Current Cardiology Reports (2019) 21:133
https://doi.org/10.1007/s11886-019-1226-5

MYOCARDIAL DISEASE (A ABBATE AND G SINAGRA, SECTION EDITORS)

On the Road to Regeneration: "Tools" and "Routes" Towards Efficient Cardiac Cell Therapy for Ischemic Cardiomyopathy

Francesca Pagano[1] · Vittorio Picchio[1] · Isotta Chimenti[1,2] · Alessia Sordano[1] · Elena De Falco[1,2] · Mariangela Peruzzi[2] · Fabio Miraldi[3] · Elena Cavarretta[1,2] · Giuseppe Biondi Zoccai[1,2] · Sebastiano Sciarretta[1,4] · Giacomo Frati[1,4] · Antonino G. M. Marullo[1]

Abstract

Purpose of Review Cardiac regenerative medicine is a field bridging together biotechnology and surgical science. In this review, we present the explored surgical roads to cell delivery and the known effects of each delivery method on cell therapy efficiency. We also list the more recent clinical trials, exploring the safety and efficacy of delivery routes used for cardiac cell therapy approaches.

Recent Findings There is no consensus in defining which way is the most suitable for the delivery of the different therapeutic cell types to the damaged heart tissue. In addition, it emerged that the "delivery issue" has not been systematically addressed in each clinical trial and for each and every cell type capable of cardiac repair.

Summary Cardiac damage occurring after an ischemic insult triggers a cascade of cellular events, eventually leading to heart failure through fibrosis and maladaptive remodelling. None of the pharmacological or medical interventions approved so far can rescue or reverse this phenomenon, and cardiovascular diseases are still the leading cause of death in the western world. Therefore, for nearly 20 years, regenerative medicine approaches have focused on cell therapy as a promising road to pursue, with numerous preclinical and clinical testing of cell-based therapies being studied and developed. Nonetheless, consistent clinical results are still missing to reach consensus on the most effective strategy for ischemic cardiomyopathy, based on patient selection, diagnosis and stage of the disease, therapeutic cell type, and delivery route.

Keywords Regenerative medicine · Ischemic cardiomyopathy · Cardiac surgery · Cardiac cell therapy · Heart failure

This article is part of the Topical Collection on *Myocardial Disease*

✉ Francesca Pagano
francesca.pagano@uniroma1.it

Vittorio Picchio
vittorio.picchio@uniroma1.it

Isotta Chimenti
isotta.chimenti@uniroma1.it

Alessia Sordano
alessia.sordano@gmail.com

Elena De Falco
elena.defalco@uniroma1.it

Mariangela Peruzzi
mariangela.peruzzi@uniroma1.it

Fabio Miraldi
fabio.miraldi@uniroma1.it

Elena Cavarretta
elena.cavarretta@uniroma1.it

Giuseppe Biondi Zoccai
giuseppe.biondizoccai@uniroma1.it

Sebastiano Sciarretta
sebastiano.sciarretta@uniroma1.it

Giacomo Frati
giacomo.frati@uniroma1.it

Antonino G. M. Marullo
antoninogm.marullo@uniroma1.it

[1] Department of Medical Surgical Sciences and Biotechnologies, Sapienza University of Rome, Corso della Repubblica 79, 04100 Latina, Italy

[2] Mediterranea Cardiocentro, Naples, Italy

[3] Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological, and Geriatric Sciences, Sapienza University of Rome, Latina, Italy

[4] Department of AngioCardioNeurology, IRCCS Neuromed, Pozzilli, Italy

Published online: 31 October 2019

Introduction

Congestive heart failure (CHF) is one of the most common diseases in western countries, mostly due to ischemic cardiomyopathy. Its incidence and prevalence are continuously rising along with the aging population and parallel improvements in primary prevention and medical management. Due to progress in interventional and medical treatments, nowadays able to reduce overall acute mortality, an increased number of ischemic patients chronically develop substantial myocardial scarring, with unfavorable left ventricular remodelling and consequent CHF.

The cardiac damage occurring after an ischemic insult, namely myocardial infarction (MI), triggers a cascade of cellular events, eventually leading to fibrosis and maladaptive remodelling [1, 2]. None of the medical or pharmacological interventions approved so far can rescue or reverse these phenomena, and cardiovascular diseases are indeed the leading cause of death in the western world [3]. According to the most recent epidemiologic data reported by the American Heart Association, nearly 5 million Americans are currently diagnosed with CHF with a constant increment of its prevalence, especially among patients over 65 years old. In fact, data projection weight up that the prevalence of heart failure (HF) will increase by 46% from 2012 to 2030, thus reaching more than 8 million adult people affected [4]. Notably, CHF is considered as malignant as most cancer types, and costs of medical care are continuously rising, with most patients undergoing repeated hospitalization. Although pharmacological advancements have led to better treatments, 50% of the patients with an acute MI die within 1 month, and 50% of the patients in the most advanced stage of HF die within 1 year. Orthotopic cardiac transplantation and, more recently, ventricular assist devices (VADs) are considered the gold standard treatments for CHF, but shortage of donors in the first case and invasiveness, limited durability, and high costs in the second case still constitute a major limit to their application. Therefore, in parallel with improved prevention and medical treatments, in the last two decades, new approaches have been proposed specifically for the management of the post-ischemic patients' subpopulation.

Cardiac regenerative medicine is a promising translational research field, which has been moving rapidly towards clinical testing since the early 2000s. Cardiac cell therapy (CCT) [5•] is based on isolation, expansion, and delivery to the heart of different populations of stem/progenitor cells and seems to be a valid tool to promote heart regeneration and counteract fibrosis. Several endogenous or extra-cardiac progenitor cell populations have been identified, with the ability to exert different therapeutic effects once transplanted into the failing heart [6], with at least two major benefits: preservation and/or reduction of the compromised cardiac function, and shrinkage of the scar with restoration, at least in part, of healthy heart muscle.

