

## Contemporary perspective on endogenous myocardial regeneration

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### Abstract

Considering the complex nature of the adult heart, it is no wonder that innate regenerative processes, while maintaining adequate cardiac function, fall short in myocardial jeopardy. In spite of these enchaining

limitations, cardiac rejuvenation occurs as well as restricted regeneration. In this review, the background as well as potential mechanisms of endogenous myocardial regeneration are summarized. We present and analyze the available evidence in three subsequent steps. First, we examine the experimental research data that provide insights into the mechanisms and origins of the replicating cardiac myocytes, including cell populations referred to as cardiac progenitor cells (*i.e.*, c-kit<sup>+</sup> cells). Second, we describe the role of clinical settings such as acute or chronic myocardial ischemia, as initiators of pathways of endogenous myocardial regeneration. Third, the hitherto conducted clinical studies that examined different approaches of initiating endogenous myocardial regeneration in failing human hearts are analyzed. In conclusion, we present the evidence in support of the notion that regaining cardiac function beyond cellular replacement of dysfunctional myocardium *via* initiation of innate regenerative pathways could create a new perspective and a paradigm change in heart failure therapeutics. Reinitiating cardiac morphogenesis by reintroducing developmental pathways in the adult failing heart might provide a feasible way of tissue regeneration. Based on our hypothesis "embryonic recall", we present first supporting evidence on regenerative impulses in the myocardium, as induced by developmental processes.

**Key words:** Cardiac regeneration; Cardiac development; Embryonic recall; Pressure-controlled intermittent coronary sinus occlusion; Heart failure; Myocardial infarction

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**Core tip:** Unlike in primitive vertebrates, any regenerative effort in adult mammalian hearts after an acute event remains unsatisfactory. Most efforts to repopulate failing hearts with functioning and integrated cardiomyocytes have not achieved clinical importance. In this overview, after describing several options for endogenous myo-

cardial repair, we support the notion of a paradigm change towards inducible developmental processes in regeneration research. Major efforts have been made to convert tissues upstream in the Waddington scheme. Recently, stress transformed acquired pluripotency raised enormous expectations, but results and proof of concept were seriously questioned. We want to introduce pressure-controlled intermittent coronary sinus occlusion as a potential resource to decipher the unsolved equation of re-inducing the developmental processes in the human heart.

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## INTRODUCTION

Advances in therapeutic approaches to acute life-threatening cardiovascular conditions, such as early revascularization in patients with acute myocardial infarction (AMI), have contributed to a dramatic decrease in short-term mortality. Hence prolonged life expectancy coupled with the residual impairment of heart function and the progressive nature of cardiovascular diseases<sup>[1]</sup> have exponentially increased the number of patients with chronic insufficiency of heart function. While aggressive medical therapy of severe chronic heart failure (HF) may alleviate symptoms, the ultimate treatment option for this growing patient group remains heart transplantation. However, due to infrastructural and technical constraints, heart transplantation could barely meet the needs of the vast population of patients with HF<sup>[2]</sup>, who have deleterious prognosis comparable to malignant diseases with the mortality rate as high as about 25% within the first year of diagnosis<sup>[3,4]</sup>. In the United States the estimated prevalence of HF is > 5 million patients, annual incidence is > 500000 patients and annual mortality with HF as underlying cause is > 50000<sup>[5]</sup>, which makes paramount the development of a therapy regimen effective beyond the acute phase and available to a broad population of patients.

The pathophysiological substrate of heart failure is the loss of functioning cardiomyocytes during the overall process of remodeling of the heart tissue, which in turn leads to diminishing of the contractile function<sup>[6]</sup>. Between the two ends of contemporary clinical reality represented by acute therapies, such as early revascularization, which are available to many, but effective mainly in improving short- and mid-term clinical outcomes, and heart-transplantation as ultimate treatment strategy for a failing heart, but available to only few, there is an unmet need to reverse the process of remodeling and foster myocardial regeneration.

The notion of replenishing lost cardiomyocytes is founded on the triad of rejuvenation, replacement and regeneration<sup>[7]</sup> with the common goal of structural and functional heart repair. While the term rejuvenation refers to activation of myocardial self-renewal processes, replacement and regeneration are based on transplantation into the damaged host myocardium, of functional tissues and progenitor cells, respectively<sup>[8]</sup>.

First studies that tested the effects of transplantation of progenitor cells in patients with AMI or chronic ischemic heart disease while proving safe, showed modest improvement in clinical outcomes<sup>[9,10]</sup>. The translation of this luring concept of repopulating heart with transplanted stem cells into every-day clinical practice has been challenged by the following issues: type and dosing of progenitor cells, *ex-vivo* processing and expansion, timing and route of administration, as well as timing of transplantation respective the stage and level of myocardial ischemia<sup>[11]</sup>. Immunological response of the host-microenvironment that impacts long-term prognosis and possible ethical implications of transplanting pluripotent cells of embryonic origin have additionally burdened the research in the field of stem-cell based approaches to heart repair.

Opposite to the transplantation-based regimens, interventions aiming at rejuvenation of damaged heart tissue by activating innate cardiac repair pathways seem to provide a more holistic approach, while avoiding possible pitfalls of cell injection into the microenvironment of an ischemic heart. The development of such therapeutic strategies is supported by the evidence of postnatal formation of new cardiomyocytes, thus indicating the potential for endogenous myocardial regeneration<sup>[12,13]</sup>. Since the effects of stem-cell therapies have been attributed not only to engraftment of transplanted cells and generation of new cardiomyocytes, by means of transdifferentiation or cell fusion, but to a considerable degree to paracrine effects<sup>[14]</sup>, the transplantation of cells could seem redundant in wake of a possible intervention succeeding in activation of endogenous repair pathways.

