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Cell therapy, 3D culture systems and tissue engineering for cardiac regeneration ☆

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ABSTRACT

Ischemic Heart Disease (IHD) still represents the "Number One Killer" worldwide accounting for the death of numerous patients. However the capacity for self-regeneration of the adult heart is very limited and the loss of cardiomyocytes in the infarcted heart leads to continuous adverse cardiac-remodeling which often leads to heart-failure (HF). The concept of regenerative medicine comprising cell-based therapies, bio-engineering technologies and hybrid solutions has been proposed as a promising next-generation approach to address IHD and HF. Numerous strategies are under investigation evaluating the potential of regenerative medicine on the failing myocardium including classical cell-therapy concepts, three-dimensional culture techniques and tissue-engineering approaches. While most of these regenerative strategies have shown great potential in experimental studies, the translation into a clinical setting has either been limited or too rapid leaving many key questions unanswered. This review summarizes the current *state-of-the-art*, important challenges and future research directions as to regenerative approaches addressing IHD and resulting HF.

Contents

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1. Introduction

1.1. Medical background

Ischemic Heart Disease (IHD) still represents the "Number One Killer" worldwide accounting for the death of numerous patients and a tremendous socio-economic impact [1]. IHD leads to myocardial infarction (heart attack) which in turn leads to myocardial necrosis and the loss/death of cardiomyocytes. Unfortunately, the capacity for self-regeneration of the adult heart is very limited. On the one hand the cardiomyocytes do not have the capability to divide and on the other hand the heart lacks a sufficient reservoir of remnant cardiac stem cells (CSCs). Although the treatment options for IHD comprising medical drug therapy, percutaneous interventions (PCI) and surgical revascularization have emerged over the past decades, the loss of cardiomyocytes in the infarcted heart leads to a continuous adverse cardiac remodeling. This includes typical phenomena such as progressive myocardial wall thinning, fibrosis and dilation leading to a significant reduction of cardiac function over time. If IHD remains untreated or is not treated timely and efficiently, IHD may lead to heart failure (HF). Approximately five million people in the U.S. are affected by HF today, with an incidence approaching 10 per 1000 population among individuals older than 65 years of age [2].

To date, heart transplantation still represents the most effective option for patients suffering from HF. However, in addition to the logistical and medical complexity of this therapeutic option, donor organ shortage represents a major limitation and up to 20% of the patients die while on the "waiting list" [3]. Therefore, there is a substantial and urgent therapeutic need to develop alternative therapy options.

In this regard, the concept of regenerative medicine comprising of cell-based therapies, bio-engineering technologies and hybrid solutions has been proposed as a promising next generation approach to address IHD and resulting HF. Numerous strategies are under evaluation for their beneficial effects on the failing myocardium comprising of cell therapy concepts, cardiac tissue engineering approaches and hybrid technologies. However, despite their great potential as demonstrated in experimental studies, the translation of such strategies into a clinical setting has either been limited or too rapid leaving many key questions unanswered.

1.2. Aim of the review

This review article provides an overview of the current *state-of-the-art* for using regenerative strategies to address IHD and resulting HF. Considering these therapeutic approaches and their modes of action they can be classified as a form of drug delivery. As shown in this review, the transplanted cells and tissue provide not only the precursors that can differentiate into functioning myocardial cells but also through paracrine effects to recruit cells, mediate inflammatory response, remodel the extracellular matrix (ECM) and prevent deleterious downstream effects of fibrotic scar tissue formation subsequent to IHD. In this light, transplanted cells and tissue may be seen as a promising and highly effective means of drug delivery.

2. Background cell based therapies and cardiac tissue engineering

2.1. The concept of cardiac regenerative therapies

Biological therapies using cells and tissue are intended to repair and regenerate the diseased heart by improving tissue structure and function. Cell based therapies also known as in situ cardiomyoplasty are defined as the application of single cells or small clusters of cells into the circulatory system (intravenous application) or directly into the myocardium (intramyocardial application). Tissue based strategies consisting of an arrangement of cells and extracellular matrix are either based on in vitro cell culture methods with subsequent implantation (e.g. epicardial application) or in situ approaches which combine cells with injectable biomaterials (intramyocardial injection) [4].

2.2. History of cell therapy and tissue replacement

To date, in-situ cardiomyoplasty has been reported to have only limited effects on patients [5,6]. While the initial cardiomyoplasty studies were motivated by the belief that the transplanted cells would primarily acquire a myocyte phenotype (by transdifferentiation) and replace lost or damaged cardiomyocytes [7], in subsequent studies evidence accumulated that most favorable outcomes could be attributed to the so called paracrine effects comprising the release of growth factors and other cytokines to promote angiogenesis, appropriate matrix remodeling and the induction of a more favorable environment for healing and functional restoration [8–10].