Since the seminal observations regarding the regenerative capacity of transplanted autologous skeletal myoblasts [7] (which can give only limited therapeutic advantages due to their propensity to generate electrical inhomogeneity with aberrant conduction within the heart muscle [4]), research has explored different cellular populations. These have been selected (at least in principle) as therapeutic agents against cardiac adverse remodelling, acting via specific beneficial mechanisms for cardiac tissue biology and function (reviewed in Pagano et al. [8, 9••]). Many different types of adult stem/progenitor cells, distinguished by their origin and differentiation capacity, have been studied, such as multipotent bone marrow–derived stem cells (BM-SCs), including hematopoietic stem cells (HSCs) and mesenchymal cells; endothelial progenitor cells (EPCs); mesenchymal stem cells (MSCs) from both bone marrow and adipose tissue; skeletal myoblasts; and, more recently, resident cardiac progenitor cells (CPCs). The mammalian heart has been considered for a long time as a terminally differentiated organ incapable of regenerating after injury. In recent years, instead, many evidences have demonstrated that cardiomyocytes during aging and after injury still possess some cell cycle re-entry capacity [2] and that a resident primitive stromal population exists in the heart, capable of cell renewal capacity, albeit limited [10]. Human CPCs can be isolated by means of different criteria, such as spheroid selection or immunophenotypic sorting, with all methods yielding cell populations with very strong transcriptomic similarity [11].

Among all adult progenitor cell types, only three are currently being tested as future cell-based commercial therapies. Two of them are in phase III testing for HF with reduced ejection fraction, which are (i) autologous bone marrow mononuclear cells (BM-MNCs) (CardiAMP trial, NCT02438306), (ii) allogeneic mesenchymal precursor cells (DREAM HF-1 trial, NCT02032004), and (iii) cardiosphere-derived CPCs, which are being tested for various types of HF, including Duchenne muscular dystrophy–associated HF (CADUCEUS trial NCT00893360 now closed and HOPE-2 trial NCT03406780 still recruiting).

In the last decades, the results obtained in several clinical trials have demonstrated the therapeutic (indirect) potential of non-cardiac stem cell sources [12, 13, 14•]. However, many evidences underlined that resident CPCs represent an optimal candidate for CCT [15, 16], and cardiosphere-derived cells are the unique cell population yet used in clinical trials which can be qualified as CPCs, being of intrinsic cardiac origin. In fact, starting from explant cultures of percutaneous endomyocardial biopsy specimens, cardiac stromal primitive cells, i.e., CPCs, can be isolated through their capacity to spontaneously form niche-like 3D spherical clusters, called cardiospheres (CSs) [17–19]. CSs can be expanded in

monolayer as CS-derived cells, defined by clonal growth capacity, intrinsic cardiovascular commitment, and strong beneficial paracrine potency [20–24].

The Road to a Broken Heart: Cell Therapy Delivery Options

Considering the complex anatomy, mechanics, and physiology of left ventricular function, well described by Torrent-Guasp [25, 26], some of the major concerns of CCT are related not only to the choice of the therapeutic cell type, but also to the delivery strategy, which may affect their survival, engraftment, paracrine potential, and functional linkage with host tissue. A recent editorial in the journal *Nature Biotechnology* [27] highlighted the importance of rigorous description of cell-mediated heart repair mechanisms before clinical translation. Given the negative results obtained by the largest US clinical trial on bone marrow–derived CD34+ cells (PreSERVE-AMI), the same attention should be given to the different delivery methods, as the right choice could give unique therapeutic advantages to selected cell types. The results of completed trials revealed that stem/progenitor cells often fail to engraft and survive long-term after transplantation [28]; therefore, defining the best delivery method for preserving their viability and therapeutic capacity becomes crucial. Many efforts have been made within the scientific community, aiming at potentiating the cellular products and describing the response to the milieu the cells would experience once transplanted. Yet, not as many addressed specifically what are the most suitable surgical delivery methods to be used for efficient transplantation of the specific cellular product. In addition, the identification of different approaches for the treatment of patients, according to the stage of the disease, is of paramount importance in the post-ischemic population. This perspective mirrors the contemporary concept of "personalized medicine." As a general thought, in fact, acute phase treatment strategies, associated to percutaneous/intracoronary delivery, presume the use of autologous cell sources that do not require prolonged ex vivo manipulation; alternatively, previously cultured allogeneic cell products from biobanking could be used upon necessity. On the other hand, treatment of patients in the chronic stage might take advantage of allowing longer pre-implant cell isolation, differentiation, and expansion techniques, as well as several routes of cardiac delivery. Moreover, since acute and sub-acute infarcted myocardium might be at higher risk of perforation, intramyocardial cell injections are probably more suitable for patients with chronic ischemic cardiomyopathy.

From the numerous preclinical and clinical studies published in the last 20 years, it is obvious that CCT strategies are still under close investigation and that no clear-cut evidence is yet available to clinicians for a licensed and standardized therapy, in terms of both cell source choice and best surgical delivery techniques. In fact, different delivery techniques have been described and approached with the aim to identify a reliable and safe protocol for regenerative cardiac therapy.

The main delivery routes can be classified as either trans-vascular or intramyocardial, and all different techniques used in large animals or human trials have shown pros and cons related to possible complications, cell survival, differentiation, homing, and engraftment (Fig. 1).

Trans-vascular Routes

The trans-vascular delivery of cells can be achieved through systemic venous injection, direct injection in the coronary artery, or through coronary sinus venous injection.

Systemic delivery has shown significant limitations related to the possible entrapment of cells in the lungs, spleen, and other organs, as well as in the peripheral microvasculature. Furthermore, even with successful delivery, less than 10% of the cells survive stress, inflammation, and hypoxia, thus even more rarely trans-differentiate into cardiac cell types [29, 30]. For these reasons, this technique has been questioned and discarded, despite promising preliminary in vivo experience.

Direct coronary injection, instead, has been described and used with the possible advantage of trans-catheter delivery in the culprit coronary, but it has shown several limitations and possible complications, as well as marginal results in terms of myocardial repair and functional improvement. This delivery route has been specifically employed to avoid invasive surgical approaches and to minimize shedding to non-target organs. Intracoronary delivery of cells into a recanalized infarct-related artery is safe and convenient and has the inherent advantage that cells are infused into myocardial regions with preserved oxygen and nutrient supply, thus ensuring in theory a favorable environment for cell survival. Cell delivery has been achieved using a dedicated catheter and the stop-flow technique by inflating an angioplasty balloon in the proximal segment of the coronary artery, in order to facilitate trans-endothelial migration into the targeted myocardium and to limit the possible washout effect. However, retention of cells is suboptimal (most cells are washed out before they can migrate into the surrounding tissue), and non-perfused regions of the myocardium remain inaccessible. Indeed, it has been described that cell transmigration, chemotaxis, and adhesion can be promoted by the intrinsic signalling of the ischemic myocardium [31–33], as evidenced by the local concentration of stromal-derived factor 1 (SDF-1) and C-X-chemokine receptor 4 (CXCR4), confirming the advantage of this approach in the post-ischemic subset population [33–36]. One of the major limitations of this technique is related