Our aim was to review the literature on the mechanisms of endogenous myocardial regeneration and to analyze the contemporary therapeutic approaches to activation of these inborn pathways of myocardial repair. We proceed in three subsequent steps by examining (1) the evidence for the self-regenerative capacity of the heart; (2) the initiation of endogenous myocardial repair mechanisms in a clinical setting such as acute or chronic myocardial ischemia; and (3) the results of the hitherto conducted clinical studies that investigated different methods of triggering endogenous myocardial regeneration in failing human hearts.

## SELF-REGENERATIVE CAPACITY OF THE HEART

The axiom of regenerative medicine has been that

apart from intestinal epithelium, skin and bone marrow, no other tissue contained stem-cells capable of self-renewal<sup>[15]</sup>, with the predominant belief that in postnatal life cardiomyocytes respond with hypertrophy when exposed to stress<sup>[16]</sup>. According to this view, from the birth on cardiomyocytes could only grow in size without any significant reproduction capacity, which is how > 20 fold increase in heart mass from birth to adulthood has been explained<sup>[15,17-19]</sup>.

The described axiom that cardiac myocytes could only grow in size, has been challenged by an early work that lists myocyte hyperplasia as mode of adaption to microenvironmental stimuli within the heart<sup>[20]</sup>. Several contemporary experiments have contributed evidence in favor of a more dynamic view of myocardial cellular homeostasis, attributing cardiac myocytes the potential to increase in number<sup>[21-24]</sup>. Of note, pathologic stimuli like acute myocardial infarction<sup>[21]</sup> and chronic ischemia<sup>[23]</sup> that lead to destruction and functional deterioration of myocytes, seem to concurrently provide the groundwork for their replenishment. It has been shown that during the initial stages of pressure overload hypertrophy the DNA synthesis in cardiomyocytes takes place<sup>[25]</sup>. Cardiomyocyte hypertrophy and hyperplasia appear to be in no way mutually exclusive and both part of heart's defense strategy, with myocyte proliferation commencing with severe functional deterioration of the heart<sup>[26]</sup>.

In order to assess the clinical relevance of the posited myocardial self-regeneration capacity two issues need to be clarified. First, what is the expected magnitude of cardiomyocyte proliferation? Second, what is the origin of the newly formed cardiomyocytes?

### Quantification of cardiomyocyte replication

A landmark study, that investigated cardiomyocyte proliferation based on carbon-14 integration in the DNA, reported that fewer than 50% of cardiomyocytes are renewed during a normal life span<sup>[12]</sup>. The annual myocyte turn-over rate decreased from 1% at the age of 25 to 0.45% at the age of 75<sup>[12]</sup>. On contrary, another study found aging to be associated with increased rates of cardiomyocyte turnover<sup>[27]</sup>. In chronically failing hearts the presence of the proliferating cell nuclear antigen, a marker of DNA synthesis and cell proliferation, was documented in 49% ± 22% of the left ventricular myocytes<sup>[26]</sup>. Quantitative analysis from the same study described 11 cardiomyocytes nuclei with mitotic images out of a million<sup>[26]</sup>. After myocardial infarction, two out of a million of cells were observed to have images of mitotic divisions, predominantly in the adjacent zones<sup>[26]</sup>. Another study documented the mitotic index after acute myocardial infarction of 520 cardiomyocytes out of a million, thus accounting for approximately 2 million cardiac myocytes in the mitosis within the left ventricle wall early after AMI<sup>[24,28]</sup>. Further calculation that assumed the existence of  $5.2 \times 10^9$  cardiac myocytes

in the left ventricle and the mitosis duration of approximately 1 h, showed that the presumed mitotic index of 11 per million<sup>[26]</sup> would produce 10% new left ventricular myocytes in a year and would double the number of cardiac myocytes in the left ventricle over a period of 10 years<sup>[29]</sup>. Of note, different studies estimated annual rates of cardiomyocyte turnover to be 1%-10%<sup>[12,15,30,31]</sup>. The reported variability in estimated rates of cardiomyocyte replication translates into uncertainty regarding the true magnitude of myocardial self-regeneration and fuels the skepticism regarding the clinical relevance of the observed findings. The observed variability may have originated from different morphometric methodologies and different experimental settings (normal vs diseased heart).

### Criticism of the theory of cardiomyocyte replication:

Although increased DNA synthesis in cardiomyocytes has been postulated as part of their adaptation to myocardial injury, many researchers remained skeptical of cardiomyocyte replication<sup>[32]</sup>. Chien *et al.*<sup>[33]</sup> reinforced the old tenet that cardiomyocytes were terminally differentiated and that the response to myocardial damage is mainly hypertrophy. Thereby, the experimentally obtained images of myocytes with clearly replicating DNA were ascribed to the fact that around 20% of heart muscle cells are multinucleated but still do not divide<sup>[24,28]</sup>.

