Engineering Cardiac Stem Cells for the Treatment of the Damaged Heart

Abstract

Heart disease remains one of the leading causes of mortality in humans. Current pharmacological treatment is only palliative, not curative; and the massive loss of cardiac tissue after acute myocardial infarction (AMI) is mainly substituted by a non-functional scar. Since there are not enough donors available for heart transplantation, new approaches have been explored, and the investigation efforts of many groups in the last decade have been directed towards the use of stem cells. Unfortunately, the stem cells used in most of the clinical trials, mainly bone marrow-derived stem cells (BMSC), have not shown a consistent and meaningful long-term benefit. Therefore, some recent clinical studies have included different type of cells derived from cardiac-tissue that still retain certain stem cell properties and, in contrast to BMSC, cardiomyogenic potential. On the other hand, cell reprogramming technologies have allowed the creation of novel cardiac cells, including multipotent cardiac stem cells (CSC) with similar features and potential to embryonic CSC. Here, we will review the main CSC populations that are being investigated in clinical trials and in preclinical models with a special focus on the CSC obtained through different cell reprogramming strategies and the recent tissue engineering approaches used to improve the retention, survival and function of CSC.

Keywords: Cardiac stem cells; Stem cell therapy; Myocardial infarction; Cardiac reprogramming; Tissue engineering

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Javier Linares[1#], Leyre Lopez-Muneta[1#], Felipe Prosper[1,2]* and Xonia Carvajal-Vergara[1]*

[1]*Cell Therapy Program, Foundation for Applied Medical Research, University of Navarra, Spain*

[2]*Cell Therapy Area, Clínica Universidad de Navarra, University of Navarra, Spain*

[#]*These authors contributed equally to this work*

***Corresponding author:** Felipe Prósper, Cell Therapy Area, Clínica Universidad de Navarra, Av. Pío XII 36, Pamplona 31008, Navarra, Spain, Email: fprosper@unav.es

Xonia Carvajal Vergara, Cell Therapy Program, Center for Applied Medical Research (CIMA), Av. Pío XII 55, Pamplona 31008, Navarra, Spain, Email: xcarvajal@unav.es

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Introduction

Acute myocardial infarction (AMI) is one of the main causes of mortality and morbidity in developed countries. Advances in medical and catheter-based therapy have improved the survival and prognosis of patients with AMI, nonetheless mortality remains as high as 13% and the 5-year mortality for patients with heart failure remains as high as 50% [1].

AMI is most often caused by the breakage of an atheroma plaque and the subsequent formation of a thrombus and occlusion of a coronary vessel, producing an acute reduction of blood supply to a portion of myocardium. Unfortunately, in adult mammals the resident cardiac cells are not able to regenerate heart tissue and restore efficiently the cardiac function in response to injury, thus, ischemia induces an irreversible damage leading to the death of cardiac tissue. It is calculated that a loss of approximately one billion cardiomyocytes occurs, which are replaced by a non-functional scar. This leads to a decrease in heart function, hypertrophy being the main compensation of the loss of cardiomyocytes, which eventually produces heart failure [2].

Currently the principal treatment for heart failure is pharmacological therapy, which is not curative but palliative. Heart transplant is the only option for most severe cases; however, it is very limited due to low availability of immunocompatible heart donors. In addition, transplanted patients must undergo a lifetime immunosuppressive therapy. To solve the lack of available hearts some alternatives have been developed in recent years, such as xenotransplantation [3], a procedure that will need to overcome significant challenges such acute rejection, cross-species infections and ethical issues.

Another alternative for the treatment of AMI and chronic heart failure is the use of cell-based therapy to improve cardiac function and, at best, regenerate damaged cardiac tissue.

