2

Cardiac Muscle Engineering: Strategies to Deliver Stem Cells to the Damaged Site

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1. Introduction

In healthy human hearts, only 10-20% of the total cells are contractile cardiomyocytes and, at the age of 25 years, no more than 1% of them are annually substituted by progenitor cells, this percentage reducing to less than 0.5% at the age of 75. In total, less than 50% of cardiomyocytes are renewed during a normal human life span [1]. For this reason, the topic of cardiac repair is among the major challenges for the tissue engineers worldwide. In fact, cardiac diseases are a predominant cause of mortality and morbidity in industrialized countries, despite the recent advancements achieved in pharmacological treatment and interventional cardiology procedures. Nonetheless, end-stage heart failure management still relies on organ transplantation as unique approach, and, notwithstanding the use of massive immunosuppressive drugs, still a percentage falling within 20%-40% of patients encounters immune rejection during the first year post-transplant [2]. Among the patients not facing severe immune rejection, almost 70% is forced to retire or reduce their working activity, their survival rate falling below 70% during the first five years post organ transplantation [3]. Last, but not least, the economic impact of cardiovascular diseases and stroke has been estimated in 2010 at $503.2 billion [4].

Currently, post-infarction myocardial revascularization protocols include the administration of raw bone marrow stem cells, while a number of clinical trials have been performed or are currently in progress in which different cell subsets are implanted in the damaged tissue by means of surgical techniques. The results of such trials are still controversial. In fact, when autologous skeletal myoblasts were injected into the heart of patients suffering from ischemic cardiomyopathy, the modest functional improvement obtained was impaired by the arising of arrhythmia events, thus requiring the adoption of a pacemaker [5]. On the other side, intracoronary administration of bone marrow mesenchymal stem cells resulted in minimal improvements in cardiac contractile function in patients with dilated cardiomyopathy [6]. These mild results were mostly ascribed to a paracrine effect exerted on host tissue, rather than to a direct contribution of stem cells to the contractile activity.

Thus, among the criticisms to be challenged before efficient cell therapy protocols for cardiac diseases can be setup, the choice of the appropriate cell subset to generate new vessels and contractile cardiomyocytes, as well as the route of cell delivery remain key steps. The solution of such problems requires additional efforts in basic research to clarify the processes leading to stem cell differentiation as well as technological advancements to setup efficient protocols to implant the cells.
In principle, adult stem cells could be extracted from patient's own tissues and expanded in culture by means of well-known techniques (Figure 1).