It is clear that in order to prove the potential for self-regeneration of the myocardium one must show the actual mitosis and quantify the increase in actual cardiomyocyte numbers and not only show DNA synthesis, the more so as the ploidy formation in the hypertrophied heart has already been documented<sup>[34]</sup>. Due to the fact that heart harbors a proportion of multinucleated myocytes accounting for ¼ of the human cardiac myocytes<sup>[29]</sup>, it is important to mention that aging, cardiac hypertrophy and ischemic cardiomyopathy do not alter the proportion of mononucleated and multinucleated cardiomyocytes<sup>[35]</sup>. It may thus be assumed that if the proportion is not changed under stress conditions, then cardiomyocyte hyperplasia could not be excluded. In this respect it has been pointed out<sup>[25]</sup> that two studies have documented mitotic images in an experimental model of myocardial injury<sup>[36,37]</sup>.

Despite presented evidence for cardiomyocyte hyperplasia<sup>[37,38]</sup>, due to the methodological constraints and the fact that myocardial ischemia is followed by a vast loss of cardiomyocytes, the actual cardiomyocyte turnover may hardly be reliably assessed. Nevertheless, the contention that cardiomyocyte proliferate appears to be further strengthened by the aging-related telomeric shortening<sup>[39]</sup>. On the other side, experiments with dog hearts exposed to acute heart failure documented increase in telomerase action<sup>[40]</sup>, which was interpreted as sign of ongoing regeneration<sup>[41]</sup>.

**Endogenous origin of replicating cardiomyocytes**

Similar to the true magnitude of the rate of cardiomyocyte renewal, uncertainty exists regarding the origin of the replicating cardiomyocytes. Besides circulating extracardiac progenitor cells that are homed into myocardium only upon a strong pathologic signal and to a very limited extent, two possible endogenous mechanisms have been postulated: (1) mitotic replication of pre-existing cardiac myocytes; and (2) development of heart tissue cells from a pool of multipotent stem cells residing within the heart<sup>[15]</sup>.

The long-standing contention that postnatal cardiomyocytes were unable to re-enter cell-cycle and undergo mitosis has been challenged by a study that posited the division of pre-existing cardiomyocytes to be the cornerstone of the observed myocardial renewal<sup>[30]</sup>. On contrary, it has been reported that in response to injury, myocardium regenerates on the basis of residing progenitor cells with no significant contribution by division of pre-existing cardiomyocytes<sup>[42]</sup>. Another study concluded that both resident stem cells and division of pre-existing cardiac myocytes contribute to myocardial regeneration, albeit the progenitor cells were attributed the leading role<sup>[31]</sup>. In wake of the opposing results, it has been argued that different experimental techniques may have caused the controversy, while the most likely source for myocardial repopulation remain resident cardiac stem cells<sup>[15]</sup>.

**Cell types:** Endogenous cardiac stem cells appear to be a highly heterogeneous population of cells, each being defined by expression of surface markers. The hitherto described types include c-kit<sup>+</sup> cardiac stem cells, Isl 1<sup>+</sup> cardiac progenitors, cardiospheres and cardiosphere-derived cells, cardiac mesoangioblasts, side-population cells and cardiac resident colony-forming unit-fibroblast cells<sup>[15,43,44]</sup>.

In the heart, c-kit<sup>+</sup> are located in significantly higher numbers in atria, as compared to left and right ventricles<sup>[45]</sup>. Initial experiments showed the ability of c-kit<sup>+</sup> cardiac stem cells to differentiate into cardiomyocytes, smooth muscle and endothelial cells<sup>[46]</sup>. A recent study confirmed the potential of c-kit<sup>+</sup> lineage to give rise to new cardiomyocytes in response to aging or myocardial injury, albeit not to a significant extent that could have an impact on heart function<sup>[47]</sup>.

A subgroup of CSCs, Isl 1<sup>+</sup> progenitor cells have been identified as key factors in cardiac development, particularly concerning atria, the right ventricle and outflow tract<sup>[48]</sup>. Despite their ability to differentiate into all three cardiac lineages, the role of Isl 1<sup>+</sup> cells in myocardial regeneration is limited due to rapid decrease in number with the increasing age, with high levels exclusively during fetal and neonatal life<sup>[49,50]</sup>.

Cardiospheres are generated from endomyocardial biopsies and represent a heterogeneous population of endogenous cardiac origin<sup>[51]</sup>. Primary cardiospheres are enzymatically digested and cultivated on fibronectin-coated dishes, to eventually give rise to cardiosphere-

derived cells (CDCs), which in turn form secondary cardiospheres when treated with epidermal growth factor (EGF)<sup>[43]</sup>. The main characteristic of the myocardial tissue-derived cardiospheres is high heterogeneity of cells that mimics cardiac microenvironment, which apart from multipotency of so derived CSCs accounts for paracrine effects leading to generation of new cardiomyocytes<sup>[52]</sup>. CDCs were shown to have superior paracrine effects when compared to exogenous stem cell types, such as bone-marrow derived mononuclear or mesenchymal and adipose-tissue derived mesenchymal cells<sup>[53]</sup>.

Recently, it has been debated that seemingly different types of CSCs essentially belong to a single cell type, based on a study that failed to detect differences between CDCs and c-kit<sup>+</sup> cells in respect to surface-antigens and gene expression pattern<sup>[54]</sup>.

In any case, the observed potential to initiate cardiac regeneration seems not to translate into a clinically relevant effect on myocardial repair, due to limited number of residing CSCs<sup>[44]</sup>.

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## MYOCARDIAL INJURY AS THE SIGNAL FOR SELF-REGENERATION

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Clinical applicability of endogenous myocardial regeneration is founded on the observation that myocardial injury serves as a trigger for activation of CSCs. In this context, progenitor cells within myocardium represent the prime therapeutic target, with an aim of boosting the heart's potential for self-renewal.