The field of cardiac tissue engineering has continuously moved beyond static two-dimensional (2D) culture systems to three-dimensional (3D) systems that are more representative of living systems. However, many shortcomings do remain, and importantly, a key hurdle is reflected in the limited ability to provide appropriate tissue structures for current in-vitro and in-vivo studies [11]. Some of these shortcomings are related to the need for next generation culture systems that can provide the appropriate scaffold, soluble factors, mechanical stimulation and electrical environment to direct the development of phenotypically appropriate tissue [11]. Furthermore, there is also a need for measurement and characterization techniques that can be used to assess and characterize the structure and function of both native and engineered tissues such that quality criteria can be defined that can match that of the native tissue [12]. Fig. 1 provides insight into the nature of cardiac tissue as it matures from an early postnatal to an adult phenotype. The figure shows the

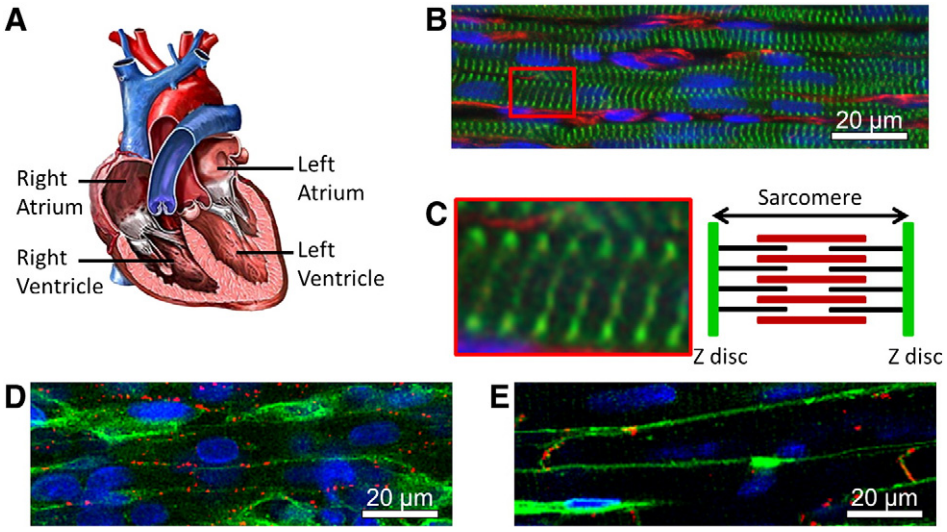

Fig. 1. Cardiac structure. Cross-section showing four chambers of the heart (A). Staining of postnatal day 12 left ventricular cardiac tissue of rat with α-sarcomeric actinin (green) to identify Z-discs of myocytes and vimentin (red) to identify non-myocytes (e.g. fibroblasts) (B). Zoomed region from B showing sarcomeres which are defined from Z-disc to Z-disc (C). Postnatal day 12 (d) and adult (e) left ventricular cardiac tissue of rat stained with wheat germ agglutinin (green) to identify cell borders and Cx43 (red) to identify gap junctions. Myocytes appear dark with Cx43 plaques located on the lateral sarcolemma in postnatal day 12 and concentrated at cell ends in adult tissue (D and E).
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characteristics of mature rat cardiac tissue where the myocytes are aligned, the z-bands are regularly spaced and the Cx43 proteins are distributed mainly at the ends of the cells. In early postnatal tissue however this arrangement has not yet been realized. This early development phenotype is similar to in-vitro cultured myocardium. Therefore it is necessary to consider the structural and functional properties of these engineered tissues as they are cultured, transplanted and then mature in-vivo to functional myocardium.

2.3. Need for bioreactors

In-vitro culture of cells for cardiomyoplasty rely on specialized cell culture environments to control environmental conditions in order to expand and even differentiate progenitor cells. These specialized cell culture environments are very different depending on the final product but all must provide a harmonized environment where the cells can remain alive in a state where they can be used for therapy or moved to another environment for subsequent processing. In addition to static culture environments such as t-flasks and multi-layer vessels, spinner-flasks, rotating wall vessels and direct perfusion systems are used to culture cells prior to subsequent use as an injectable therapeutic. Spinner-flask bioreactors allow higher cell densities and overcome mass-transfer limitations of static culture environments. They have also been shown to improve cell seeding and development of cardiac tissue constructs [13,14]. Rotating wall vessels produce lower levels of fluid shear due to mixing than spinner flasks and can also improve mass transfer rates. These vessels have been used for both cell suspensions and small engineered tissue constructs and have demonstrated the ability to form tissue with more elongated myocytes, improved intercellular connections and form mechanically competent tissue structures [15–18]. Systems that employ direct perfusion overcome limitations associated with poor cell infiltration and non-uniform seeding/distribution and have been shown to enhance cell survival, growth, and function [14,19–21].