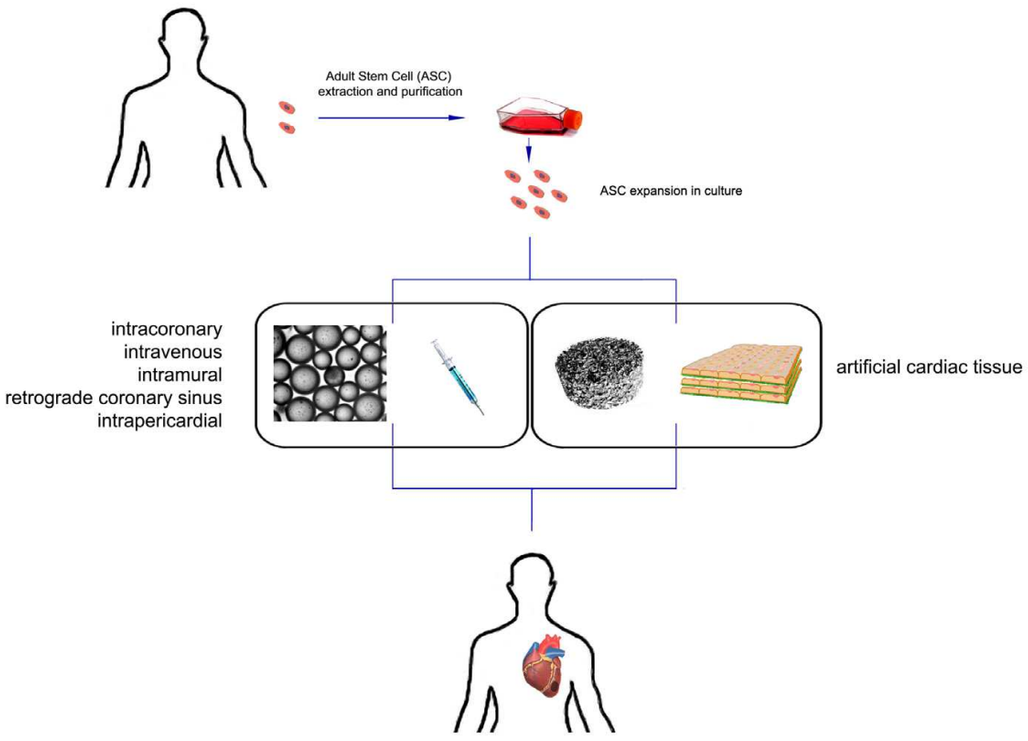

Fig. 1. Cardiac Tissue Engineering paradigm. Adult stem cells can be harvested, purified from the patient and expanded in culture. Such cells can be delivered to the injured heart by injection (intramural or through bloodstream with or without injectable carriers), or in the form of solid bio-constructs. Stem cell-derived bio-constructs can be obtained by culturing the cells on scaffolds or by scaffold-free technology

Nonetheless, a number of issues should be challenged before safe procedures to manipulate stem cells *in vitro* for cardiac transplant can be setup. In fact, stem cells should be amplified *in vitro* to reach a critical number (Figure 1). During this passage, malignant transformation is likely to occur in *ex vivo* cells when standard culture conditions are adopted to expand stem cells [7, 8]. On the other side, stem cells could encounter senescence after a short number of passages *in vitro* [9]. Moreover, the use of animal-derived supplements during the phase of cell expansion would hinder the use of stem cells for cardiac cell therapy.

The employment of autologous stem cells would avoid the problem of immune rejection and the need for immune-suppressive drugs, while, in the treatment of pathologies for which a genetic basis is suspected the use of autologous cells is hampered. As far as the use of autologous cells is concerned, the possibility that a significant patient-to-patient variability in stem cell quality exists should be taken into account [10]. Finally, the use of cellular and tissue-based products in human disease therapy is subjected to regulations issued by the European Union and Food and Drug Administration (FDA) aimed at establishing classification criteria for advanced therapy medicinal products (ATMP). In particular, the European Regulation states that human cells to be used in cell therapy have to comply with the principles of Good Manufacturing Practice (GMP) protocols [11, 12].

2. Adult stem cells for cardiac repair

A number of stem cells and progenitors have been so far proposed for cardiac repair, due to the inability of cardiomyocytes to proliferate after birth [1]. Among the cell sources challenged for the possibility to produce new cardiomyocytes, skeletal myoblasts have proven to be able to acquire a contractile phenotype *in vitro* [13]. Moreover, when implanted *in vivo* in a canine model of dilated cardiomyopathy (DCM), they attenuated cardiac remodeling [14]. This result is likely to be due to the fusion of skeletal myoblasts with the surrounding myocardium rather than to direct cell differentiation, as suggested by *in vitro* experiments [15]. As discussed in the following section, clinical trials demonstrated that skeletal myoblasts are not able to couple electrically with host tissue, leading to arrhythmia events [5].

The role of hematopoietic stem cells (HSC) in cardiac repair has been investigated by several research groups and their contribution to cardiac regeneration *in vivo* has been heavily debated, being the ability of HSC to transdifferentiate to other lineages still questionable. Indeed, evidence of the ability of bone marrow-derived c-kit+ HSC to help cardiac tissue healing has been given using two different approaches: c-kit+ cells were (i) either delivered to the infarcted site by intramural injection [16] or (ii) mobilized from bone marrow through growth factor administration [17]. More recently, elegant experiments compellingly clarified that HSC are not able to acquire contractile phenotype *in vivo* [18-20]. Nonetheless, a subset of bone marrow hematopoietic precursors expressing CD34 and CD133 has been proven to contain endothelial progenitors. Thus, they have been tested for revascularization protocols in hind limb ischemic animals and could be proposed for cardiac infarction therapy [21]. On the other hand, the results obtained in preliminary investigations in which another bone marrow-derived stem cell subset, mesenchymal stem cells (BM-MSC or MSC) were challenged as a candidate for cellular cardiomyoplasty, raised great enthusiasm for such a cell subpopulation. Recent studies clarified that the direct contribution of MSC to cardiac repair in terms of production of new contractile cells is minimal if any, while a paracrine effect on the diseased tissue of such cells is universally recognized [22]. Such cells are also appealing for their ability to induce a certain degree of immune tolerance [23].

