function does not necessarily mean myocardial regeneration. After transplantation of non-cardiac cells that do not appear to transdifferentiate into new beating cardiomyocytes, beneficial functional effects have been observed. The potential mechanisms leading to improvement are highly debated and might involve reduction in ventricular remodeling after MI and/or paracrine effects that stimulate cell survival and angiogenesis. Considering the experience from the published clinical trials with non-cardiac stem/progenitor cells, a step backward to basic research study needs to be performed to better understand the real potential of the cells we are handling. In this regard, extensive studies have yet to be performed to better understand and control the self-renewal and differentiation of these cells. Such knowledge could also be valuable to establish how to recruit CSCs al-

ready residing in the heart by stimulating their proliferation and differentiation.

The second concept is that several subpopulations of cardiac stem/progenitor cells reside within the adult heart. These cells are self-renewing, clonogenic and multipotent, being able to differentiate into cardiomyocytes, endothelial and smooth muscle cells both *in vitro* and *in vivo*. The relationship between the various cardiac stem/progenitor cells described awaits clarification through comprehensive characterization and correlation of their origin, maintenance, and inherent reparative potential. Even if unable of massive regeneration, nonetheless these cells seem to participate in some kind of spontaneous attempt of heart regeneration after myocardial injury. However their ability to replace dead cardiomyocytes during normal aging has been questioned by a recent genetic fate-mapping study [26].

Theoretically a true cardiac progenitor cell would be the ideal cell type for the repair of a broken heart. Stem cells derived from a patient's own heart represent an attractive source for cellular transplantation and myocardial regeneration therapy. They are easily obtained in sufficient number from biopsy specimens and are capable of proliferation and differentiation into functional cardiomyocytes and vascular cells. Additionally, autologous CSCs can be used without immunosuppression. The possibility of using cardiac biopsy specimens as a source for *ex vivo* isolation and expansion of CSCs was introduced for the first time by our group [44, 45]. The expansion *ex vivo* over a period of weeks is necessary to obtain a sufficient number of cells for experimental and clinical purposes. Human CSCs, in the features of CSps or CDCs, injected into the infarct border zone of SCID mice, promoted cardiomyocyte and vessels formation, and improvement in systolic function. Therefore, human CSps and CDCs represent attractive cell sources for cardiac repair, and display unique characteristics among CSCs. Because both c-kit-positive cells and cells that express mesenchymal markers are present in CSps, they have the potential ability to improve function through several mechanisms. Indeed, in addition to new cardiomyocyte formation, improvements in cardiac function and increased regeneration within the infarct area could be attributed to paracrine effects, increased mobilization of endogenous CSCs and formation of new blood vessels.

Future clinical trials that will use stem cells for myocardial repair must focus at least on three concerns that are related to the delivery of these cells: 1) safety, 2) cell tracking to their ultimate destination(s) and 3) cost-efficiency ratio. While cell tracking technologies allow researchers to follow their migration patterns, stem cells must safely target their destination(s) and be retained there for the required time to achieve benefit. To facilitate targeting and enable clinical use, stem cells must be delivered easily and efficiently to their sites of application. Finally, the ease and the cost necessary for cell isolation and preparation will also impact the transition to the clinic of cardiac cell therapy.

ACKNOWLEDGEMENTS

This study was supported by MIUR, by the "Istituto Pasteur – Fondazione Cenci Bolognetti", and by the Department

of Experimental Medicine, University "La Sapienza", Roma (Italy).

ABBREVIATIONS

ANP	=	Atrial natriuretic peptide
BM	=	Bone marrow
CDC	=	Cardiosphere derived cell
CM	=	Cardiomyocyte
CPC	=	Circulating progenitor cell
CSC	=	Cardiac stem cell
CSp	=	Cardiosphere
CTE	=	Cardiac tissue engineering
Cx43	=	Connexin 43
EC	=	Endothelial cell
EPC	=	Endothelial progenitor cell
EPDC	=	Epicardially derived cell
ESC	=	Embryonic stem cell
GCSF	=	Granulocyte colony stimulating factor
GFP	=	Green fluorescent protein
LV	=	Left ventricle
MC	=	Mesothelial cell
MHC	=	Myosin heavy chain
MI	=	Myocardial infarction
MSC	=	Mesenchymal stem cell
PBSC	=	Peripheral blood stem cell
Scal	=	Stem cell antigen 1
SM	=	Skeletal myoblast
SMA	=	Smooth muscle actin
SMC	=	Smooth muscle cell
SP	=	Side population
SSEA1	=	Stage specific embryonic antigen 1
TnI	=	Troponin I
UPC	=	Uncommitted precursor cell
vWF	=	von Willebrand factor

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