3. Cell types for cardiac repair

Numerous types of stem cells are currently under evaluation for their capacity to promote cardiac repair and regeneration [22]. These include crude bone marrow-derived/circulating progenitor cells (BMPCs) and their subpopulations, such as marrow stromal derived stem cells (MSCs) and endothelial progenitor cells (EPCs); skeletal myoblasts (SMs), adipose tissue derived stem cells (ATSCs), umbilical cord derived stem cells (UPCs), embryonic stem cells (ESCs), induced pluripotent stem cells (IPSCs) and resident cardiac stem or progenitor cells (CSCs) [22].

3.1. Crude bone marrow-derived/circulating progenitor cells (BMPCs)

Most researchers have been focusing on crude bone marrow-derived/circulating progenitor cells (BMPCs) or so called bone marrow mononuclear cells for their potential to repair the heart. Due to their abundant availability, easy access and particularly, their safety profile, BMPCs have a high clinical potential. Importantly, paracrine effects appear to be the major mechanism of BMPCs after transplantation, while the evidence of BMPC trans-differentiation into cardiomyocytes remains controversial. Several studies have shown that cardiac differentiation capacity of BMPCs in vivo is very low and cannot explain the therapeutic effect provided to the infarcted heart [22,23]. Being the most clinically used cell source, it was recently demonstrated that for acute myocardial infarction and chronic ischemia the long-term mortality was significantly reduced [24]. Unfortunately, it has to be mentioned that most often only the positive results are presented while the negative outcomes are neglected. Therefore, a certain publication bias may be present in several available meta-analyses and should be taken with caution [25].

3.2. Mesenchymal stem cells (MSCs)

Mesenchymal stem cells (MSCs) are traditionally isolated from the bone marrow via hip- or sternal puncture and are considered a valuable cell-source for cardiac repair [26]. These cells are defined by three criteria including adherence, the expression of specific surface markers such as CD105, CD73 und CD90 and their ability to differentiate into the adipoietic, chondrogenic and osteogenic lineage [26,27]. Major advantages of these cells are their easy accessibility as well as their strong expansion capacity representing a highly relevant clinical cell source. In addition, MSCs have repeatedly been suggested to be immunoprivileged [26]. It also has been shown that MSCs secrete large amounts of angiogenic and antiapoptotic factors in vitro including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), transforming

growth factor B, hepatocyte growth factor and adrenomedullin (an antiapoptotic peptide) [26]. In addition, MSC produces insulin-like growth factor 1 (IGF-1) which is a mediator for myocardial growth and placental growth factor (PGF) which has an effect on angiogenesis and monocyte recruitment. Therefore, MSCs have been repeatedly investigated in preclinical animal studies showing a positive effect on the ejection fraction after myocardial infarction [26–30] and thus were recently introduced into clinical safety and feasibility trials [31,32]. Although the differentiation of MSCs into cardiomyocyte-like cells expressing various cardiac markers has been demonstrated in vitro, their ability to differentiate into functional cardiomyocytes remains unclear. However, recent reports indicate that bone-marrow MSCs can be programmed into a cardiac committed phenotype increasing their clinical relevance and potential [33]. This is besides their principal suggested function through multiple paracrine effects [26] comprising the secretion of cytokines and growth factors that suppress the immune response, inhibit fibrosis and apoptosis, enhance angiogenesis and/or stimulate the endogenous precursor cells for cardiac differentiation [26].

3.3. Adipose tissue-derived stem cells (ATSCs)

The adipose tissue represents an ideal source to isolate stem cells that are comparable to those isolated traditionally from the bone-marrow. Importantly, adipose tissue-derived stem cells (ATSCs) have been reported to have even a stronger in vitro differentiation proliferation potential [34]. ATSCs can be easily isolated by a simple liposuction which is a safe and less invasive procedure than a bone marrow puncture. Recent data have demonstrated a beneficial therapeutic effect on cardiac function in small animal studies as well as large animal trials [35] after intra-myocardial delivery of ATSCs following myocardial infarction and have built the basis for the initiation of first-in-man clinical trials.

3.4. Hematopoietic stem cells (HSCs)

Hematopoietic stem cells which are defined by the surface markers CD34 and CD133 have been demonstrated to be more effective than crude bone-marrow derived mononuclear cells. In a preclinical setting, the application of human CD34 cells displayed an improved impact on the heart function which was also associated with an increased neovascularization and less fibrotic remodeling processes [36]. Based on these animal data, randomized clinical studies confirmed safety and feasibility of this approach in patients with refractory angina pectoris [37]. While the intra-myocardial injection of CD133 cells was associated with a significantly improved cardiac function in patients that underwent coronary artery bypass grafting (CABG) [38], another recent report did not show any significant benefit on the long-term after intramyocardial cell application [39] questioning the real impact on myocardial regeneration of these cells.