The presence of a small reservoir of cardiac resident progenitor cells (CPC or CSC) has been recently demonstrated in human as well as in other mammals' heart [24]. Such tissue-resident cells participate in myocardial homeostasis and retain a limited regenerative capacity throughout organism lifespan [1]. All the subsets so far identified through the expression of stemness markers (c-kit+, Sca-1+, Islet-1+) demonstrated the ability to give birth to new contractile cells *in vitro*, while only c-kit+, Sca-1+ progenitors were shown to be

involved in post-natal cardiac tissue homeostasis *in vivo* [25]. In fact, the presence of Islet-1+ cells appears to be limited to fetal life and their contribution to the endogenous program of cardiovascular repair is still unknown; on the other hand, the very low number of c-kit+ and Sca-1+ cells in the myocardium is considered the limiting factor of cardiac regeneration [26]. Furthermore, among the adult stem cells, a novel "artificial" subset can be recognized: induced pluripotent stem cells (iPSC, Figure 2). This cell type can be produced *in vitro* by transducing somatic cells with a combination of transcription factors able to induce the nuclear reprogramming of differentiated cells. These cells, which display the functional features of pluripotent embryonic stem cells, have been credited of the ability to produce new cardiomyocytes. They could thus be the source of autologous, although genetically modified, patient-specific contractile cells [27]. Moreover, the possibility to directly obtain functional cardiomyocytes by the genetic reprogramming of postnatal cardiac or dermal fibroblasts has been demonstrated [28]. Such a result was firstly obtained *in vitro* but also when the cells were transplanted into mouse hearts one day after transduction of transcription factors (GATA-4, MEF-2c, Tbx-5) known to be involved in cardiac muscle development. Nonetheless, the reprogramming and differentiation efficiency of these cells appears to be really low, thus requiring an efficient purification step before they can be implanted *in vivo*. Additionally, safety concerns due to the use of genetically modified cells and / or viral vectors remain.

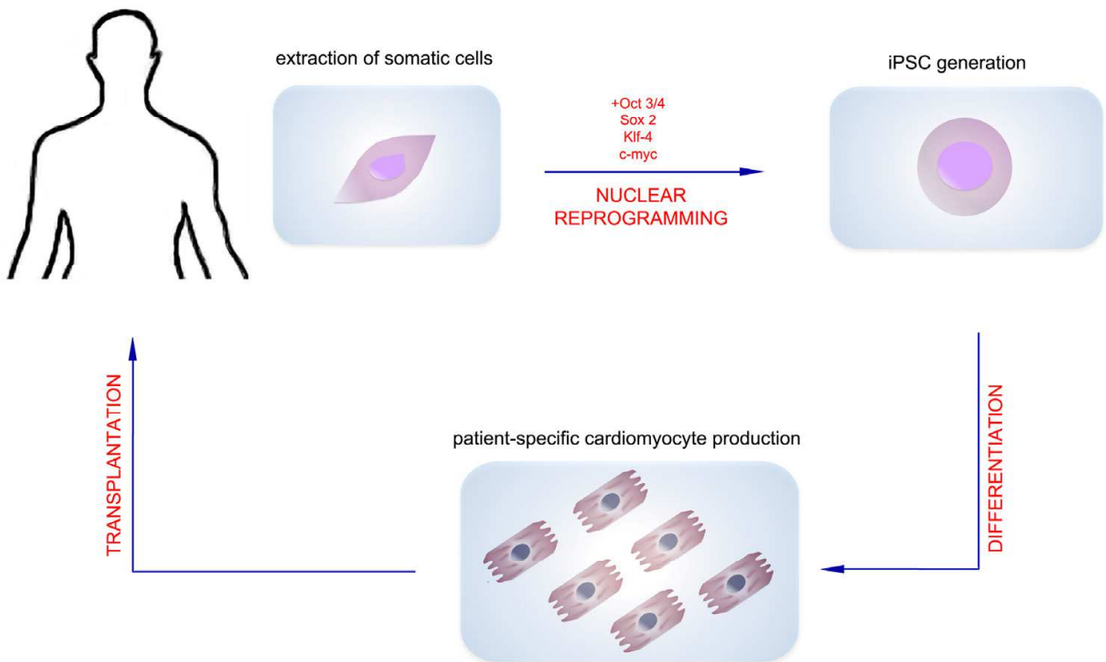

Fig. 2. Induced Pluripotent Stem Cell Generation. Induced pluripotent Stem Cells (iPSC) can be generated by reprogramming somatic cells through their transduction with four transcription factors. iPSC share functional similarities with Embryonic Stem Cells (ESC) and can be differentiated towards cardiomyocytes, thus representing an autologous source of contractile cells

3. Stem cell delivery to the injured heart

As previously said, cell route of delivery to damaged heart represents the major topic in the setup of efficient, minimally invasive techniques to treat cardiac pathologies. Recently, a number of techniques to deliver stem cells to the injured site have been proposed but questions remain regarding the optimal approach able to favor high cell retention, differentiation rate and clinically relevant improvement in cardiac performance.