Adult somatic stem cells can be isolated from different tissues and can be differentiated spontaneously *in vivo* in response to endogenous cues. Some adult stem cells have been studied in the treatment of AMI and chronic heart failure, such as bone marrow-derived stem cells (BMSC), adipose tissue-derived stem cells (ADSC), skeletal myoblasts and cardiac tissue-derived stem cells, BMSC being the most used cells in clinical trials. Unfortunately, the results from the studies using BMSC such as BOOST (Hannover Medical School, Identifier NCT00224536), REPAIR-AMI (A. M. Zeiher, Identifier NCT00279175) and TOPCARE-AMI (Johann Wolfgang Goethe University Hospital, Identifier NCT00289822) have been ambiguous and not conclusive. In C-CURE trial (Celyad, Identifier NCT00810238) an interesting approach was developed, which consists of guiding mesenchymal stem cells obtained from bone marrow towards a cardiopoietic stem cell phenotype. The main advantage of ADSC over BMSC is that larger amounts of cells can be obtained by liposuction. APOLLO trial (Cytori Therapeutics, Identifier NCT00442806) was performed in order to investigate the safety and feasibility of intracoronary infusion of ADSC in patients with AMI, however, no significant improvement was observed. All the clinical trials using this type of cells have been fully reviewed [4-6].

Cardiac stem/progenitor cells (CSC) have the potential to proliferate, self-renew and differentiate into the major cardiovascular lineages (cardiomyocytes, endothelial and smooth muscle cells); therefore, these cells may represent ideal candidates for cardiac regenerative therapy. However, under the term CSC many different cell types from different origins, expressing different surface markers and with distinct proliferative and differentiation potential have been included in the literature. Here, we will review the main CSC populations used to treat the injured heart in clinical and preclinical studies, including the CSC obtained through recent cell reprogramming strategies. The main cardiac tissue-derived stem cell populations currently used in clinical trials and the novel CSC obtained through different cell reprogramming approaches are summarized in Figure 1.

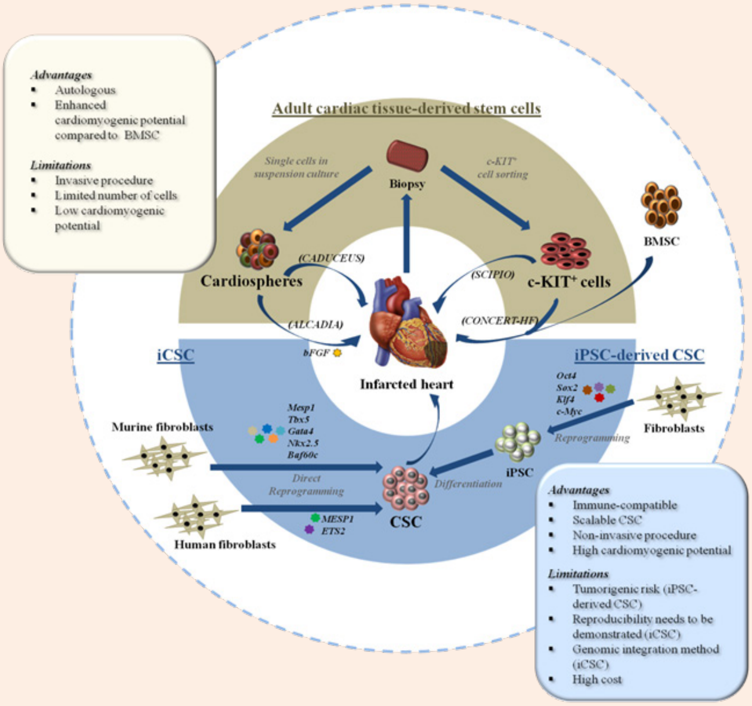

Figure 1: Schematic representation of adult stem cells derived from autologous cardiac tissue used in clinical trials (above, in beige colour) and CSC obtained through two different cell reprogramming approaches (below, in blue colour).

Adult Cardiac Tissue-Derived Stem Cells (Endogenous/ Resident Csc) in Preclinical and Clinical Studies

The adult mammalian heart has been generally considered a terminally differentiated organ. In contrast with this idea, some studies revealed that human cardiomyocytes are capable of renewal during adulthood, although the turnover rate is a controversial issue [7] New cardiomyocytes in the adult heart can derive from both the division of pre-existing cardiomyocytes and the activation of endogenous cardiac stem cells. Cardiac tissue-derived stem cells comprise different populations of cells distributed all over the adult heart. These cells show clonogenicity and multipotency, therefore they seem to be a great promise for cardiac repair. A variety of adult cardiac tissue-derived stem cells based on the expression of different markers have been reported: c-Kit+ cells, cardiosphere-derived cells, Sca-1+ cells, Isl1+cells, epicardium-derived cells, side population cells and cardiac colony-forming unit fibroblasts [8,9]. These populations have also been described in adult human heart except for Sca-1+ population cells, since Sca-1 human orthologue has not so far been identified.