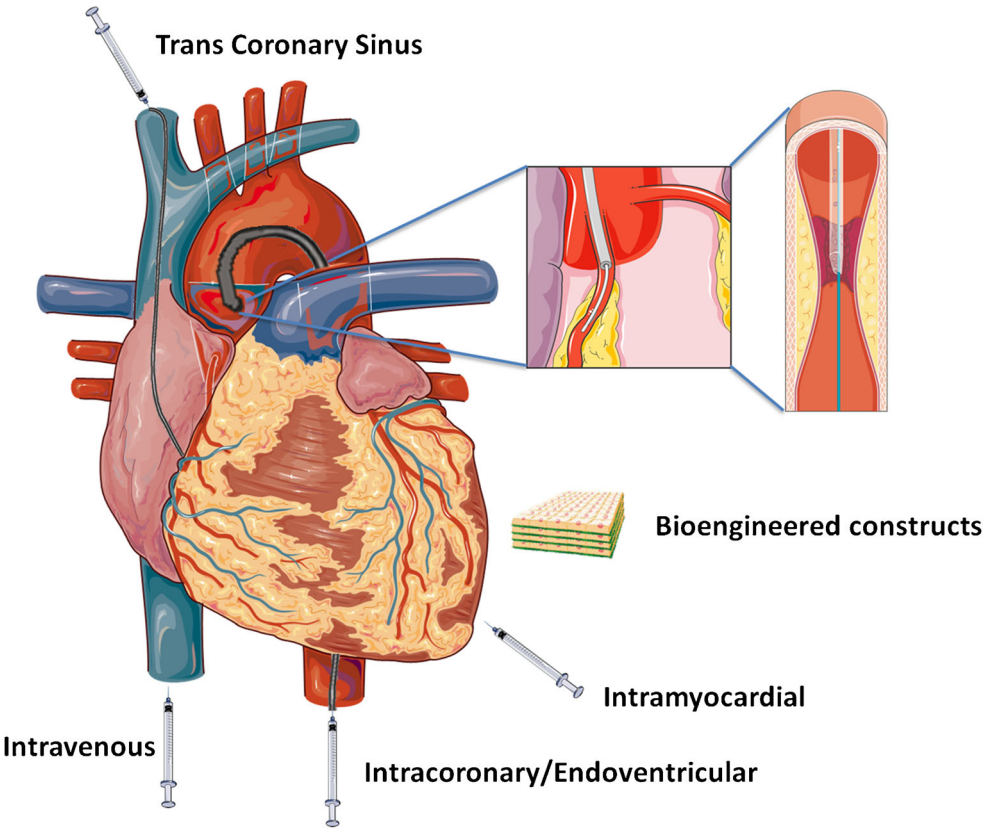

Fig. 1 Schematic representation of the surgical delivery techniques used for cell therapy protocols, discussed in the review. The main delivery options are depicted as small syringes attached to a catheter, pointing at the specific access sites used for each cell delivery method to reach the damaged heart. A generic bio-construct is also depicted. The insert shows the intravascular side of the catheter

to the possibility of coronary thromboembolic complications, and therefore, the cell type choice must be limited to small cells, such as BM-MNCs, since clusters possibly formed by cultured or adhesive cells can be responsible for acute coronary embolization and/or occlusion [37, 38].

Another described coronary trans-arterial delivery technique is the perivascular injection of cells with intravascular ultrasound (IVUS) imaging guidance. This is performed using a dedicated catheter with perpendicular microneedles able to penetrate the arterial wall, thus allowing delivery into the tunica adventitia and the perivascular space. This delivery method gives the advantage of selectively reaching the target zone under direct visualization, but the successful engraftment to this area depends indeed on the specific coronary vessel anatomy.

The retrograde coronary sinus infusion, instead, can be performed through peripheral vein access (usually through the femoral vein), by positioning a catheter into the coronary sinus followed by cell infusion with the use of a balloon. This device prevents the possible flow of the cells into the right atrium, therefore, avoiding possible systemic loss. This technique has shown advantages in terms of safety and cell retention [39–41]. The possible intra-procedural rupture of the coronary sinus and the risk of systemic embolization must be underlined among the possible complications of this approach. Moreover, despite the reported high cell retention, this technique can also be limited by the coronary vein anatomy that often does not allow the delivery in the targeted region of the myocardium.

Intramyocardial Routes

Intramyocardial approaches have been developed for their intrinsic advantage of direct delivery to the infarcted cardiac tissue. This allows to overcome the limitations due to necessary viable and pervious vascular access, as well as the requirement for trans-endothelial migration that, according to the reported data, cannot be effectively induced and controlled. This approach can be achieved either through endo-ventricular and trans-ventricular delivery or through trans-epicardial application or injection of the cells.

Endo-ventricular and trans-ventricular intramyocardial injections allow direct delivery of therapeutic cells, reducing the limitations related to vascular access and/or trans-endothelial cell migration. Intramyocardial delivery has usually been achieved through percutaneous access with dedicated catheters and fluoroscopic guidance or electromechanical mapping to facilitate delivery to the target myocardium. Clinical studies have shown the feasibility and safety of this technique, with a cell retention rate most likely superior to the vascular approach [37]. However, despite the use of dedicated catheters,

reported cell loss and migration associated with these delivery approaches are still significant, confirming the major limitations of percutaneous delivery strategies.

Trans-epicardial delivery, instead, is usually performed during open-heart surgery, with multiple injections directly into the infarct and the peri-infarct ischemic tissues. The major limitation of this approach is related to the inability to deliver therapeutic cells in all those regions that are not directly accessible, such as septal myocardial segments. Moreover, in addition to open questions related to the cellular death rate, retention percentage, washout, and extravascular migration, other major issues associated with this approach are related to the bias of coronary revascularization. In fact, concomitant surgical revascularization does not consent to dissect functional improvements directly related to cell therapy from the functional improvements ensuing the revascularization.

The Bioengineering Option

Considering the important limitations described above with all the main delivery techniques and the marginal clinical results obtained, more recently, CCT has been focusing on cell delivery associated with bioengineered composite sheets, patches, or gels, in order to improve cell survival, engraftment, and functional linkage with the host myocardium [42]. Possible advantages of this combined approach are related to the application of therapeutic cells already seeded in a scaffold or resorbable gels that might favor structural and functional connection with the endogenous ventricular myocardium [42–44].