3.5. Endothelial progenitor cells (EPCs)

Endothelial progenitor cells (EPCs) are a circulating, heterogenous cell population most probably originating from the bone-marrow [40]. Based on their capacity to differentiate from circulating mononuclear cells, the EPCs have been grouped into “late” and “early” EPCs [41]. While early EPCs are mainly considered to support endothelial regeneration and angiogenesis, late EPCs are suggested to differentiate into endothelial cells [42]. The differentiation potential of early EPCs into cardiomyocytes is controversially discussed [43]. Interestingly, EPCs of patients carrying cardiovascular risk factors such as hypertension and diabetes displayed an impaired capacity of re-endothelialization in a murine model [44]. Translating these experimental results into the clinical setting, this EPC dysfunction may represent a major limitation of EPC based therapies as most of the anticipated patients present with multiple cardiovascular risk factors.

3.6. Skeletal myoblasts (SMs)

Skeletal myoblasts were also one of the first cell types tested for cardiac repair [45] and displayed beneficial effects on cardiac function after transplantation into the infracted myocardium [46,47]. It has been demonstrated that transplanted skeletal myoblasts integrate and differentiate into myotubules after transplantation improving the cardiac performance in animal models. However, major concerns do remain, as these cells do not electro-physiologically couple to the hosting cardiomyocytes via gap junction proteins such as connexin-43 and others resulting in an increased risk for ventricular arrhythmia after cell transplantation [45,48]. Although the concept of intramyocardial transplantation of skeletal myoblasts made it into the clinical arena and even displayed beneficial effects in small feasibility studies, these results could not be confirmed in a first placebo-controlled trial [49].

3.7. Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) are derived from the inner wall cell mass of the blastocyst and represent the preclinical gold-standard for producing cardiomyocytes. They display the highest plasticity and represent the only pluripotent stem cell source for generation of cardiomyocytes with a complete phenotype that is free from reprogramming-induced genomic alterations and mutations [22]. In experimental studies, ESCs have been shown to improve cardiac function and survival after myocardial infarction [50,51]. However, despite their high regenerative potential ESCs have several limitations and are therefore still in preclinical evaluation: the transplantation of undifferentiated ESCs into the myocardium may lead to the in-vivo development of cardiac teratomas [52] and furthermore human ESCs are associated with ethical issues that have limited their progress in the clinical setting [53]. Until these important problems of tumorigenicity and ethics, along with immunogenicity are resolved, their clinical utilization will be rather limited.

3.8. Induced pluripotent stem cells (IPSCs)

Similar characteristics to ESC are shown by the so-called ESC-like cells, also known as induced pluripotent stem cells (iPSCs). They can be generated via retroviral transduction of stemness transduction factors such as Oct 3/4, Klf4, Sox2 and c-Myc [50,54–56]. They are able to differentiate into cells of all three germ layers including functional cardiomyocytes [57]. In preclinical studies, the transplantation of iPSCs in the infarcted myocardium led to their differentiation into cardiac myocytes where they formed gap junction [58,59]. Furthermore, it was shown that iPSCs can also be generated using reprogramming proteins without the need for DNA vectors [60]. However, despite all enthusiasm, while autologous-derived iPSCs show the advantage over ESCs by avoiding ethical and immunological issues for autologous transplantation, significant safety issues remain unresolved and limit their clinical applicability [61].

In addition, in another set of recent experimental proof-of-concept studies the feasibility of direct reprogramming of cardiac fibroblasts into functional cardiomyocytes in vitro and in-vivo has been reported and may thus even represent a novel strategy for myocardial regeneration without the need for stem or progenitor cells [62].

3.9. Cardiac stem cells (CSCs)

Over the last years, there has been accumulating evidence about the heart harboring its own regenerative potential [63–69]. After identification of resident cardiac stem cell populations, there was a paradigm shift that may lead to the unforeseen opportunity for myocardial repair. CSCs