Cardiac tissue-derived stem cells in preclinical studies

Stem cells isolated from heart tissue expressing the tyrosine kinase receptor c-Kit have shown to possess the potential to differentiate into the main cardiac lineages (cardiomyocytes, vascular smooth muscle and endothelial cells), self-renew and improve cardiac function [10]. However, the degree to which c-Kit-expressing progenitors generate cardiomyocytes is a controversial issue and recently several studies have elucidated with genetic cell mapping that resident c-Kit+ cells do not have a cardiac origin [11,12].

Cardiosphere-derived cells are a natural mixture of stromal, mesenchymal and progenitor cells obtained from an endomyocardial biopsy grown in suspension as clusters, known as cardiospheres [13]. Preclinical studies demonstrated that cardiosphere-derived cells are superior to BMSC or ADSC in terms of ischemic tissue preservation, anti-remodeling effects and functional benefits [14].

Isl1 is expressed in embryonic CSC during cardiomyogenesis

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[15], however, Isl1 do not serve as a marker of adult CSC since its expression is restricted to cells in the sinoatrial node and these cells are not recruited to the infarct zone in mouse models [16]. Side population cardiac progenitors are a subpopulation of cardiac tissue-derived stem cells that specifically express the ABCG2 gene and have significant cardiomyogenic potential *in vitro*. Side population cardiac progenitor cells can be isolated from the heart and show multipotency and regenerative potential in response to cardiac injury [17]; however, a significant fraction of this population originates from the bone marrow [9]. Epicardium derived cells are predominantly formed by mesothelial cells and dense connective tissue, and are known to play a crucial role in the development of the embryonic heart. Some preclinical studies have shown that these epicardium derived cells may get activated after AMI and they can be mobilized and promote neovascularization of the damaged heart suggesting that these epicardial cells may retain certain regenerative potential [9]. Another cardiac progenitor population named cardiac colony-forming unit fibroblasts (c-CFU-Fs) has been characterized by the expression of PDGFRα and identified in murine and human hearts. These PDGFRα+ cells have self-renewal potential and are multipotent *in vitro*. Some recent studies show that PDGFRα+ cells are able to differentiate into vascular cells, fibroblasts and to a less extent into cardiomyocyte-like cells [8,9].

Cardiac tissue-derived stem cells in clinical trials

The role of endogenous CSC in heart regeneration therapy has not been fully investigated in clinical studies. The only populations of autologous cardiac tissue-derived stem cells from human adult heart which are currently being used in clinical studies arec_KIT+ cells and cardiosphere-derived cells (Table 1).

Table 1: Clinical trials using CSC in ischemic heart disease.

Study Name	NCT Identifier (Sponsor)	Study Design	Number of Patients	Deliver Route/ Cell Type	Cell Dose	Follow-Up Period	Outcomes	Adverse Effects
Autologous cardiac tissue derived stem cells								
SCIPIO	NCT00474461 (University of Louisville)	Phase I, first-in-human, open-label, randomized controlled study	Treated=20; Controls=13	Intracoronary /c-KIT+ CSC	1 million cells	4 and 12 months	Increased LVEF (+13.7%) Decreased infarct size (-30.2%)	No major adverse cardiac events
CADUCEUS	NCT00893360 (Cedars-Sinai Medical Center)	Phase I, open-label, randomized controlled study	Treated=17; Controls=8	Intracoronary / Cardiosphere derived cells	12.5-25 million cells (dose escalation)	6 and 12 months	No changes in LVEF Incresed Viable myocardium (+22.6±9.4g Decreased scar size (-11.1%±4.6)	Seven-cell treated patients had serious adverse effects within 12 months of infusion
ALCADIA	NCT00981006 (Naofumi Takehara)	Phase I, open-label, single group assignment (non randomized)	Treated=6; No Controls	Intramyocardial /cardiosphere derived cells with controlled release of bFGF	0.5 millions cells/kg (patient body weight) and 200µg bFGF	6 and 12 months	Increased LVEF (+9-12%) Decreased scar size after 6months (-3.3%)	One major adverse cardiac effect (congestive heart failure)
CONCERT-HF	NCT02501811 (The University of Texas Health center, Houston)	Phase II, randomized, placebo-controlled	144 estimated enrolled patients (recruiting participants)	Transendocardial/ c-KIT+ CSC and /or autologous BMSC	5 million CSC and/or 150 million BMSC	6,12 and 24 months	N/A (in progress)	N/A (in progress)
Allogenic cardiac tissue derived stem cells								
ALLSTAR	NCT01458405 (Capricor Inc.)	Phase I/II, randomized, double blind, placebo-controlled	134 estimated enrolled patients	Intracoronary / Cardiosphere derived cells	25 million cells	6 and 12 months	N/A (in progress)	N/A (in progress)