Scaffolds can be either pre-formed, with a specific morphology set up during their production, or capable of state-transition from liquid to solid (so-called hydrogels), based on physical properties of the environment (e.g., pH, temperature) or biochemical reactions (e.g., polymerization) [45]. Concerning pre-formed scaffolds, they must be seeded with therapeutic cells and then transplanted as patches, therefore, requiring open-heart interventions for epicardial application. Moreover, it is necessary to fix the patch to the heart by sutures or biocompatible glues (e.g., fibrin glue), in order to avoid movement or detachment of the bio-scaffold from the selected area on the epicardial position, which might be induced by the physiologic contractile activity of the cardiac muscle [].

Concerning the delivery of hydrogels, dedicated catheters are needed based on their composition and possibility of acutely pre-mixing different solutions upon delivery [46, 47]. A main concern is also the need for appropriate guiding and imaging systems to avoid dangerous intra-ventricular leakage, possibly leading to embolization.

Delivery Options for Therapeutic Cells: What Is the Best Combination?

Completed clinical trials on different sources of therapeutic cells show that a variety of approaches have been tested individually (Table 1), making it hard to directly compare them and draw conclusions on which route is the best, for which cell type, and why. We will focus on the data available regarding the delivery options tested in both experimental and preclinical settings, of either cardiac or non-cardiac cell types, that are currently under clinical evaluation [5, 48] and their effects on the cells used.

BM-MNCs

When isolated cells are used for cell therapy approaches, the surgical delivery method has been proven to affect cell viability and engraftment capacity. For example, a comparison study of local administration methods using labelled BM-MNCs revealed that intracoronary infusion allows higher engraftment in the infarct region, compared with intravenous injection. The study on cell biodistribution was performed on three subjects using 2-[^{18}F]-fluoro-2-deoxy-d-glucose (^{18}F-FDG)–radiolabeled cells, either infused into the infarct-related coronary artery or injected intravenously. 3D PET imaging monitoring after 50 to 75 min from cell infusion revealed that 1.3 to 2.6% of labelled BM-MNCs delivered by intracoronary infusion homed at the damaged myocardium site, while only background signal was detected with intravenous transfer [49]. This observation was confirmed also in another study, where the authors compared two different delivery techniques for the infusion of BM-MNCs in patients with ST-elevation myocardial infarction (STEMI): the anterograde intracoronary artery (ICA) delivery and the retrograde intracoronary vein (ICV) approach. BM-MNC retention into the damaged heart tissue was apparently higher when the ICA delivery was used [50]. Overall, no exhaustive evidence of heart regeneration has been reported in any of the clinical trials involving BM-MNCs [5,13,50,51]. A recent study designed for the evaluation of the cardiac repair induced by intramyocardial injection of BM-MNCs showed no change in both cardiac function and clinical parameters in patients with CHF over a follow-up period of 12 months [52]. At the moment, a prospective randomized clinical trial is ongoing (CardiAMP trial NCT02438306) assessing the effects of BM-MSC in ischemic systolic HF.

Despite the heterogeneous results obtained, BM-MSCs usage has been proven to be safe for AMI treatment in all of phase I–II trials completed so far. For this reason, the phase III BAMI trial (FP7-HEALTH ID: 278967) was set up in 2011, and the results will be available after

Table 1 Summary of the delivery routes presented, the specific pros and cons addressed, and the therapeutic cell lines tested using each delivery method

Delivery routes		Pros	Cons	Cells tested
Trans-vascular	Systemic venous injection	Safety	Extra-cardiac entrapment of cells (lung, spleen, peripheral microvasculature)	CPCs
		Promising preliminary experience in vivo	Stress-dependent low cellular survival	
			Rare trans-differentiation into cardiac cell types	
	Direct injection in the coronary artery	Safety	Suboptimal cell retention	BM-MNCs
		Non-invasiveness of the procedure	Inaccessibility of non-perfused heart regions	BM-MSCs ADMSCs
		Minimum shedding to non-target organs	Thromboembolic complications	CPCs
		Preservation of oxygen and nutrient supply	Only small cells can be used.	
	IVUS perivascular Injection	Selective to the target zone	Successful delivery dependent on specific artery anatomy	CPCs
	Retrograde coronary sinus infusion	Safety	Systemic embolization	BM-MNCs
		High cell retention	Limited target region for coronary vein anatomy	BM-MSCs
		Systemic loss avoided with balloon		
Intramyocardial	Endo-ventricular	Safety	Cell loss	MSCs
	Trans-ventricular	High cell retention	Surgical thromboembolic complications	BM-MSCs
		Direct delivery to the infarcted cardiac tissue		
	Trans-epicardial	Safety	Inaccessibility of heart regions (septal myocardial segments)	
		High cell retention	Cell washout	
			Extravascular migration	

completion of the ongoing follow-up phase in autumn 2019. The trial is designed for standardizing the procedure and testing a definitive endpoint for AMI therapy using BM-MSCs and includes a detailed timeframe for bone marrow aspiration and cell processing and delivery, which was performed by intracoronary infusion only [53].

Despite the target of 3000 patients being not reached (only 375 patients were recruited and randomized), BAMI remains the largest phase III trial in this specific cardiology area. The number of patients enrolled will not reach the power needed to provide definitive endpoint results, but it will still provide valuable evidence on the successful standardization of the bone marrow procurement and of the cell manufacturing technique, both achieved so far in the study. In addition, it will provide a good estimate of the efficacy of the standardized delivery technique used once the follow-up results will be available (a partial report of the trial is available at https://cordis.europa.eu).

The use of hydrogels and matrix as scaffolds greatly increases the therapeutic potential of BM-MSCs. The MAGNUM clinical trial explored the feasibility and safety of MI treatment using BM-MNCs seeded onto collagen scaffolds, grafted onto the infarcted ventricle. The study enrolled 20 patients: half of them were treated with cells only and the other half with collagen matrix seeded with autologous BM-MSCs. The results showed a general improvement in the efficiency of cellular cardiomyoplasty. Moreover, the use of a collagen scaffold increased viable tissue within the infarct scar. This generated a lower degree of cardiac remodelling with the improvement of diastolic function, supported by a better response to cardiac wall stress in patients treated with the cell-seeded collagen scaffolds [54].