a) Direct injection

Stem cell direct *intramural injection*, including trans-epicardial and trans-endocardial cell injection, is the elective strategy for patients with severe occlusion of coronary vessels. In particular, trans-epicardial approach consists in the direct injection of a high number of cells into the infarcted area or around the border zone. Endocardial stem cell injection is performed using catheters such as MyoStar™ injection catheter (Biosense Webster) integrated with imaging systems like NOGA® system (Cordis Corp., Warren, NJ, USA), which allows real-time three-dimensional reconstruction of left ventricle as well as the targeting and functional assessment of specific myocardial area [29]. Such procedures are highly invasive since they require open-heart surgery and gave contrasting results so far. For example, pre-clinical studies performed on experimental animals demonstrated that, although a certain extent of cardiac repair was achieved when bone marrow Stro-3+ perivascular cells are implanted *in vivo*, the cells vanished from the application site within few days [30]. In other reports, when Sca-1+ cardiac resident stem cells were injected in infarction border zone, a modest but significant improvement in cardiac function was reported, with evidence of cell engraftment and differentiation [31]. Finally, in another pre-clinical study, bone marrow-derived c-kit+ cells were shown to repair entire ventricular areas while massively engrafting and differentiating in contractile and vascular figures *in vivo* [32]. Of interest, independent groups already demonstrated that c-kit+ bone marrow-derived hematopoietic stem cells fail to acquire contractile phenotype when implanted in diseased myocardium [19, 20]. Such discrepancies are not surprising since different stem cell subsets or preparation protocols were probably used in these studies.

Stem cells can be delivered *intravenously* to the heart, through *coronary arteries* or even through *retrograde coronary sinus*. The major drawback of stem cells being infused through peripheral venous system seems to be the low retention of cells into infarcted area. Results obtained in pre-clinical animal models showed that this minimally invasive approach results in a significant percentage of injected cells being sequestered in lungs, liver or spleen, due to blood flow [33]. On the other hand, intracoronary or retrograde coronary sinus infusion of the cells are mainly performed after acute myocardial infarction using an angioplasty balloon and high pressure to deliver cells to heart muscle [34]. The coronary route was proven to be free of stem cell systemic delivery, while a limited number of cells could be found in the infarcted area [35].

Finally, an interesting attempt with stem cells being injected into the *pericardial cavity* has been proposed. By this means, a higher number of cells could be deposited and retained in the pericardial cavity, while migration across the visceral pericardium is required (Table 1).

DELIVERY METHOD	ADVANTAGES	DRAWBACKS
intravenous	No invasive technique	Cells can be sequestered in lung, liver, spleen
intracoronary	No risk of systemic delivery Direct delivery to the target site	Few cells delivered
intramyocardial	Direct delivery	Risk of perforation
retrograde coronary sinus	Homogeneous cell delivery	Endothelial wall transmigration required
intrapericardial	Large number of cells delivered	Visceral pericardium transmigration required

Table 1. Advantages and disadvantages of injecting stem cells by intravenous, intracoronary, intramyocardial, retrograde coronary sinus or intra-pericardial route

b) Injectable scaffolds

Injectable scaffolds are defined as materials offering the unique solution of replacing damaged myocardial ECM and/or delivering cells directly to the infarcted region while holding the potential for minimally invasive delivery [36]. Such scaffolds can be composed of biocompatible microspheres or in situ gelling materials having reasonable dimensions as to surpass capillary barrier. They are considered a promising tool for stem cell delivery to damaged myocardium. In situ gelling materials are generally made of components of extracellular matrix (ECM), which are induced to a transition after being implanted *in situ*. Complex injectable gelling materials have been prepared by decellularization technique out of ventricular or epicardial ECM, thus possibly avoiding animal-derived components and paving the way to the definition of patient-specific treatments.