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DYNAMIC	NCT02293603 (Capricor Inc.)	Phase I, randomized, double blind, placebo-controlled	42 patients	Intracoronary / Cardiosphere derived cells	37.5-75 million cells	6 and 12 months	Increased LVEF (+17.5%) in the short term (in progress)	No preliminary major adverse cardiac events (in progress)
CAREMI	NCT02439398 (Coretherapix)	Phase I/ II, first-in- human, randomized, double-blind, placebo-controlled	55 patients	Intracoronary / allogeneic human CSC	11, 22, 35 million cells (dose-escalation)	6 and 12 months	Safety	No preliminary major adverse cardiac events
Embryonic stem cells derived CSC								
ESCORT	NCT02057900 (Assistance Publique-Hopitaux de paris)	Phase I, open-label, single group assignment (non randomized)	6 estimated enrolled patients (recruiting participants)	Epicardial delivery/Human embryonic stem cell-derived SSEA1+Isl1+CSC in fibrin patch	4 million cells within a fibrin patch	3,6 and 12 months	Increased LVEF (+10%) in first clinical case (in progress)	No preliminary major adverse cardiac events (in progress)

N/A: Not Available; NCT: National Clinical Trial

Based on the preclinical results with c-Kit+ cells, SCIPIO trial (University of Louisville, Identifier NCT00474461) was carried out to analyze the feasibility, safety and efficacy of an intracoronary infusion of cardiac tissue-derived c-KIT+ cells into patients with sustained myocardial infarction. A significant increase in left ventricular ejection fraction (LVEF) was reported compared to baseline [18]. However, the integrity of certain data related to this study has been questioned [19]. CONCERT-HF (The University of Texas Health Science Center, Houston, Identifier NCT02501811) is a clinical trial designed to assess feasibility, safety and effect of autologous c-KIT+ cells and BMSC, individually or in combination, for the treatment of patients with ischemic cardiomyopathy.

In CADUCEUS trial (Cedars-Sinai Medical Center, Identifier NCT00893360), cardiosphere-derived cells were delivered in patients with recent AMI. No changes could be observed in LVEF in patients treated with cardiosphere-derived cells compared to patients treated with conventional therapy, however infarct size was significantly reduced in the cell treated group [20]. ALCADIA trial (NaofumiTakehara, Identifier NCT00981006) has demonstrated the safety and efficacy of transplantation of autologous human cardiosphere-derived cells with the controlled release of bFGF. Patients showed increased LVEF and decreased scar size after 6 months; however, the number of patients treated in ALCADIA trial is small (n=6) and there is no control group.

Allogeneic cells may offer many advantages in cardiac regenerative medicine regarding scalability and reproducibility. Allogeneic CSC could resolve some limitations relating to the age and health of the patient that can affect autologous cell transplantation. In this sense, ALLSTAR (Capricor Inc., Identifier NCT01458405) and DYNAMIC (Capricor Inc., Identifier NCT02293603) are ongoing clinical trials which expect to determine the safety and efficacy of allogeneic cardiosphere-derived cells for the treatment of myocardial infarction and dilated cardiomyopathy respectively. CAREMI trial (Coretherapix, Identifier NCT02439398) has demonstrated the safety and efficacy of intracoronary infusion of allogeneic human CSC in patients with AMI six months after treatment (Table 1).