MSCs

Mesenchymal stem cells (MSCs) represent a good candidate for both autologous and allogeneic transplant as they are naturally non-immunogenic and easily sourced from adipose tissue or bone marrow, with a relatively low risk for the patient. MSCs are one of the options for CCT because of their beneficial paracrine and immunomodulatory properties [55]. Similar to other cell sources, MSCs show poor engraftment efficiency when injected directly, and the benefits observed in the clinical settings can be attributed to their high paracrine activity and immunomodulatory function. Moreover, genetic engineering approaches on these cells have been explored, aiming at overexpressing pro-survival factors (reviewed in [56]).

Currently, there are over 25 registered clinical trials assessing the effects of MSC delivery for cardiac regeneration for acute MI and ischemic cardiomyopathy treatment (http://www.clinicaltrials.gov, [57]). Overall, BM-MSCs have been used in many clinical trials, and the route of delivery, rather than intracoronary or intravenous, has been predominantly intramyocardial. The intramyocardial delivery is the riskiest and most invasive method, as previously discussed; despite the hypothesis that it should grant higher engraftment rates, experimental evidence in preclinical studies surprisingly showed no advantages of this delivery route in comparison with the other. For example, a randomized study showed no significant difference in cell delivery efficiency to the myocardium in a large animal model between intracoronary and trans-endocardial delivery [58].

Conversely, a review article has summarized a few studies comparing intracoronary and endo-ventricular approaches and showed that there is indeed some difference between the two methods in terms of efficacy and overall efficiency of the CCT protocol used [59]. About MSC delivery options, Perin et al. [60] compared intracoronary and trans-endocardial delivery of allogeneic MSCs in a canine model of AMI and found that trans-endocardial injection improved heart function, while intracoronary infusion did not. In contrast, Rigol et al. [61] compared intracoronary versus trans-endocardial administration of adipose tissue–derived MSCs in a porcine model of AMI and found that the intracoronary route significantly increased neovascularization compared with the trans-endocardial route, despite both delivery strategies resulted in a similar engraftment rate.

MSCs therapeutic usage is also greatly improved by delivering them using scaffolds, which enhance their therapeutic potential [62]. In particular, the combination of MSCs with hydrogels (both fibrin and collagen based) has been tested in a rat MI model of intramyocardial treatment and showed increased cell survival, with consequent improvement of the overall cardiac function recovery [63]. Furthermore, in a study performed on adipose-derived stromal cells (ADSCs) in rats, both fibrin and collagen polymers showed increased cardiac retention, but cell survival was higher with collagen [64].

CPCs

Since 2009, several clinical trials have been performed using resident CPCs to treat different heart dysfunctions, such as ventricular dysfunction (NCT00893360), myocardial infarction (NCT01458405), ischemic and non-ischemic cardiomyopathy (NCT02293603), dilated cardiomyopathy (NCT03129568), and HF (NCT02941705). In all cases, CPCs were administered by intracoronary infusion. As discussed above, the coronary route is easy and safe and has the advantage that cells are delivered through a natural space without artificial tissue disruption [65]. Recently, CPCs were also introduced in genetic disease therapy to treat cardiomyopathy in patients with Duchenne muscular dystrophy (DMD) with two clinical trials: HOPE (NCT02485938) and HOPE-2 (NCT03406780). In the HOPE trial, a single dose of allogeneic CPCs was delivered by intracoronary infusion in three coronary arteries supplying the three major cardiac territories of the left ventricle (anterior, lateral, inferior/posterior). It was shown to be generally safe and well tolerated, and the authors have demonstrated significant and sustained improvement in cardiac, as well as skeletal muscle function, compared with patients who received usual care only. The ongoing HOPE-2 trial, instead, is evaluating the safety and efficacy of repeated doses of CPC delivery trough intravenous infusion (the least invasive delivery method) every 3 months, for a total of 4 doses in boys and young men with DMD.

The results obtained by CCT clinical studies on ischemic cardiomyopathy using CPCs have shown that these cells can reduce scar formation but cannot change the ventricle pump efficiency (no detectable changes in ejection fraction). Moreover, the intracoronary delivery of these cells allows local conditioning of the damaged tissue but does not permit remote myocardium to be reached efficiently. Surprisingly, the recently completed ALLSTAR trial (NCT01458405) testing the effects of allogeneic CPCs for the treatment of ventricular remodelling post MI showed no therapeutic effect of cell therapy. The cells were isolated from donor heart tissue and delivered through intracoronary infusion in the infarct-related artery [66]. Apparently, the extent of myocardial coverage achieved through single-vessel intracoronary infusion allows only limited myocardial coverage. Instead, a sequential infusion into each of the three major coronary vessels achieves a widespread distribution of viable CPCs [65]. This delivery method is being currently tested in the "Dilated cardiomYopathy iNtervention With Allogeneic MyocardIally-regenerative Cells" trial (DYNAMIC - NCT02293603) on the use of CPCs in HF patients that will be terminated on April 2020. Interestingly, a recent work showed in a porcine model of ischemia that a global infusion of CPCs, compared with local delivery, could greatly improve the efficacy of the therapy and allow LVEF increase [67]. These results seem encouraging for a positive outcome of the DYNAMIC trial.

Similar to other therapeutic cells, also CPC efficiency through multiple cardiac repair mechanisms might be improved by increasing their retention at the infarct site or enhancing their paracrine mechanism of action [42, , 68]. CPCs have been tested for their viability and differentiation potential after encapsulation in hydrogels. This material has been shown to be useful for efficient and

minimally invasive delivery of adult stem/progenitor cells used in cell therapy applications (see above). Mouse CPCs were successfully encapsulated in a pH-sensitive hydrogel, which was liquid at blood pH (7.4), allowing safe and efficient delivery of the cells through the blood flow, and then solidified in the cardiac infarct zone (pH 6–7), increasing cell retention in the myocardium [69].

Together with synthetic gels, the use of decellularized matrix and cell-derived extracellular matrix (ECM) has been tested in preclinical settings. In particular, in vitro experiments performed on CPCs seeded on cardiac fibroblast-derived ECM, named cardiogel, revealed that this ECM can induce secretion of specific factors and a peculiar gene expression pattern. In particular, matrix derived from healthy hearts could improve CPCs therapeutic potential [70]. The ECM microenvironment is also very important for CPCs viability and therapeutic potential [71, 72]. To date, a few scaffolds have been tested in vitro for their ability to effectively host CPCs, such as hydrogels [73, 74], RGD-modified collagen, and gelatin solid foams [44]. Also, cells seeded on tissue construct with similar cell adhesion properties, but different biomechanical features (e.g., global modulus, single fiber modulus, fiber density, and fiber alignment) have shown different degrees of viability and cardiac differentiation, with the relatively low modulus scaffold being the best scaffold inducing increased CPCs cardiac differentiation [75], consistently with mechanosensing and microenvironment signalling [71, 76].