The use of injectable, synthetic microspheres has already been proven promising in the treatment of neurological diseases *in vivo* [37]. Recently the possibility of using injectable scaffolds in cardiac cell therapy has been explored by interfacing murine mesenchymal (mMSC) and cardiac stem cell (mCSC) lines with poly-lactic acid (PLA) microspheres having a diameter of 30 and 100 m. Preliminary *in vitro* experiments demonstrated that such cells can be grown onto PLA microspheres while preserving their phenotype, but the formation of cell clumps can hamper the application of this technique [38]. The use of dynamic seeding techniques (i.e. bioreactors) would favor a more homogeneous distribution of the cells. An interesting approach has been recently proposed to deliver human mesenchymal stem cells to the injured myocardium: RGD-modified alginate microsphere (diameter: 200-700 m) encapsulation of hMSC was setup. *In vitro* experiments showed that hMSC could survive, proliferate and migrate through the porous material. When intramyocardially injected in a rat model of myocardial infarction by left anterior descendant coronary (LAD) ligation, cell-loaded alginate microspheres promoted angiogenesis and prevented LV negative remodeling [39]. Nonetheless, few human cells were found in the injection area after few days, while microbead remains were still present

within host myocardium 10 weeks after the injection. The aspect of microbead resorption should thus be addressed before clinical perspectives could be foreseen.

c) Scaffold-based technology

The possibility of using biocompatible scaffolds to deliver stem cells to the injured heart has been explored by a number of independent research groups so far. The scaffolds proposed are natural of synthetic but when designing cardiac-specific constructs, a number of requirements should be fulfilled. For example, it cannot be neglected that myocardial contractile function relies on the transmission of electrical and mechanical forces throughout a functional syncytium. So, the integrity of the tissue has to be preserved. For this reason, a cardiac-specific scaffold should comply with tissue architecture and thus be deformable enough to indulge and, if possible sustain cardiac contraction. Moreover, as far as stem cell engraftment is concerned, scaffolds should be able to start at least cell alignment and commitment to favor stem cell electromechanical coupling with host tissue. In this respect, the work of Mandoli and collaborators using Cerium Oxyde nanoparticles to affect poly-lactic acid film surface and obtain a controlled nanorugosity appears intriguing [40]. In fact, far from being a noxious compound for stem cells, ceria was able to induce cardiac stem cell alignment and growth. Nonetheless, cardiac tissue is extremely complex and highly demanding in terms of blood supply and catabolite removal, so that porous scaffolds that could allow microvascular branches formation and oxygen perfusion are to be preferred. To fulfill such requirements, the first attempts were performed by the group of Thomas Eschenhagen. Neonatal cardiomyocytes were seeded in Collagen I + Matrigel to produce Engineered Heart Tissue (EHT). Continuous contractile activity up to 1 week *in vitro* as well as cell survival and integration *in vivo* in singenic rat hearts were reported [41]. In another attempt, anisotropic accordion-like honeycomb scaffolds were prepared by excimer laser microablation using poly(glycerol sebacate) as an elastomeric tool to mimic anisotropic cardiac muscle stiffness distribution [42]. Although the authors demonstrated that such scaffolds promote neonatal rat cardiomyocyte alignment and contraction, *in vivo* testing has not been performed so far. The same material has been utilized to produce elastomeric patches on which human embryonic stem cell-derived cardiomyocytes were grown, showing that it is indeed possible to observe spontaneous beating activity *in vitro* up to 3 months [43]. Such patches were shown to be suitable as delivery systems and, when sutured in the absence of cells onto healthy rat left ventricle, they did not affect cardiac contractile activity. More basic studies were also conducted to study the ability of stem cells to interface with different synthetic and natural materials. In this respect, few research groups focused on the possibility to drive a certain extent of stem cell commitment through tailoring scaffold physical and chemical properties, independently of biological cues. In this respect, a common agreement on the ability of stem cells to sense substrate rugosity and elasticity has been reached [44]. Thus, in order to rule out the occurrence of spontaneous events of differentiation in implanted cells, the possibility to induce *in vitro* stem cell commitment on scaffolds towards a desired phenotype is being investigated. Indeed, Engler and collaborators compellingly demonstrated that the possibility to affect stem cell fate determination by simply tuning substrate elasticity as to match tissue-specific stiffness, exists. Recently, this concept has been corroborated by other research groups, showing that cardiac resident progenitors (Sca-1+ CPC) can be committed to cardiac phenotype by the physico-chemical signals arising from matrix, but biological factors are needed to complete the differentiation process [45, 46].