Several studies showed that under stress conditions such as ischemia or volume overload, myocardial tissue responds with initiating the pathways of regeneration<sup>[13,21,25,55-57]</sup>. The experiments with bromodeoxyuridine (BrdU) labeled rat cardiomyocytes unveiled the tendency of myocytes to remain mitotically inactive once the adult values of the volume and pressure loads on the ventricle were reached, thus 3 or 4 wk after birth<sup>[58]</sup>. The pressure and volume burden seemed to be strongly related to the cell cycle of the cardiomyocytes. In relation to this finding, Insulin growth factor (IGF-1) was found to be down-regulated within the postnatal myocardium. Thereafter the hypothesis was introduced that the IGF-1 and IGF-1 receptor (IGF-1R) system plays a crucial role in proliferation but not hypertrophy of cardiomyocytes<sup>[58]</sup>. The upregulation of mRNA for IGF-1 and IGF-1R in cardiac myocytes was shown to be the direct consequence of ischemia. Interestingly, it was also documented that after the myocardial infarction the remaining viable myocytes expressed mRNA for IGF-1R. The fact that IGF-1/IGF-1R system ceases to be as abundantly represented within the myocardium as the time progresses and myocytes grow in volume, and the fact that the system seems to be reactivated during myocardial stress inspired the hypothesis of postnatal cardiomyocyte proliferation signaled in part by IGF-1<sup>[25]</sup>. Interestingly, an over-

expression of IGF-1 in infarcted mice hearts was shown to attenuate the ventricular dilatation, wall stress and myocardial hypertrophy<sup>[59]</sup>.

### **Self-regenerative capacity of myocardium suffering from acute ischemia**

Acute myocardial ischemia appears to set a strong proliferative impulse with a around 90% increase in DNA replication 2 d after the infarction and over 300% after 7 d. This marked up-regulation of the DNA synthesis was mainly observed in the myocytes adjacent to the necrotic myocardium<sup>[25]</sup>. The authors commented that due to the consequent elevation of myocardial mass (hypertrophy), reduced "loading condition" on the myocardium temporarily takes away the trigger for the myocyte proliferation until the global functional deterioration reaches certain critical point where the DNA replication commences<sup>[25]</sup>.

The analysis of patients who died shortly after AMI revealed proliferating myocytes that were identified by the presence of Ki-67, a nuclear antigen associated with cell division and detectable during the phases G1, G2 and S of the cell cycle<sup>[21]</sup>. The rate of Ki-67 presence was 4% in the border zones and 1% in the remote zones of myocardial infarction<sup>[21]</sup>. Subsequent analysis showed that post-infarction myocytes from the border zones that express Ki-67 antigen are 84 times higher in number compared to the healthy myocardium. At the same time, remote myocardial portions contained 28 times more myocytes expressing Ki-67 in their nuclei as compared to the myocytes of a normally perfused heart<sup>[21]</sup>. However, the extent of myocardial proliferation remains disputable due to different methodological approaches<sup>[12,24]</sup>.

### **Self-regenerative capacity of myocardium suffering from chronic ischemia**

An experimental model of ischemic cardiomyopathy, produced by narrowing but not occluding coronary arteries, yielded similar observations as the described experiments with acute ischemia. One week after the intervention, the BrdU-labeled myocyte nuclei expended 7.31-fold, thus indicated an augmentation in DNA synthesis. After 3 mo this increase in DNA synthesis fell back to virtually the same rate as before the intervention<sup>[25]</sup>.

Cardiomyocyte loss, cellular hypertrophy and ventricular scarring are immediate histological sequelae of chronic myocardial ischemia leading to functional impairment of the left ventricle. Fibrosis is formed where the most severe myocyte loss occurs. Even though end stage ischemic heart failure would imply around 90% decrease in left ventricular cardiomyocytes only 30%-decrease was documented<sup>[56,60]</sup>. In order to supply more evidence for the capacity of cardiomyocytes to proliferate, a study<sup>[56]</sup> analyzed tissue samples of hearts of patients with chronic myocardial ischemia. Cardiomyocyte analysis of the failing myocardium

revealed higher mitotic indices of roughly 140 myocyte nuclei per million as compared to cardiomyocytes from the healthy myocardium.

Both acute and chronic myocardial injury may provide stimulus for myocardial self-regeneration. However, the magnitude of cardiomyocyte proliferation does not seem to compensate for the structural and functional myocardial damage.

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## **FROM BENCH TO THE BEDSIDE:**

### **RESULTS FROM CLINICAL TRIALS**

The inadequacy of myocardial self-regenerative response has led to the development of therapeutic techniques that are based on isolation, *ex vivo* expansion and finally transplantation of endogenous cardiac stem cells.

Hitherto, three clinical trials assessing the effects of transplantation of cardiac-derived stem cells have been conducted (Table 1): the CARDiosphere-Derived autologous stem CELLS to reverse ventricular dysfunction (CADUCEUS) study<sup>[61]</sup>, cardiac Stem Cell In Patients with Ischemic cardiomyopathy (SCIPIO) trial<sup>[62]</sup> and AutoLogous Human CARDiac-Derived Stem Cell To Treat Ischemic cARDiomyopathy (ALCADIA) trial<sup>[63]</sup>.