are defined by various surface markers including C-kit, Sca-1 and others [70]. Messina et al. were the first to describe the isolation and expansion in vitro of adult cardiac stem cells [69]. These clonogenic, so-called cardiospheres (CSPs) were successfully isolated from human heart samples [69]. Furthermore, the authors were able to demonstrate the differentiation capacity of CSPs into vascular cells and cardiomyocytes upon subcutaneous or intramyocardial transplantation (after MI) in an immune-suppressed murine model [69]. In a landmark study of the Anversa group, the authors isolated and expanded human c-kit-positive CSCs from small cardiac samples and demonstrated that human heart has a pool of clonogenic stem cells that acquire cardiac and vascular lineages in vitro and in vivo [67]. Other studies focused on another novel population of cells residing in the heart and being able to proliferate and to differentiate into cardiac cells from rat, mouse and human post-natal hearts. These cells express the cardiac progenitor marker islet-1 but not the stem cell markers Sca-1 or c-kit [71]. Whereas isl1$^+$ cells also express early cardiac differentiation markers like Nkx2.5 and GATA-4, they lack transcripts of mature myocytes. However, when co-cultured in vitro with differentiated myocytes, they spontaneously acquired myocyte characteristics including electromechanical coupling [71]. From the clinical point of view, CSCs may be isolated from human heart using a minimally invasive biopsy procedure [63,66]. Importantly, an autologous approach would therefore minimize the risk for immune rejection or teratoma formation. To address the safety and feasibility first-in-man trials have been initiated [72,73]. However, to date, the exact origin and frequency of CSCs in the heart remains to be elucidated. Furthermore long-term efficacy and the mechanism by which such cells contribute to myocardial repair and regeneration needs to be demonstrated.

4. Experimental and preclinical cardiac cell therapy research

4.1. Experimental research

In the last years tremendous effort has been undertaken to evaluate different stem cell types for their capability of cardiac repair and regeneration in the preclinical setting, comprising of in vitro studies, small animal experimental studies as well as preclinical large animal trials [63,64,74–84].

Small animal models of myocardial infarction (MI) have been used to study the effects of mesenchymal stem cells on post MI ventricular remodeling. These types of studies are particularly important to investigate how changes to the cells during in-vitro processing can influence their downstream physiologic effects. The cells can be modified by altering culture conditions or through genetic modification. Pretreatment of MSCs with melatonin was shown to increase ejection fraction, decrease wall thinning and decrease collagen accumulation in a rat MI model [85]. MSCs have also been preconditioned with TGF-β and cultured on decellularized human tissue patches for subsequent transplantation into a nude rat MI model. This treatment led to fractional shortening of the heart as well as increased angiogenesis [86]. A mouse model was used to investigate the effects of genetically modified MSCs. The MSCs were modified to increase prostaglandin I synthase (PG1S) transcription. When injected into mice post-MI, the increased PG1S production resulted in increased fractional shortening and ejection fraction [87]. Another study modified MSCs by inserting the gene for ATK-1, a cyto protective factor encoding gene. When injected into a rat MI model, these cells were shown to result in a smaller infarct size and decreased myocyte apoptosis [88]. These studies highlight the importance of in-vitro manipulations of the MSCs and how these changes can influence the course of events when the cells are transplanted subsequent to MI. While pre-clinical studies are a powerful tool to investigate the effects of cell manipulations, their most important role is to elucidate the basic biologic and physiologic mechanisms behind the use of transplanted cells and tissue for cardiac disease therapy.

4.2. Preclinical research

In the setting of large animal trials, a recent meta-analysis performed by van der Spoel et al. [84] evaluated the human relevance of stem cell therapy in large animal models of Ischemic Heart Disease. Summarizing, 52 studies with a total of almost 900 animals the authors made interesting findings for future stem cell therapy concepts. The report showed that most of the evaluated studies addressed principal feasibility and safety but also focused on important procedural questions such as timing for cell application, cell numbers, and the definition of appropriate follow-up times. The meta-analysis showed that bone marrow derived mononuclear cells and mesenchymal stem cells represent the most often used cell source in large animal models [28–30,89–91]. In addition, skeletal myoblasts, cardiac progenitors, embryonic and iPSCs have been used [77,92–97]. The number of cells used in most of the studies was between 1×10^6 and 1×10^9, and most of the studies presented with a follow-up between 4 and 8 weeks post transplantation [84]. The meta-analysis revealed significant observations: first, the analysis showed a mean ejection fraction improvement of 7.5% at time of follow-up in the treated animals. Importantly, to achieve this benefit it required at least the injection of 1×10^7 or ideally $1 \times 10^8 - 10^9$ cells, while on the other hand, an increased number of applied has been repeatedly suggested to carry an increased risk of arrhythmia. Next, the selection of cell-type was of high importance. In regard to optimal timing of therapy, the highest therapy effect was seen at 5–8 weeks follow-up and when the cell application was timed at least >7 days post infarction. While this meta-analysis systematically provided evidence that cardiac cell therapy appears to be safe and effective in a large animal setting it has to be acknowledged that outcomes may significantly differ in the clinical scenario.

5. The concept of 3D culture systems for cardiac repair

Stem cells that are directed towards cell therapy or engineered tissue therapy must be harvested, isolated, expanded and stored. Specialized bioreactors have been developed to control environmental process parameters such as mass transfer, pH, temperature and oxygen tension to provide the appropriate environments for processing [98–101].