d) Preparation of thick cardiac substitutes by Scaffold-free technology

To overcome the problem of poor cell retention reported in cell injection experiments in the heart [30] and avoid the release of possibly harmful scaffold byproducts, scaffold-free technology has been developed, in which cells are grown in a monolayer onto thermo-responsive surfaces and easily detached in the form of cell sheet by lowering the temperature [47]. Such technology takes advantage of the ability of polymers like poly-N-isopropylacrylamide (PNIPAAm) to shift between hydrophobic and hydrophilic status when the temperature ranges from 37ºC to 32ºC. Cell sheets can be serially stacked to obtain multilayered scaffoldless constructs (Figure 3). Such an approach has already been applied to obtain cell sheets composed of rodent [48, 49] and human [50] cells. Given the need for thick cardiac substitutes suited to comply with cardiac muscle continuous contractility, thermo-responsive technology has been envisaged as a possible answer to the lack of heart donors. Pre-clinical trials performed onto experimentally infarcted animals demonstrated that when a murine adipose-derived monolayer sheet is leant onto injured myocardium, it can be retained and help tissue repair [48]. Similarly, striking results are obtained when a Sca-1+ cardiac progenitor cell-derived sheet is used [49]. Finally, an interesting approach has been recently proposed to deliver cardiac stem cells cultured in the form of cardiospheres to the injured heart: cardiospheres were embedded into a cardiac stromal cell-derived sheet obtained by using poly-lysine/ collagen IV-coated dishes [51]. The formation of mature vessels as well as new cardiomyocytes *in vivo* was reported after 3 weeks.

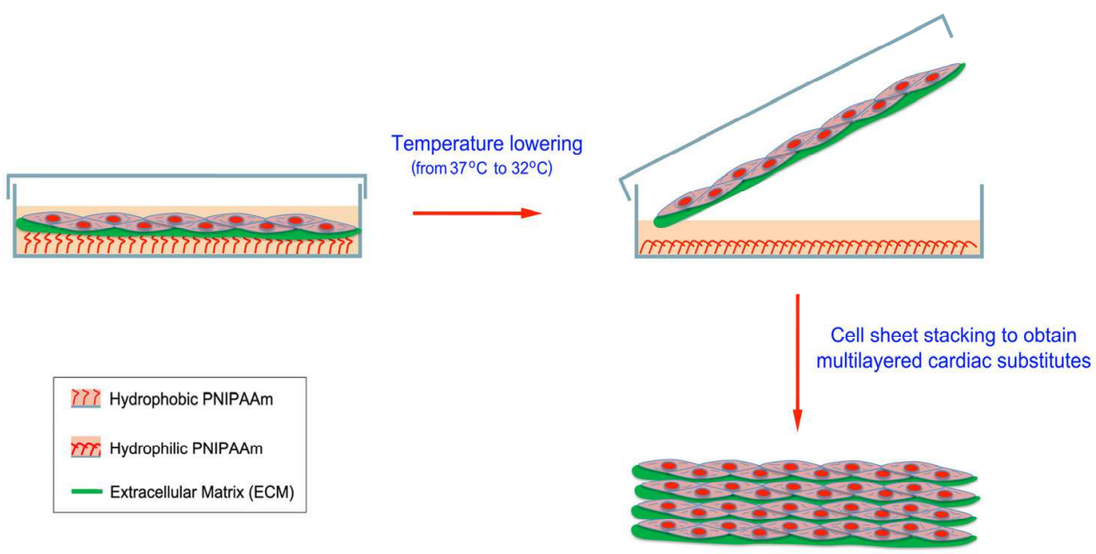

Fig. 3. Generation of scaffoldless multilayered bio-constructs by means of thermo-responsive technology: cells grown in a monolayer onto thermo-responsive poly-N-isopropilacrylamide (PNIPAAm)-coated dishes can be detached by lowering the temperature below 32°C. At 37°C the surface is highly hydrophobic and allows cell adhesion. When the temperature is lowered, PNIPAAm becomes hydrophilic, the cell sheet is detached and extracellular matrix (ECM) preserved. Multilayered cell sheets can be obtained by serially stacking monolayered sheets

4. Clinical trials

In the attempt to transfer bench experience to bedside, a number of clinical trials in which different stem cell or progenitor subsets are used have been approved (see http://www.clinicaltrials.gov). Most of them are still in the recruitment phase while some already gave indications and preliminary results. Since most of the ongoing trials are based on the injection of raw stem cell preparations (mostly bone marrow-derived cells), the time and route of cell application remain the key problems to be addressed before proceeding to routine clinical practice. In this respect, recent animal experiments demonstrated that the acute phase of myocardial infarction is probably not suitable for stem cell engraftment and differentiation [52]. Therefore, the right moment in which stem cells should be delivered is to be studied. An overview on some of the ongoing clinical trials is given below.