CADUCEUS was a two-center randomized trial that assessed the effect of intracoronary infusion of cardiosphere-derived cells on infarct size reduction in patients after myocardial infarction, with left ventricular ejection fraction (LVEF) 25-45%. The autologous cardiac stem cells, cultivated from endomyocardial biopsies, were injected 1.5-3 mo after acute MI in 17 patients (control group consisted of 8 patients). At six months, magnetic resonance imaging (MRI) showed significant reduction in scar mass and increase in viable heart mass and regional contractility<sup>[61]</sup>.

SCIPIO was an open label trial that randomized post-infarction patients with left ventricular dysfunction who were scheduled for coronary artery bypass grafting (CABG) to either intracoronary infusion of autologous c-kit<sup>+</sup> cardiac stem cells or placebo. In total, 16 patients received infusion of stem cells while 7 patients were controls. In 14 patients who had undergone follow-up four months after cell infusion there was a significant increase of LVEF (absolute difference vs baseline was about 8%), compared to no change in the control group. At one year the increase in LVEF was sustained (absolute difference vs baseline about 12%)<sup>[62]</sup>.

ALCADIA is an open label, non-randomized safety/feasibility study that evaluates intramyocardial injection of autologous cardiac-derived progenitor cells in combination with administration of basic fibroblast growth factor (bFGF), in patients with ischemic cardiomyopathy and heart failure. Cardiac progenitor cells were derived from endomyocardial biopsies and expanded for a month, prior to intramyocardial injection

**Table 1 Clinical studies with endogenous cardiac stem cells**

Study	Design	Population	Intervention	No. of patients receiving treatment	Primary endpoint	Conclusion
CADUCEUS	RCT	Post-MI with LVEF 25%-45%	Intracoronary infusion of CDCs	17	Occurrence of death, new MI, new cardiac tumour on MRI and/or admission for HF	Safety proven; MRI-scar reduction and increase in viable myocardium
SCIPIO	RCT	Post-MI with LVEF ≤ 40%	Intracoronary infusion of autologous c-kit+ CSCs	16	Short-term safety	Safety proven; LVEF improvement, infarct size reduction
ALCADIA	Non-randomized	Ischemic CMP with LVEF 15%-35%	Intramyocardial injection of autologous CSCs with bFGF	6	-	No adverse events; improvement of NYHA class and LVEF

RCT: Randomized controlled trial; CDCs: Cardiosphere-derived stem cells; MRI: Magnetic resonance imaging; HF: Heart failure; CSCs: Cardiac stem cells; bFGF: Basic fibroblast growth factor; CADUCEUS: CARDiosphere-Derived aUTologous stem CELls to reverse ventricUlar dysfunction; SCIPIO: Stem Cell In Patients with Ischemic cardiomyopathy; ALCADIA: AutoLogous Human CARDiac-Derived Stem Cell To Treat Ischemic cARDiomyopathy.

during CABG<sup>[64]</sup>. So far 6 patients have been enrolled, one patient was excluded due to graft occlusion and one developed worsening heart failure symptoms.

Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR) study is ongoing (ClinicalTrials.gov Identifier NCT01458405) and will randomize 274 patients to intracoronary infusion of either allogenic cardiosphere-derived cells or placebo, 4-12 wk after anterior MI and successful primary percutaneous intervention for LAD coronary artery, if LVEF ≤ 45%.

The combined enrollment for all three trials has been 39 patients, with no major safety concerns. Common denominator of the three trials was the use of autologous cardiac stem cells that have been cultivated from endomyocardial biopsies. In SCIPIO trial, the so cultivated cells were then filtered to obtain the c-kit<sup>+</sup> lineage. The number of cardiosphere-derived cells, used in CADUCEUS study, was limited due to their size (about 20 microns, as compared to the size of coronary capillaries of about 7 microns)<sup>[64]</sup>. ALCADIA trial evaluated an interesting concept that combined cardiac stem cell transplantation with administration of a growth factor, with the purpose of enhancing the retention of the transplanted cells<sup>[64]</sup>. Principal limitations of the hitherto conducted trials are the small number of included patients and the non-standardized cell therapy that varied both in respect to the cell type (cardiosphere-derived cells vs isolated c-kit<sup>+</sup> cells) and the route of delivery (intracoronary vs intramyocardial). In addition, one study (ALCADIA) used bFGF to boost the retention of the transplanted cells.