5.1. Cell based therapies

Once harvested and isolated, the stem cells are expanded in-vitro. Current expansion systems for multiplying these cells can vary but all have the requirement that they not push the cells down an unintended differentiation pathway. Many variables during expansion can influence cell behavior such as initial seeding density [102,103], excessive passaging [104], changes in oxygen tension [105] and physical forces such hydrodynamic shear forces [106,107]. These environmental parameters guide processing requirements for expanding stem cells.

5.2. Tissue based therapies

Engineered cardiac tissue must be grown in environments that take into account (1) the substrate, (2) the environmental culture conditions, (3) the culture medium, and (4) the cells themselves [11]. These requirements build from the basic requirements of mammalian cell culture which were developed using flat two-dimensional (2D) substrates. Three-dimensional (3D) cell culture models are being used which take advantage of newly developed substrates and bioreactors in order to more appropriately direct tissue growth and speed clinical translation [108].

Engineering myocardial tissue requires a matrix for the attachment and arrangement of the anchorage dependent cells. This 3D substrate or scaffold can be provided in one or a combination of four ways: 1) naturally occurring extracellular matrix materials, 2) hydrogels with entrained cells, 3) laminated cell sheets with accompanying matrix,

and 4) synthetic, porous substrates [109–112]. The goal of these scaffolds is to provide the appropriate material chemistry and substrate architecture — including porosity, alignment and mechanical properties. These scaffold properties play a large role in dictating the form and function of the engineered tissue. Fig. 2 shows the extracellular matrix of healthy human heart tissue. This matrix supports and connects myocytes in a composite network that is synthesized and remodeled on an ongoing basis by the resident population of fibroblasts. This architecture can serve as an ideal model for the structure of in-vitro scaffolds and is part of the motivation for using decellularized myocardium as a tissue engineering substrate [86].

Scaffold fabrication technique and materials determine microarchitecture which affects cultured cardiac stem cells. Electrospinning is used to create scaffolds with control over material, porosity and alignment. In this technique a polymer is deposited on a target surface in long thin strands [113,114]. These nonwoven materials are comprised of a series of fibers whose diameters can be on the order of nanometers and can be spun using multiple materials [115] and varying porosities [116].

Porous biomaterials can also be fabricated using a technique known as phase separation. This technique takes advantage of differential solubility to precipitate a polymer from a solvent solution by using a miscible non-solvent to coerce the polymer out of solution. By varying processing conditions such as material, temperature and technique a variety of scaffolds can be fabricated [117–119]. If the underlying substrate is patterned then the precipitated polymer can assume the shape of the substrate and this type of micropatterning can be used to direct cell organization [120]. Sheets of phase inversion polymers can be laminated, used to construct thicker scaffolds [121] and to impart channels for improved hydraulic permeability [122].

Scaffold anisotropy influences not only bulk mechanical properties but also the way by which the cells attached and arrange. In a series of studies on microabraded coverslips coated with fibronectin, Bursac et al. reported that 2D cultures could have varying degrees of functional electrophysiological properties in response to changes in substrate architecture [123]. Scaffold orientation is also seen to alter the excitation–contraction mechanism as seen in a series of experiments where intracellular calcium was influenced depending on degree of tissue anisotropy [124]. The mechanical properties of the cell scaffold can also be used to direct cellular activity [125,126]. Scaffold mechanical property can be manipulated using polymer blending [117] and geometric arrangement [116,127]. Post precipitation elongation has been used on phase inversion sheets to impart anisotropic structure and properties. This anisotropy is transferred throughout the 3D substrate to the seeded cells [128].

A significant hurdle in tissue engineering is overcoming the challenge of providing metabolically appropriate conditions inside of a 3D tissue construct more than approximately 1 mm thick. At this distance, diffusion alone cannot support the metabolic demands of healthy cells. If the cells are metabolically active, such as cardiomyocytes, then the