Embryonic CSC

The heart derives from the anterior mesoderm which arises from the anterior primitive streak. Mesodermal pre-cardiac cells migrate toward the cephalic pole of the embryo to form the cardiogenic crescent or first heart field (FHF) that will form the primary linear heart tube. The second heart field (SHF) is a second population of CSC that is present at the medial and ventral parts of the FHF. The SHF is located within the pharyngeal mesoderm, and contributes to the development of the heart chambers [21].

The induction of cardiac mesoderm is controlled by critical signals including Nodal, Wnt and BMP4 in a dose- and time-dependent manner. The same signaling pathways can also induce the differentiation of embryonic stem cells into cardiac cells *in vitro* [22].

One clinical case report related to human ESC-derived CSC was carried out in 2015 [23]. ESC were committed toward a cardiac lineage by BMP2 and a FGF inhibitor, and the SSEA-1+ fraction (enriched in CSC) was sorted and embedded into a fibrin patch that was surgically delivered into the infarcted area. The feasibility and good tolerance of the procedure was demonstrated. The clinical use of these cells was encouraged by the observed effects on cardiac differentiation and improvement of cardiac function in rat and non-human primate models of myocardial infarction [24]. However, the number of transplanted cells which survived in the host tissue after transplantation was very low, and grafted cells were absent after 4 months in rat models. These observations suggest a paracrine mechanism of action for the enduring functional benefits, in accord with other studies that reveal similar functional recovery effects driven by CSC-derived extracellular vesicles in mice with chronic heart failure [25].

Reprogramming Strategies to Obtain CSC

Cell reprogramming approaches emerged as an alternative to obtain CSC a decade ago, circumventing the limited cardiomyogenic potential [11,12,26] of cardiac tissue-derived stem cells, and ethical concerns and immune rejection problems associated to the use of ESC. Specifically, CSC can be obtained by two different reprogramming strategies:

A. The reprogramming of somatic cells into induced pluripotent stem cells (iPSC) and the further differentiation into CSC, or

B. Direct reprogramming of somatic cells into induced CSC (iCSC).

iPSC-derived CSC

The group of Yamanaka demonstrated in 2007 that human somatic cells can be reprogrammed to iPSC by ectopic expression of the reprogramming transcription factors OCT4, SOX2, KLF4 and c-MYC (OSKM) [27]. iPSC, as ESC, have the potential to give rise to any cell type of the human body, and constitute an unlimited source of cells. Many laboratories worldwide have established iPSC from different tissues and diseases, demonstrating the high reproducibility of this technology. Moreover, genome integration-free iPSC can be established as a step toward safety and their clinical use [28].

The same procedures used in ESC have been used to differentiate iPSC into CSC. BMP, Activin and Wnt signalling contribute to the induction of mesendoderm in early differentiation stages, whereas the inhibition of these pathways is required for cardiac specification in late stages [29,30]. Multipotent human CSC can be isolated using reporters regulated under specific transcription factors such as Mesp1, Isl1 or Nkx2.5 [15,31,32], or the expression of surface markers such as SSEA1+, KDR+/PDGFR-α+ or GFRA2+ [29,33,34]. Taking into account the rapid transition of CSC from multipotency to commitment, the isolation of CSC is a real challenge. In order to solve this issue, several groups have used Wnt pathway modulators to promote pluripotent stem cell-derived CSC preservation and expansion [35-37], a crucial requisite for future clinical applications.

iCSC

Direct transdifferentiation of fibroblasts towards a diverse range of cell types has been already demonstrated [38]. Specifically, Islas et al. [39] achieved direct reprogramming of human fibroblasts into KDR+/Nkx2.5+ iCSC in 2012 by ectopic expression of ETS2 and MESP1 factors. However, these iCSC were not characterized in depth since these iCSC spontaneously differentiated into immature cardiomyocytes.

Very recently, Zhang et al. [40] and Lalit et al. [41] reported two different strategies for reprogramming adult mouse fibroblasts into highly expandable CSC [42].