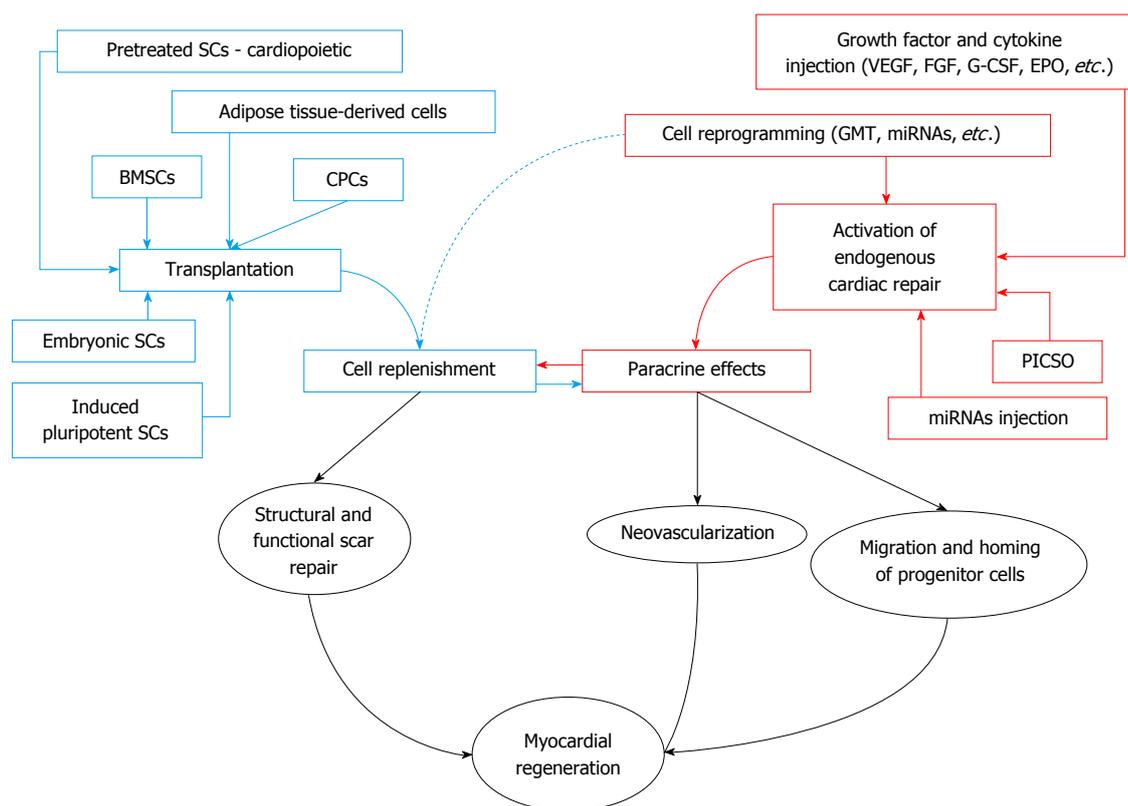
The transplantation of cardiac-derived stem cells appears to at least in theory upgrade the hitherto developed cell therapies for treatment of myocardial injury (*i.e.*, bone-marrow derived cells), inasmuch as CSCs resemble the host myocardial microenvironment. However, the endogenous pathways of regeneration still remain largely enigmatic. As has been presented in the above sections of this review, there is still controversial evidence regarding the origin of the

newly formed cardiac myocytes (myocardium-specific progenitor cells vs self-replicating cardiomyocytes). The ambivalence in respect to the key factors behind the process of endogenous myocardial regeneration hampers clinical research, inasmuch as it makes the standardization of the therapeutic approach virtually impossible - the variety of scientific theories that are aiming at deciphering the endogenous signals is mirrored by the variety of clinical interventions and techniques (different cell types, routes of delivery, target populations, *etc.*, Table 1). In addition, there are significant constraints that are intrinsic to any process of cell transplantation, irrespective of how well the transplanted cells may fit to the host microenvironment and that could limit the effects of endogenous regeneration.

## ACTIVATION OF MYOCARDIAL SELF-REGENERATION WITHOUT CELL TRANSPLANTATION

The process of cell transplantation presupposes stem cell isolation from the tissue of origin (*i.e.*, myocardium or bone marrow), *ex vivo* expansion and ultimately cell delivery into the host myocardium<sup>[65]</sup> (Figure 1). Hence, the use of autologous stem cell renders acute treatment impossible, even though the issue of timing may be the crucial component of a successful myocardial regeneration. It has been shown that mice heart could regenerate if partial surgical resection is performed on day 1 after birth, while the ability of regeneration is lost by day 7<sup>[66]</sup>. Genetic fate mapping showed that mainly the replication of preexisting cardiomyocytes was responsible for the myocyte replenishment<sup>[66]</sup>.

*In situ* activation of endogenous myocardial repair without cell transplantation (Figure 1) would help to avoid the inherent problematic issues associated with the transplantation of stem cells, such as long time periods and high cost of the preparation of cells, non-standard route of delivery (*i.e.*, intramyocardial



**Figure 1 Transplantation of stem cells vs initiation of endogenous myocardial regeneration without cell transplantation.** Depicted are two basic approaches to myocardial regeneration: (1) via transplantation of different types of stem cells into the damaged myocardium (on the left, in blue); and (2) by activation of endogenous pathways of cardiac repair, without cell transplantation (on the right, in red). Both sets of techniques are thought to ultimately exert their effects on the paracrine route and by replenishing lost cardiomyocytes. SCs: Stem cells; BMSCs: Bone marrow-derived stem cells; CPCs: Cardiac progenitor cells; VEGF: Vascular endothelial growth factor; FGF: Fibroblast growth factor; G-CSF: Granulocyte colony stimulating factor; EPO: Erythropoietin; GMT: Gata4, Mef2c, Tbx5; miRNAs: microRNAs; PICSO: Pressure-controlled intermittent coronary sinus occlusion.

injections) and insufficient regenerative response due to problematic retention of the transplanted cells. Thus, four criteria for successful regenerative therapy have been proposed: (1) available at all times; (2) safe and routine application, compatible with gold standard therapies, such as primary PCI; (3) affordable; and (4) effective in salvaging myocardium (at least 50-60 g of tissue) and thus preventing heart failure<sup>[15]</sup>.

The existence of endogenous reservoir of stem cells within myocardium warrants the development of therapeutic strategies directed at activating dormant salvage pathways and thus avoiding the costly and laborious process of cell transplantation.