1. **MAGIC (Myoblast Autologous Grafting in Ischemic Cardiomyopathy).** In one of the first phase II clinical trials setup to study the possibility to use stem cells to treat cardiac pathologies, ninety-seven (97) patients undergoing coronary artery bypass grafting (CABG) were enrolled. 400-800 X 10^6 autologous myoblasts harvested from patient muscle biopsy were implanted in the akinetic area of ventricular wall 21 days after *in vitro* culture. The follow-up after 30 days and 6 months demonstrated the arising of arrhythmia events, thus requiring the implantation of pacemaker. Moreover, no cardiac function improvement was reported. Such negative results were ascribed to the inability of skeletal myoblasts to balance cell death and achieve complete electromechanical integration with the recipient myocardium. Finally, skeletal myoblast administration was reported to determine no enhancement in major cardiac adverse events and mild effects on left ventricular remodeling process [53, 54]. More recently, final results from **SEISMIC** [Safety and Effects of Implanted (Autologous) Skeletal Myoblasts (MyoCell) Using an Injection Catheter] Trial, a phase II-a study encompassing 40 patients experiencing congestive heart failure and receiving percutaneous intramyocardial injection of autologous skeletal myoblasts, reported the feasibility and safety of this procedure without significant arrhythmogenic events recorded at 6-month follow-up with respect to control groups, although left ventricular ejection fraction did not result significantly improve. These encouraging results suggest that myoblast cell therapy could be considered as a potential effective treatment when associated with standard medical therapy in patients with previously implanted cardiac defibrillators [55].
2. **TOPCARE-CHD, -AMI, -DCM (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction, Chronic Stable Ischemic Heart disease or Dilated Cardiomyopathy)**. In this complex clinical trial, a total of 346 patients were classified to CHD, AMI or DCM pathologies and infused either with bone marrow cells (BMCs), blood-derived stem cells, or no infusion. In TOPCARE-CHD, 121 patients (mean age: 59) with chronic stable ischemic heart disease (CHD) were treated. Although complications occurred in 21% of the patients during 3 months follow-up, BMC intracoronary administration was related with a reduction of both brain and atrial natriuretic peptide (NTP) serum levels (indicators of LV remodelling process) in the remaining population (79%), especially in patients with higher NTP levels at baseline and receiving a greater BMC number with a high functional capacity. Moreover, these results were also correlated with a left ventricular ejection fraction (LVEF) increase and better survival during the further follow-up, suggesting that cell therapy could be

associated with cardiac function enhancements in patients with advanced chronic post-infarction heart failure [56]. Similarly, two hundred and four (204) patients were treated using bone-marrow-derived progenitor cells directly into the infarct artery three to seven days after an acute myocardial infarction (AMI). A statistically significant 2.5% improvement in left ventricular ejection fraction at four months was reported for patients randomized to the bone marrow injection [57]. Finally, intracoronary infusion of bone marrow cells was performed in 33 patients with dilated cardiomyopathy (DCM) by using an over-the-wire balloon catheter. Three month follow-up demonstrated an improvement in left ventricular pump function while a modest improvement in Brain Natriuretic Peptide (BNP) levels was reported after 1 year [6]. Importantly, the conditions chosen in the present clinical trial were representative of different conditions (acute, chronic phase) encountered in the clinic. Unfortunately, no clear indication on stem cell characterization or on their actual ability to regenerate contractile cells is available.

3. **TRACIA STUDY (Intracoronary Autologous Stem Cell Transplantation in ST Elevation Myocardial Infarction).** The phase II/ III clinical trial aimed at evaluating the effects of intracoronary administration of adult stem cells on LV ejection fraction and major adverse cardiovascular events (MACE) after 6 months follow-up. For this reason, 1-2 million CD34+ cells were injected through the infarct-related artery few days after post-infarct angioplasty using an "over-the-wire" catheter in 80 patients aging from 20 to 75 years. The results of this study are still to be published.
4. **Combined CABG and Stem-Cell Transplantation for Heart Failure.** Intramyocardial delivery of autologous bone marrow cells extracted from iliac crest and purified by Ficoll centrifugation, during cardiac surgery for CABG intervention in 30 patients, as compared to 30 patients undergoing CABG without cell infusion. Although information on the number and characteristics of cells to be injected has not been given, the trial is currently ongoing and the follow-up is scheduled in 6-12 months (http://clinicaltrials.gov).
5. **POSEIDON-Pilot Study (The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis Pilot Study)** Poseidon-pilot Study is a phase I/ II multi-center trial in which the trans-endocardial injection of autologous Mesenchymal Stem Cells (20-, 100-, 200 X 10^6) is compared to autologous non-purified bone marrow cells and to allogeneic human Mesenchymal Stem Cells. The implant is performed during cardiac catheterization using the Biocardia Helical Infusion Catheter in fifty (50) patients suffering from chronic ischemic left ventricular dysfunction secondary to myocardial infarction. The data collection is currently ongoing.
6. **SCIPIO (Cardiac Stem Cell Infusion in Patients With Ischemic CardiOmyopathy).** This phase I clinical trial is aimed at assessing the safety and effectiveness of intracoronary autologous cardiac stem cell therapy. As such, forty (40) patients suffering from ischemic cardiomyopathy are exposed to intracoronary injection of cardiac resident stem cells (CSC). Cardiac stem cells are harvested from right atrial appendages and selected for c-kit expression, cultured and expanded in vitro prior to injecting them via intracoronary route, three to five months after CABG surgery. The hypothesis is that CSC infused into nonviable myocardial segments will regenerate infarcted myocardium by differentiating into cardiomyocytes and vascular cells. The preliminary results are encouraging: in the nine patients treated at four months after