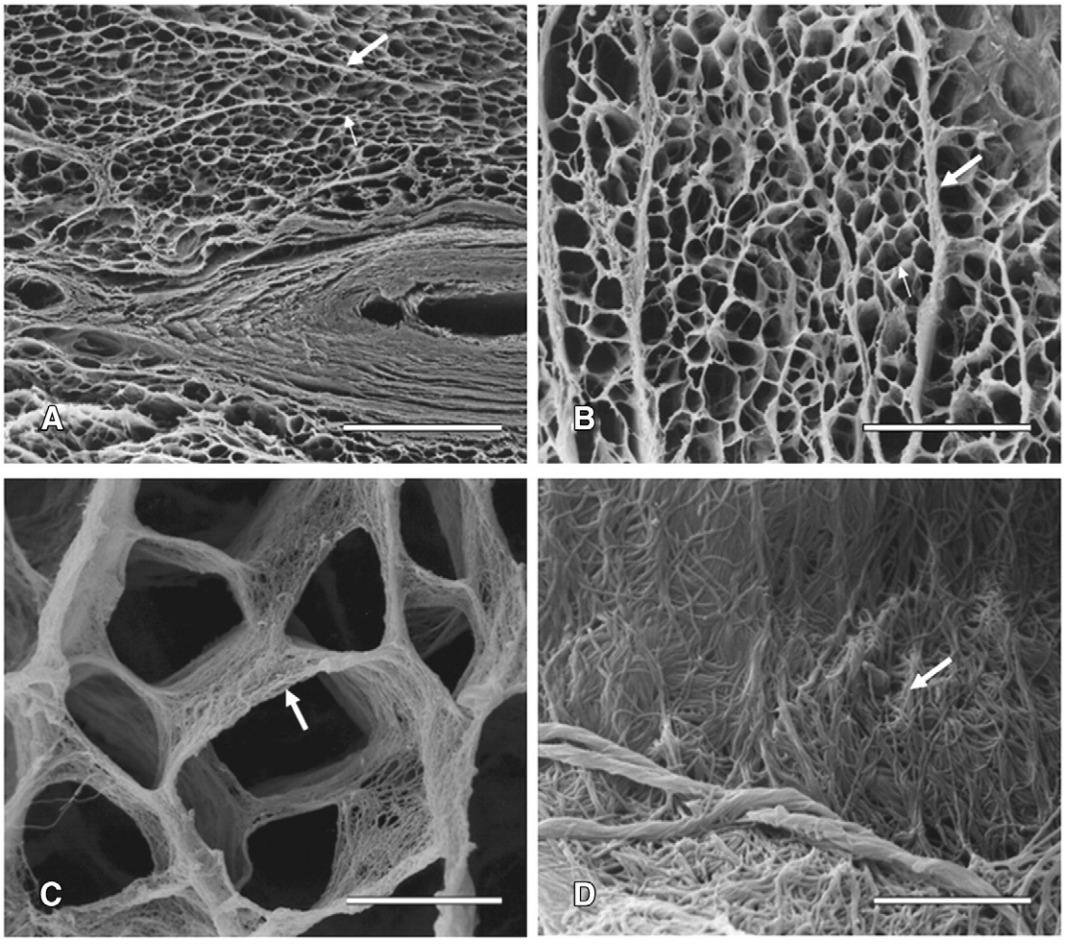

Fig. 2. Connective tissue skeleton of human heart (transverse section). A, The collagen network around cardiomyocytes and small vessels is clearly observed. Bar = 200 μm; magnification ×150 (thick arrow, perimysium; thin arrow, endomysium). B, The interstitial connective tissue consisting of perimysial and endomysial components presents a honeycomb shape. The perimysium (thick arrow) surrounds groups of cardiomyocytes, and the endomysium (thin arrow) surrounds each cardiomyocyte. Bar = 100 μm; magnification ×300. C, The endomysium supports and connects individual cardiomyocyte fascicles. Bar = 10 μm; magnification ×3000. D, At higher magnification, collagen fibers show interconnections on the surface of cardiomyocytes. Thin collagen strands are probably collagen III (arrow). Bar = 3 μm; magnification ×10,000.
Adapted from Kanzaki Y. et al. Circulation 2010;122:1973–1974; with permission from the American Heart Association.

demand is even greater. Several groups have reported on scaffold materials that promote hydraulic permeability with the thought that an additional means to perfuse nutrients into and remove waste products from the interior of the engineered tissue construct can better meet the metabolic demands of the cells. Perfusion has been shown to increase spatial uniformity of cardiac cells in a synthetic 3D substrate [14] increase the density of MSCs on polyurethane substrates [129] and decrease excitation threshold of neonatal rat cardiomyocytes in channeled elastomeric scaffolds [130]. Scaffold designers have used channels, corrugations, laminations and various combinations to alter hydraulic permeability for perfusion tissue culture [122,131–134]. As cultured tissue becomes more sophisticated and the promise of cardiac tissue engineering is realized there is a motivation to develop culture systems that are more purposeful and controllable. The design of sophisticated bioreactors is a crucial step towards the clinical realization of engineered, replacement cardiac tissue [11].

5.3. Systems in use and development

Environmental features of bioreactors for cardiac tissue engineering include mechanical conditioning such as cyclic strain [110,135–137] and electrical stimulation [137–140]. Mechanical conditioning was found to enhance cell proliferation and matrix organization of human heart cells [141], and structural organization and force of contraction in neonatal rat cardiac constructs [110,135,136,142–144]. Electrical stimulation was shown to induce myocyte alignment, increase electrical coupling via Cx43 up-regulation, and increase conduction velocity [138,145]. However, only little is known about the specific mechanical force and pacing regime that is responsible for promoting development to a specific tissue phenotype [100,146]. Although several approaches have been used to engineer cardiac tissue with some level of success, many shortcomings remain [11]. Most current bioreactor designs are limited in function, complex, unreliable and difficult to set up and operate [100,147]. They have not been designed for low cost, ease of use, and reliability.