### Growth factor injection

The idea behind treating the diseased myocardium with growth factor injection rests on the observations that transplanted CSCs impact the host tissue microenvironment twofold, by differentiating into cardiac lineages and by exerting paracrine effects<sup>[44,67]</sup>. Growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), placental growth factor (PIGF),

stem cell factor (SCF) and angiopoietin (Ang) have been postulated as activators of endogenous stem cells within the myocardium and so as initiators of its self-regeneration<sup>[68]</sup>.

Intracoronary administration of the combination of IGF-1 and HGF in the infarct-related coronary artery has been associated with reduced ventricular remodeling and improved myocardial regeneration<sup>[69]</sup>. However, the fact that IGF-1/HGF stimulated cardiomyocyte generation is a slow process may hamper the overall improving of the heart's function and the clinical implementation of this strategy<sup>[15]</sup>.

Initial trials on the effects of FGF in patients with coronary artery disease (CAD), *via* intracoronary delivery of adenoviruses, showed potential of this method to improve regional blood flow<sup>[70]</sup>. However, further clinical studies failed to produce reliable favorable clinical outcomes in CAD patients, albeit the effect of FGF seemed to have been gender-specific with improvement in outcomes in women<sup>[71]</sup>.

Experimental studies have demonstrated the potential of G-CSF to improve LV function after MI, mainly by fostering arteriogenesis<sup>[72]</sup>. Initial clinical application of G-CSF in MI patients indicated the potential to improve echocardiographic LV functional parameters<sup>[73]</sup>, but subsequent randomized trials

did not show improvement in clinical outcomes<sup>[68,74]</sup>. Similarly, in patients with chronic occlusive CAD, subcutaneous injection of G-CSF improved neither myocardial perfusion nor LV function, albeit it appeared to reduce anginal symptoms<sup>[75]</sup>.

GM-CSF has been associated with mobilization of endothelial progenitor cells (EPCs) and ultimately neoangiogenesis<sup>[68]</sup>. A clinical trial showed enhanced collateral flow following the subcutaneous injection of GM-CSF in CAD patients<sup>[76]</sup>. However, the study raised a safety concern due to occurrence of acute coronary syndrome in patients on treatment with GM-CSF<sup>[76]</sup>.

The use of VEGF as a potential mediator of neovascularization has been extensively studied both experimentally and in clinical trials. Animal models of myocardial ischemia indicated the potential of VEGF to induce angiogenesis<sup>[68]</sup>. Subsequent trials showed tendency toward improvement of functional parameters such as regional myocardial perfusion or wall motion, but with no significant effect on clinical outcomes<sup>[77,78]</sup>.

EPO is secreted in tissues under hypoxia and is posited to foster angiogenesis and progenitor cell development, while inhibiting apoptosis<sup>[68]</sup>. Whereas animal studies indicated that EPO administration was associated with the decrease in infarct size<sup>[79]</sup>, a clinical trial in patients with acute MI did not show improvement of heart's function<sup>[80]</sup>.

Further growth factors such as PIGF, Ang and the ligand of c-kit, SCF, which have been proposed to induce neovascularization and mobilization of progenitor cells, showed their potential to regenerate damaged myocardium in animal models, but solid clinical evidence is still lacking<sup>[68]</sup>.

A common characteristic of the described growth factors and cytokines is that their potential to induce myocardial regeneration, mainly *via* paracrine effects, has been established in experimental models, but with no direct translation in the improvement of clinical outcomes.

### Cell reprogramming

The idea of cell reprogramming to achieve structural and functional renewal of the damaged myocardium has been substantiated through the concept of induced pluripotency, whereby fibroblasts could be reprogrammed to assume pluripotency and further directed into the state of cardiac lineage cells<sup>[81-83]</sup>. However, this process implies transplantation of such induced cells into the damaged myocardium (Figure 1). On the other hand, direct reprogramming of the scarred myocardium involves conversion of fibroblasts into functioning cardiomyocytes by delivery of the following transcription factors into the cells of heart tissue: Gata4, Mef2c, Tbx5 (GMT)<sup>[83,84]</sup>. Despite its luring perspective, cardiomyocyte replenishment *via* GMT reprogramming has been hampered by a purported lack of efficiency, relating primarily to

the degree of molecular and electrophysiological development of the newly formed cardiomyocytes<sup>[83,85]</sup>.

Cell reprogramming has been enhanced by the delivery of microRNAs<sup>[86]</sup> or in combination with transcription factors<sup>[87]</sup>. Furthermore, the delivery of sets of microRNAs that have been associated with cardiac developmental pathways may present another option of initiating myocardial regeneration without the need for cell transplantation<sup>[88]</sup>.

### Pressure-controlled intermittent coronary sinus occlusion

Pressure-controlled intermittent coronary sinus occlusion (PICSO) is a technique that is based on repeated cycles of coronary sinus occlusion. PICSO alters the expressional pattern of endo- and perivascular cells by exerting shear stress and cyclic strain on endothelial cells. It has been shown that PICSO up-regulates the expression of VEGF and heme oxygenase-1 (HO-1)<sup>[89]</sup>. In an animal model of myocardial infarction PICSO was shown to upregulate VEGF and VEGF-receptor 2 in the capillary endothelial cells of the remote zones of myocardial infarction<sup>[90]</sup>. ICSO procedure, which differs from PICSO by not being controlled by coronary sinus pressure, was able to significantly reduce infarct size in experimental trials<sup>[91]</sup>. In a clinical trial, PICSO significantly reduced occurrence in MACE in patients with acute MI treated with thrombolysis<sup>[92]</sup>.