The reprogramming approach described in 2011 by Ding laboratory [40] is based on transient expression of the four Yamanaka factors (OSKM) in combination with JAK inhibitor JI1 and BACS (BMP4, activin A, CHIR99021, and SU5402) to induce partial reprogramming of fibroblasts into CSC, and the later culture of these cells in BACS conditions to promote the maintenance and expansion of CSC. By this protocol, expandable CSC (Flk1+, PDGFRa+, Isl1+ and Nkx2.5+) could be derived from fibroblasts in two weeks.. They demonstrated that CSC were tripotent when differentiated under cardiomyocyte, endothelial and smooth muscle cell induction conditions, and the transplantation of CSC improved cardiac function in infarcted mice. Although this reprogramming approach was described as a direct conversion initially, it has been demonstrated that it generates a pluripotent intermediate state [43,44].

Lalit et al. [41] demonstrated that the ectopic expression of at least 5 cardiac factors (Mesp1, Tbx5, Gata4, Nkx2.5 and Baf60c), in combination with LIF (JAK/STAT activator) and BIO (a GSK3β inhibitor), can reprogram adult mouse fibroblasts from different tissues of origin (cardiac, lung and tail-tip) into proliferative and multipotent iCSC. This group used a Nkx2.5 cardiac reporter mouse model expressing enhanced yellow fluorescent protein (EYFP) crossed with a transgenic mouse expressing a reverse tetracycline transactivator (rtTA) to enable dox-inducible transgene expression. The generated iCSC were able to differentiate into cardiomyocytes, endothelial and smooth muscle cells *in vitro* and *in vivo*; however, the iCSC-derived cardiomyocytes only started contracting when co-cultured with mESC-derived cardiomyocytes, not spontaneously. The injection of the iCSC into the border zone of infarcted hearts in mice improved the survival of animals from 11% in control animals to 75%.

These methods have enabled the generation of billions of CSC without losing their differentiation potential, which is critical for clinical use.

CSC-Based Tissue Engineering

The biggest barrier current stem cell-based therapies face is the poor engraftment of the transplanted cells. To counteract the problems associated with low retention of transplanted cells, diverse biomaterials and bioengineering approaches have entered the research arena for optimizing therapeutic benefits with promising results. Tissue engineering methodologies that combine stem cells and different biomaterials (cell sheets, porous scaffolds, injectable hydrogels, cell surface engineering and microcapsules) have been reported to improve cardiac repair [45].

Multiple stem cell types have been bioengineered in order to improve cardiac regenerative therapy, but the effects on cardiac function have been modest [45,46]. The classic heart tissue engineering approach is the combination of cardiomyocytes with biomaterials to generate a beating cardiac tissue. One of the critical complications affecting cardiac tissue engineering is a low mechanical and electrical integration of the cells into the host myocardium. In this sense, the most important challenges of cardiac tissue engineering are the acquisition of mature cell phenotype to avoid arrhythmias (immature cardiomyocytes beat spontaneously) and the incorporation of a vascularized network to permit the survival of the transplanted cells into the ischemic host myocardium. Thus, the use of multipotent CSC for cardiac tissue engineering has attracted a great research interest.

The transplantation of cells with injectable biomaterials as a suspension provides a favorable microenvironment to the cells, increasing trapping and survival of the cells at the injection site. Hydrogel and nanoparticles are the most used injectable biomaterials for heart regeneration. Embedding human biopsy-derived CSC within matrix-enriched hydrogel capsules, composed of integrin-binding proteins, positively affects long-term cell survival, retention and cardiac function in post-ischemic events [47]. A recent study has shown that intramyocardial injection of mESC-derived Islet1+ CPC in combination with fibrin gel allows the differentiation of CPC into the three cardiovascular lineages after transplantation, reduces infarct size and improves cardiac function in infarcted mice [48]. However, the mechanical properties of the bioengineered hydrogel prevent correct distribution and cell coupling.