CSC infusion, LVEF increased from 31.3 + 2.5 percent before CSC infusion to 38.8 + 3.2 percent four months after CSC infusion. Moreover, in the five patients in whom data are available at 12 months after stem cell infusion, the improvement in LVEF observed at four months was even greater, averaging 15% at 12 months. The follow-up is scheduled in 1,5 years.

7. **ALCADIA (AutoLogous Human CArdiac-Derived Stem Cell to Treat Ischemic cArdiomyopathy).** In this phase I, multicenter clinical trial, a rather different approach is followed. In fact, patients' own cardiac stem cells obtained by endo-myocardial biopsies are delivered by a single intramyocardial injection. The cells injected are 0.5 million cells/kg (patient body weight) and their engraftment should be favored by the concomitant implantation of gelatin hydrogel sheet releasing human recombinant beta Fibroblast Growth Factor (bFGF), during CABG surgery. The study has been designed to treat refractory heart failure, ischemic cardiomyopathy or ventricular dysfunction cases. Importantly, this is the first clinical trial, to our knowledge, in which a human recombinant growth factor is used. Unfortunately, the number of enrolled patients is limited to six (6).
8. **REGEN-IHD (Bone Marrow Derived Adult Stem Cells for Chronic Heart Failure).** In this phase II/ III study, granulocyte-colony stimulating factor (G-CSF) is subcutaneously administered for 5 days to patients with heart failure secondary to ischemic heart disease to mobilize CD34+ bone marrow stem cells. A concomitant intracoronary or intramyocardial administration of bone marrow derived stem cells is performed. The number of enrolled patients is high (165) and the aim of the study is to compare the effects of G-CSF and autologous bone marrow progenitor cell infusion on the quality of life and left ventricular function in the patients. The follow-up timepoint is scheduled in 6-12 months.

A number of papers reporting statistical analyses and comparisons among the clinical trials in which stem and progenitor cells have been adopted are currently available. [For further information, please refer to www.clinicaltrials.gov].

5. Conclusions

The possibility to treat cardiac diseases by cell therapy techniques is an extraordinary promise. While a number of different approaches has been so far proposed to setup minimally invasive techniques for cardiac repair, few of them being already in the clinical experimental phase, basic questions still need to be addressed. In fact, the molecular processes leading to cardiac differentiation still need to be fully clarified, while the impact of novel, genetically modified cell types obtained from adult differentiated cells on cardiac microenvironment deserve further investigations. More importantly, the seek to identify suitable delivery systems (i.e. scaffolds) able to foster stem cell survival, growth and differentiation, while degrading without negative effects as the formation of new tissue occurs is still open. A look at the literature reveals that an impressive effort to translate the information obtained by *in vitro* and pre-clinical studies to the bedside is being produced. In particular, a number of stem cell subsets, which have been previously tested *in vitro* and in animal models, are currently being tested in phase I, II clinical trials. As expected, the predominant delivery system used in the ongoing clinical trials is intracoronary or intramural injection of stem cells. The possibility to adopt tissue engineering techniques to

design patient-specific cardiac substitutes containing synthetic or natural scaffolds is still far from being taken into consideration for clinical application, since any single formulation will have to be approved before clinical testing.

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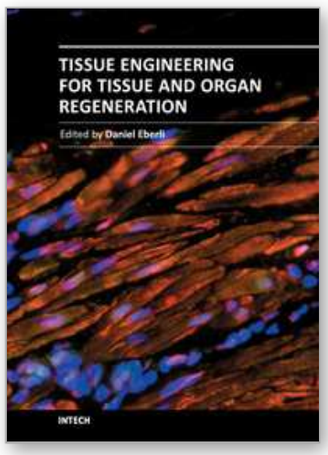

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Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

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