Conclusions and Perspectives

After nearly 20 years of preclinical and clinical research, it is clear that the road to cardiac regeneration is anything but a straight line and that the nature of the cardiac damage itself requires tools that are specific and precise. Research has focused on evaluating the many options available, and efforts across the world have led to a fairly deep understanding of the cellular and molecular characteristics of the different therapeutic cell types [77]. Ischemic cardiomyopathy treatment needs personalized medicine approaches, taking into account the numerous variables of the disease onset and progression, together with technical issues concerning therapeutic cell types and delivery strategies of choice. This clinical need, together with the biological complexity of heart regeneration and repair mechanisms, has made the journey hard and full of uncertainty and non-conclusive evidence so far.

The outcomes of clinical as well as experimental tests on the different cell therapy approaches collected so far mined the confidence into cell therapy as a valid therapeutic approach to CHF. The lack of a consensus and possibly the difficulties in the definition of the real regenerative capacity of cell therapy approaches inspired the institution of the "Working Group on Cardiovascular Regenerative and Reparative Medicine", established in September 2017 by the European Society of Cardiology. The main scope of the group is to bring together scientists across Europe to improve knowledge and develop therapeutic approaches through the synergy of basic and translational science.

Besides therapeutic cell administration, we must remember that heart regeneration can be also induced through the stimulation of endogenous mechanisms of repair, albeit with a limited and not jet standardized efficacy, as well. Cardiomyocytes can be stimulated to cycle via different strategies, including transgene expression, small molecules, and microRNAs [2]. In addition, reprogramming of non-myocyte cells to a myocyte phenotype has been proposed as an alternative to cell therapy approaches [78].

In conclusion, future research should include efficient crosstalk between bench and bedside scientists, in order to speed up the development of efficient personalized treatments, where cells and delivery options are developed in parallel and in synergy, to achieve increasingly significant advancements in cardiac regenerative medicine.

Funding Information This work has been supported by a Sapienza University of Rome grant Prot. RM11715C7852D47E to GF.

Compliance with Ethical Standards

Conflict of Interest Francesca Pagano, Vittorio Picchio, Isotta Chimenti, Alessia Sordano, Elena De Falco, Mariangela Peruzzi, Fabio Miraldi, Elena Cavarretta, Giuseppe Biondi Zoccai, Sebastiano Sciarretta, Giacomo Frati, and Antonino G.M. Marullo declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as: • Of importance •• Of major importance

1. Schirone L, Forte M, Palmerio S, Yee D, Nocella C, Angelini F, et al. A review of the molecular mechanisms underlying the development and progression of cardiac remodeling. Oxidative Med Cell Longev. 2017;2017:1–16.
2. Leach JP, Martin JF. Cardiomyocyte proliferation for therapeutic regeneration. Curr Cardiol Rep. 2018;20:63.

3. Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388:1459–544.
4. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics—2019 update: a report from the American Heart Association. Circulation. 2019;139:e56–528.
5.• Marbán E. A mechanistic roadmap for the clinical application of cardiac cell therapies. Nat Biomed Eng. 2018;2:353–61 **Provides a thorough overview of cardiac cell therapies currently used in clinic, underlining the importance of a mechanism-supported efficacy, achieved through the identification and description of those factors allowing superior therapeutic efficacy.**
6. Forte E, Chimenti I, Barile L, Gaetani R, Angelini F, Ionta V, et al. Cardiac cell therapy: the next (re)generation. Stem Cell Rev. 2011;7:1018–30.
7. Menasché P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, et al. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial. Circulation. 2008;117:1189–200.
8. Pagano F, Picchio V, Angelini F, Iaccarino A, Peruzzi M, Cavarretta E, et al. The biological mechanisms of action of cardiac progenitor cell therapy. Curr Cardiol Rep. 2018;20:84.
9.•• Fernández-Avilés F, Sanz-Ruiz R, Climent AM, Badimon L, Bolli R, Charron D, et al. Global position paper on cardiovascular regenerative medicine. Eur Heart J. 2017;38:2532–46 **Up-to-date summary of the shared vision of leading expert teams in the field.**
10. Ahuja P, Sdek P, MacLellan WR. Cardiac myocyte cell cycle control in development, disease, and regeneration. Physiol Rev. 2007;87:521–44.
11. Gaetani R, Feyen DAM, Doevendans PA, Gremmels H, Forte E, Fledderus JO, et al. Different types of cultured human adult cardiac progenitor cells have a high degree of transcriptome similarity. J Cell Mol Med. 2014;18:2147–51.
12. Golpanian S, Wolf A, Hatzistergos KE, Hare JM. Rebuilding the damaged heart: mesenchymal stem cells, cell-based therapy, and engineered heart tissue. Physiol Rev. 2016;96:1127–68.
13. Jeevanantham V, Afzal MR, Zuba-Surma EK, Dawn B. Clinical trials of cardiac repair with adult bone marrow- derived cells. Methods Mol Biol. 2013;1036:179–205.
14.• Menasché P. Cell therapy trials for heart regeneration — lessons learned and future directions. Nat Rev Cardiol. 2018;15:659–71 **Summary of the main outcomes of completed cell-based regenerative trials, with a perspective indication on how to draw future testing in order to establish standardized and functional therapies.**
15. Nguyen PK, Rhee J-WW, Wu JC. Adult stem cell therapy and heart failure, 2000 to 2016: a systematic review. JAMA Cardiol Am Med Assoc. 2016;1:831–41.
16. Li T-S, Cheng K, Malliaras K, Smith RR, Zhang Y, Sun B, et al. Direct comparison of different stem cell types and subpopulations reveals superior paracrine potency and myocardial repair efficacy with cardiosphere-derived cells. J Am Coll Cardiol. 2012;59:942–53.
17. Messina E. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res. 2004;95:911–21.
18. Li T-S, Cheng K, Lee S-T, Matsushita S, Davis D, Malliaras K, et al. Cardiospheres recapitulate a niche-like microenvironment rich in stemness and cell-matrix interactions, rationalizing their enhanced functional potency for myocardial repair. Stem Cells. 2010;28:2088–98.
19. Chimenti I, Massai D, Morbiducci U, Beltrami AP, Pesce M, Messina E. Stem cell spheroids and ex vivo niche modeling: rationalization and scaling-up. J Cardiovasc Transl Res. 2017;10:150–66.
20. Chimenti I, Gaetani R, Barile L, Forte E, Ionta V, Angelini F, et al. Isolation and expansion of adult cardiac stem/progenitor cells in the form of cardiospheres from human cardiac biopsies and murine hearts. Methods Mol Biol. 2012;879:327–38.
21. Stastna M, Chimenti I, Marbán E, Van Eyk JE. Identification and functionality of proteomes secreted by rat cardiac stem cells and neonatal cardiomyocytes. Proteomics. 2010;10:245–53.
22. Davis DR, Zhang Y, Smith RR, Cheng K, Terrovitis J, Malliaras K, et al. Validation of the cardiosphere method to culture cardiac progenitor cells from myocardial tissue. Leri A, editor. PLoS One. 2009;4:e7195.
23. Chimenti I, Smith RR, Li T-S, Gerstenblith G, Messina E, Giacomello A, et al. Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice. Circ Res. 2010;106:971–80.
24. White AJ, Smith RR, Matsushita S, Chakravarty T, Czer LSCC, Burton K, et al. Intrinsic cardiac origin of human cardiosphere-derived cells. Eur Heart J. 2013;34:68–75.
25. Buckberg GD. Basic science review: the helix and the heart. J Thorac Cardiovasc Surg. 2002;124:863–83.
26. Torrent-Guasp F, Ballester M, Buckberg GD, Carreras F, Flotats A, Carrió I, et al. Spatial orientation of the ventricular muscle band: physiologic contribution and surgical implications. J Thorac Cardiovasc Surg. 2001;122:389–92.
27. Nature Biotechnology Editorial Board. A futile cycle in cell therapy. Nat Biotechnol. 2017;35:291.
28. Wernly B, Mirna M, Rezar R, Prodinger C, Jung C, Podesser BK, et al. Regenerative cardiovascular therapies: stem cells and beyond. Int J Mol Sci. 2019;20:1–13.
29. van der Bogt KEA, Sheikh AY, Schrepfer S, Hoyt G, Cao F, Ransohoff KJ, et al. Comparison of different adult stem cell types for treatment of myocardial ischemia. Circulation. 2008;118:S121–9.
30. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007;25:1015–24.
31. Loffredo FS, Steinhauser ML, Gannon J, Lee RT. Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell. 2011;8:389–98.
32. Sussman M. Hearts and bones. Nature. 2001;410:640–1.
33. Szilvassy SJ, Bass MJ, Van Zant G, Grimes B. Organ-selective homing defines engraftment kinetics of murine hematopoietic stem cells and is compromised by ex vivo expansion. Blood. 1999;93:1557–66.
34. Jiang Q, Song P, Wang E, Li J, Hu S, Zhang H. Remote ischemic postconditioning enhances cell retention in the myocardium after intravenous administration of bone marrow mesenchymal stromal cells. J Mol Cell Cardiol. 2013;56:1–7.
35. Elmadbouh I, Haider HK, Jiang S, Idris NM, Lu G, Ashraf M. Ex vivo delivered stromal cell-derived factor-1alpha promotes stem cell homing and induces angiomyogenesis in the infarcted myocardium. J Mol Cell Cardiol. 2007;42:792–803.
36. Kamota T, Li T-S, Morikage N, Murakami M, Ohshima M, Kubo M, et al. Ischemic pre-conditioning enhances the mobilization and recruitment of bone marrow stem cells to protect against ischemia/reperfusion injury in the late phase. J Am Coll Cardiol. 2009;53:1814–22.
37. Steinhoff G, Nesteruk J, Wolfien M, Große J, Ruch U, Vasudevan P, et al. Stem cells and heart disease - brake or accelerator? Adv Drug Deliv Rev. 2017;120:2–24.
38. Furlani D, Ugurlucan M, Ong L, Bieback K, Pittermann E, Westien I, et al. Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy. Microvasc Res. 2009;77:370–6.