Engineered cardiac graft is another approach which allows electromechanical forces and vascularization, assuring the retention and organized distribution of the cells. Human adult cardiac progenitors-derived cardiospheres embedded in gelatin and collagen scaffolds are biocompatible and allow selective commitment of cells towards cardiomyocyte fate [49]. Extracellular matrix-mimicking nanofibrous poly(L-lactic acid) scaffolds have also been reported to support attachment and proliferation of mESC-derived CSC, as well as differentiation towards cardiomyocytes, endothelial cells and smooth muscle cells after subcutaneous implantation in mice [50].

Menasché et al. [23] published a case report in 2015 which showed the feasibility of the application of human ESC-derived CSC (Islet1+/SSEA-1+) combined within a tissue-engineered fibrin patch in a patient suffering from severe ischemic left ventricular dysfunction. The cell-loaded patch was surgically delivered onto peri-infarcted epicardium in addition to coronary artery bypass surgery. Improved functional and clinical outcomes of the patient with severe ischemic heart failure were observed with no complications such as arrhythmias, tumor formation or immunosuppression-related adverse events. The first clinical trial using hESC-derived cardiac progenitors is ongoing and it will allow further data about feasibility, safety and efficacy of hESC-derived CSC in cardiac regenerative medicine (ESCORT trial, Assistance Publique-Hôpitaux de Paris, Identifier NCT02057900, Table 1).

Tissue printing technology offers the possibility to deliver scaffolding materials in combination with cells in a defined and controlled manner, preserving a precisely defined 3D structure that supports the formation of cardiac structures. Human cardiac tissue-derived progenitor cells printed in alginate scaffolds promoted cell survival, proliferation and differentiation into cardiac lineages. Moreover, the cells were able to migrate out of the matrix to form tubular-like structures [51].

Repopulation of decellularized heart provides a promising strategy for regenerative medicine. Decellularized whole hearts preserve the original 3D architecture, natural matrix components and local niches providing intact heart scaffolds. Yang's group succeeded in engineering for the first time a bioartificial human heart by repopulating decellularized mouse hearts with human iPSC-derived multipotent CSC; however, the engineered heart did not generate the sufficient mechanical force for pumping blood and the electric conduction was too slow [52]. Pericardium-derived scaffolds, obtained by decellularization of pericardium membranes, have also been reported to be useful as 3D macroporous scaffolds that enabled human Sca1+ CSC to survive, proliferate, migrate and differentiate toward cardiovascular fates [53].

Future Perspectives and Challenges

Although the turnover of cardiomyocytes in the adult heart occurs at a very low rate, in contrast to other species, human heart cannot be healed naturally after AMI, and the loss of myocardial tissue is replaced by fibrous tissue. Stem cell-based therapy could palliate adverse heart remodeling events, improve cardiac function and in the best case scenario regenerate the lost cardiac tissue. Since the results from clinical trials using adult extracardiac stem cells have not demonstrated a substantial long-term benefit, other stem cells with enhanced cardiomyogenic potential are being explored.

Some endogenous cardiac progenitors with regenerative capability after AMI have been recently identified in preclinical studies, but it would be necessary to know further about their mechanisms of activation, mobilization and expansion to be clinically effective.

The novel reprogramming approaches have enabled a way to obtain CSC with higher differentiation and proliferative potential than adult somatic stem cells. However, before these CSC can be translated into clinical practice, many critical issues need to be addressed. To circumvent the tumorigenic risk related to human iPSC-derived cells it will be necessary to isolate and transplant pure CSC, and in the case of iCSC, to modify the procedure with a non-integrative method. In addition, the reproducibility of this direct reprogramming approach needs to be demonstrated by different laboratories. Moreover, a cost- and time-effective large-scale production of reprogrammed CSC and studies in large animal will be necessary before beginning clinical trials.

Nevertheless, the most accepted hypothesis is that any beneficial effect observed after cell transplantation is mediated through paracrine release of anti-apoptotic, immunomodulatory and proangiogenic factors derived from transplanted and resident cells, since cell grafts are not observed shortly after transplantation, regardless of cell type. Thus, long-term success of cardiac cell therapy will be determined by both the development of methods to improve engraftment and the optimal cell type that ensures not only safety but also the regeneration of the failing heart. Heart tissue engineering holds great promise but further research is needed to improve electrical, chemical and mechanical properties of the engineered cardiac constructs.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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