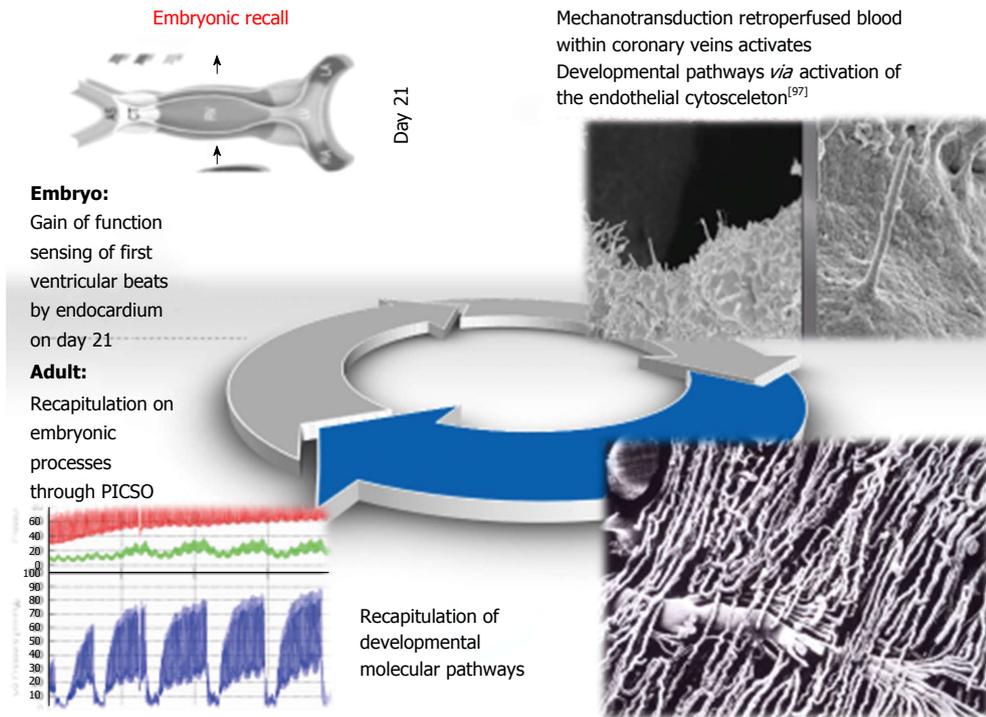
It has been postulated that PICSO has the potential to elicit myocardial regeneration by activating innate regenerative pathways<sup>[93,94]</sup>. Intermittent pressure elevation in the venous system of the heart results in shear stress and pulsatile stretch of the vessel wall, which in turn translates into gene expression on the way of mechanotransduction, thereby mimicking the pulsatile signal on the developing heart tube (Figure 2)<sup>[95-98]</sup>.

Intermittent pressure elevation in the coronary venous system may thus serve as a signal for the activation of endogenous cardiac stem cells *via* paracrine routes and induce dormant pathways of myocardial salvage *via* mechanotransduction, which mimics the structural and functional interplay of the developing heart.

The ease of the application of PICSO without biohazards and instant availability may help to collect clinical data in heart failure patients, thus giving an example that STAP (stress transformed acquired pluripotency) in spite of the unsolved questions in hitherto collected results, might not be an illusion.

## CONCLUSION

Recent findings have overturned the classical contention that postnatal myocardium is terminally differentiated by providing evidence for the formation of new cardiomyocytes. The postulated origin of new cardiomyocytes is the population of resident cardiac stem cells that have the ability to differentiate into the



**Figure 2 Hypothesis of embryonic recall.** In embryos, the first heart beat is sensed by mechanotransduction of the endocardium, leading to a burst of developmental signals thriving cardiac morphogenesis. In analogy, elevated pressures in cardiac veins act *via* stretch and shear stress on perivascular cells, thus reiterating the same mechanotransducing signals in the adult failing heart. Pressure elevation in cardiac veins have to be pressure controlled not to increase arterial resistance to nutritive flow (PICSO).

three cardiac lineages. Myocardial injury provokes the initiation of the innate pathways of regeneration, but the extent remains insufficient to provide significant improvement of heart function. Isolation of cardiac-derived stem cells *via* endomyocardial biopsy and *ex vivo* expansion of stem cells are necessary steps in the process of cell transplantation. However, the transplantation of cardiac stem cells to repair the damaged myocardium is costly and time-consuming and of diminished efficacy due to problematic long-term retention of the transplanted cells. Hence, new therapeutic approaches such as growth factor injection and PICSO are under scrutiny with the proposed goal of activating endogenous progenitor cells and thus initiating myocardial regeneration.

Why does cardiac endogenous regeneration in mammals fail in contrast to reptiles and numerous regenerative signals? One very important aspect is the timing of these signals. Unlike morphogenesis of the developing heart, which is a controlled stepwise approach, regenerative pulses in necro-apoptosis are random in time and region. So one aspect would be to synchronize and amend the signals in order, presuming that we understand the full picture of the molecular signals involved. Therefore reviving a developmental process *via* an epigenetic stimulus like activation of vascular cells by mechanotransduction might initiate the plethora of morphogenetic pulses necessary to enforce and synchronize endogenous repair.

Morphogenesis of the heart seems to be a controlled

stepwise approach in tissue generation, alternating gene transcription and established physical functions which again initiate molecular growth signals. These "epigenetic" signals which could be explained as "morphometric windows" can be used as initiation of endogenous repair.

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