39. Zakharova L, Nural-Guvener H, Feehery L, Popovic S, Nimlos J, Gaballa MA. Retrograde coronary vein infusion of cardiac explant-derived c-Kit+ cells improves function in ischemic heart failure. J Heart Lung Transplant. 2014;33:644–53.
40. Tuma J, Fernández-Viña R, Carrasco A, Castillo J, Cruz C, Carrillo A, et al. Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. J Transl Med. 2011;9:183.
41. Patel AN, Mittal S, Turan G, Winters AA, Henry TD, Ince H, et al. REVIVE trial: retrograde delivery of autologous bone marrow in patients with heart failure. Stem Cells Transl Med. 2015;4:1021–7.
42. Gaetani R, Rizzitelli G, Chimenti I, Barile L, Forte E, Ionta V, et al. Cardiospheres and tissue engineering for myocardial regeneration: potential for clinical application. J Cell Mol Med. 2010;14:1071–7.
43. Cortes-Morichetti M, Frati G, Schussler O, Van Huyen J-PD, Lauret E, Genovese JA, et al. Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration. Tissue Eng. 2007;13:2681–7.
44. Chimenti I, Rizzitelli G, Gaetani R, Angelini F, Ionta V, Forte E, et al. Human cardiosphere-seeded gelatin and collagen scaffolds as cardiogenic engineered bioconstructs. Biomaterials. 2011;32:9271–81.
45. Rane AA, Christman KL. Biomaterials for the treatment of myocardial infarction: a 5-year update. J Am Coll Cardiol. 2011;58:2615–29.
46. O'Cearbhaill ED, Ng KS, Karp JM. Emerging medical devices for minimally invasive cell therapy. Mayo Clin Proc. 2014;89:259–73.
47. Dolan EB, Kovarova L, O'Neill H, Pravda M, Sulakova R, Scigalkova I, et al. A dvanced M aterial Cath eter (AMCath), a minimally invasive endocardial catheter for the delivery of fast-gelling covalently cross-linked hyaluronic acid hydrogels. J Biomater Appl. 2018;33:681–92.
48. Gao L, Gregorich ZR, Zhu W, Mattapally S, Oduk Y, Lou X, et al. Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. Circulation. 2018;137:1712–30.
49. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, et al. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation. 2005;111:2198–202.
50. Silva SA, Sousa AL, Haddad AF, Azevedo JC, Soares VE, Peixoto CM, et al. Autologous bone-marrow mononuclear cell transplantation after acute myocardial infarction: comparison of two delivery techniques. Cell Transplant. 2009;18:343–52.
51. Nowbar AN, Mielewczik M, Karavassilis M, Dehbi H-MH-M, Shun-Shin MJ, Jones S, et al. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): weighted regression and meta-analysis. BMJ. 2014;348:g2688.
52. Mann I, Tseng · CCS, Rodrigo · SF, Koudstaal · S, Van Ramshorst · J, Beeres SL, et al. Intramyocardial bone marrow cell injection does not lead to functional improvement in patients with chronic ischaemic heart failure without considerable ischaemia. Orig Artic Neth Hear J. 2019;27:81–92.
53. Mathur A, Arnold R, Assmus B, Bartunek J, Belmans A, Bönig H, et al. The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: rationale and design of the BAMI trial. Eur J Heart Fail. 2017;19:1545–50.
54. Chachques JC, Trainini JC, Lago N, Cortes-Morichetti M, Schussler O, Carpentier A. Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM trial): clinical feasibility study. Ann Thorac Surg. 2008;85:901–8.
55. Gnecchi M, Danieli P, Malpasso G, Ciuffreda MC. Paracrine mechanisms of mesenchymal stem cells in tissue repair. Methods Mol Biol. 2016;1416:123–46.
56. Thakker R, Yang P. Mesenchymal stem cell therapy for cardiac repair. Curr Treat Options Cardiovasc Med. 2014;16:323.
57. Ward MR, Abadeh A, Connelly KA. Concise review: rational use of mesenchymal stem cells in the treatment of ischemic heart disease. Stem Cells Transl Med. 2018;7:543–50.
58. van der Spoel TI, Vrijsen KR, Koudstaal S, Sluijter JP, Nijsen JF, de Jong HW, et al. Transendocardial cell injection is not superior to intracoronary infusion in a porcine model of ischaemic cardiomyopathy: a study on delivery efficiency. J Cell Mol Med. 2012;16:2768–76.
59. Golpanian S, Schulman IH, Ebert RF, Heldman AW, DiFede DL, Yang PC, et al. Concise review: review and perspective of cell dosage and routes of administration from preclinical and clinical studies of stem cell therapy for heart disease. Stem Cells Transl Med. 2016;5:186–91.
60. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, et al. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol. 2008;44:486–95.
61. Rigol M, Solanes N, Farré J, Roura S, Roqué M, Berruezo A, et al. Effects of adipose tissue-derived stem cell therapy after myocardial infarction: impact of the route of administration. J Card Fail. 2010;16:357–66.
62. Perez-Estenaga I, Prosper F, Pelacho B. Allogeneic mesenchymal stem cells and biomaterials: the perfect match for cardiac repair? Int J Mol Sci. 2018;19.
63. Ye Z, Zhou Y, Cai H, Tan W. Myocardial regeneration: roles of stem cells and hydrogels. Adv Drug Deliv Rev. 2011;63:688–97.
64. Danoviz ME, Nakamuta JS, Marques FLN, dos Santos L, Alvarenga EC, dos Santos AA, et al. Rat adipose tissue-derived stem cells transplantation attenuates cardiac dysfunction post infarction and biopolymers enhance cell retention. Schwartz A, editor. PLoS One 2010;5:e12077.
65. Tseliou E, Kanazawa H, Dawkins J, Gallet R, Kreke M, Smith R, et al. Widespread myocardial delivery of heart-derived stem cells by nonocclusive triple-vessel intracoronary infusion in porcine ischemic cardiomyopathy: superior attenuation of adverse remodeling documented by magnetic resonance imaging and histology. Tang Y, editor. PLoS One. Public Library of Science; 2016;11:e0144523.
66. Chakravarty T, Makkar RR, Ascheim DD, Traverse JH, Schatz R, Demaria A, et al. ALLogeneic Heart STem Cells to Achieve Myocardial Regeneration (ALLSTAR) trial: rationale and design. Cell Transplant. 2017;26:205.
67. Suzuki G, Weil BR, Young RF, Fallavollita JA, Canty JM. Non-occlusive multi-vessel intracoronary infusion of allogeneic cardiosphere-derived cells prevents remote zone myocyte loss in swine with myocardial infarction. Am J Physiol Circ Physiol. 2019;317:H345–56.
68. Pagano F, Angelini F, Siciliano C, Tasciotti J, Mangino G, De FE, et al. Beta2-adrenergic signaling affects the phenotype of human cardiac progenitor cells through EMT modulation. Pharmacol Res. 2018;127:41–8.
69. Li Z, Fan Z, Xu Y, Lo W, Wang X, Niu H, et al. pH-sensitive and thermosensitive hydrogels as stem-cell carriers for cardiac therapy. ACS Appl Mater Interfaces. 2016;8:10752–60.
70. Pagano F, Angelini F, Castaldo C, Picchio V, Messina E, Sciarretta S, et al. Normal versus pathological cardiac fibroblast-derived extracellular matrix differentially modulates cardiosphere-derived cell paracrine properties and commitment. Stem Cells Int. 2017;2017:1–9.
71. Mauretti A, Spaans S, Bax NAM, Sahlgren C, Bouten CVC. Cardiac progenitor cells and the interplay with their microenvironment. Stem Cells Int. 2017;2017:7471582.

72. Castaldo C, Chimenti I. Cardiac progenitor cells: the matrix has you. Stem Cells Transl Med. 2018;7:506–10.
73. Smith RR, Marbán E, Marbán L. Enhancing retention and efficacy of cardiosphere-derived cells administered after myocardial infarction using a hyaluronan-gelatin hydrogel. Biomatter. 2013;3: e24490.
74. Kim D-H, Kshitiz, Smith RR, Kim P, Ahn EH, Kim H-N, et al. Nanopatterned cardiac cell patches promote stem cell niche formation and myocardial regeneration. Integr Biol (Camb). 2012;4: 1019–33.
75. Xu Y, Patnaik S, Guo X, Li Z, Lo W, Butler R, et al. Cardiac differentiation of cardiosphere-derived cells in scaffolds mimicking morphology of the cardiac extracellular matrix. Acta Biomater. 2014;10:3449–62.
76. Pesce M, Messina E, Chimenti I, Beltrami AP. Cardiac mechanoperception: a life-long story from early beats to aging and failure. Stem Cells Dev. 2017;26:77–90.
77. Peruzzi M, De Falco E, Abbate A, Biondi-Zoccai G, Chimenti I, Lotrionte M, et al. State of the art on the evidence base in cardiac regenerative therapy: overview of 41 systematic reviews. Biomed Res Int. 2015;2015:613782.
78. Nam J, Munshi NV. The promise of cardiac regeneration by in situ lineage conversion. Circulation. 2017;135:914–6.