6. 3D culture small and large animal studies

6.1. Cardiomyocyte derived cardiac patches

A functional cell based cardiac patch of clinically relevant thickness (approx. 1 cm) would represent the ideal construct for a cardiac tissue engineering approach to replace the diseased myocardium. Ideally the cardiac patch would consist of autologous cardiomyocytes that minimize immunologic reactions, couple with the host myocardium and have the ability to generate active forces during the contraction process. Based on numerous in vitro studies demonstrating the feasibility of creating beating cardiac tissue in vitro [16,148] a wide range of in vivo studies has been performed to evaluate this concept.

For cardiac tissue development numerous synthetic and natural materials have been used comprising of polyglycolic acid, poly-L-lactic acid, and polyglycerol sebacate as well as alginate, collagen, and chitosan [21,149–152]. While most of the available cardiac tissue engineering studies have utilized primarily rat cardiac cells so far, more recently also the use of mouse or human ES cell or iPS cell derived cardiomyocytes has been reported [59,153]. Interestingly, recent studies using rat derived cardiac cells demonstrated that the seeded cells present with stiffness characteristics that match those in native rat hearts [154]. Furthermore, Jacot and colleagues highlighted that cardiomyocytes can generate the highest degree of contractile force when cultured on substrates that match the stiffness of the native myocardium [155].

In another concept sheets of cardiomyocyte monolayers were stacked to generate functional cardiac tissue. Thereafter, the cell sheets were transplanted onto the infarcted rat myocardium and significant improvement of cardiac performance as well as engraftment was observed [156]. Next, in a landmark study Zimmermann and colleagues generated so called engineered heart tissue (EHT) with the capability of spontaneous and synchronous contraction after 1–2 week cultivation following seeding of a mix of collagen I, ECM proteins and neonatal rat cardiomyocytes into lattices or circular molds [136]. Thereafter, in a rat MI animal model, transplanted EHTs appeared to couple with the hosting myocardium and importantly improved cardiac function indicating that the transplantation of EHT can improve cardiac performance [157].

To evaluate the integration and engraftment potential of engineered cardiac patches primarily rat or mouse MI models have been used. Importantly, the lack of sufficient vasculature in the infarcted zone limits the engraftment and survival of transplanted engineered cardiac tissues [77,158]. To overcome this problem, recent studies have evaluated scaffolds containing angiogenic growth factors or the use of porous structures to improve cellular survival and neo-vascularization post transplantation. Other strategies propose rapid vascularization of cardiac tissues by the modular approach or the co-application of endothelial cells and fibroblasts [136,159–161].

6.2. Stem cell derived cardiac patches

In parallel, the use of adult stem cells has been reported to construct tissue patches for the regeneration of the ischemic myocardium via epicardial transplantation. Stem cells derived from the bone marrow represent the most often used source comprising of crude bone marrow cells, mononuclear cells or mesenchymal stem cells to generate stem cell based tissue patches for myocardial repair. To fabricate the tissue patches various approaches with regard to the utilized scaffold have been reported including natural starter matrices, synthetic polymers, biological materials such as autologous muscle, and also decellularized biological matrices which are then re-populated with stem cells [162–164].

Several in vivo studies in mouse or rat MI models revealed a beneficial effect of such tissue patches after epicardial application [162,163,165–167]. In a recent mouse MI study, the implantation of a bone marrow cell seeded patch improved myocardial performance, induced neo revascularization and enhanced cell survival and migration to the infarction area [162]. Next, in a rabbit model, epicardial patches fabricated from bone marrow derived MSC-seeded, decellularized small intestine submucosa (SIS) were used to repair rabbit myocardium [167]. At follow-up, LV function as well as capillary density improved following implantation of the tissue patches. In another report, collagen type I scaffold was used to generate a MSC based patch for application in a rat model [166]. The results of this study showed that neovascularization was induced and left ventricular remodeling was improved. Importantly, engraftment could be observed 7 days post implantation, but was not detectable at four week follow-up.

In addition to the use of bone marrow derived cells to generate tissue patches [162,163,165–167], the use of adipose tissue derived MSCs as well as cardiac progenitors has been reported. In a recent study, adipose tissue derived MSC patches were fabricated to treat post MI heart failure in rats. After implantation, myocardial function improved and neo-vessel formation could be observed which was primarily related to the capability of the adipose tissue derived MSCs to differentiate into vascular cells and to secrete angiogenic factors [168]. Zakharova and colleagues applied scaffold-free, cardiosphere-derived cell sheets to treat the infarcted rat heart [169]. As a result, cell migration and engraftment could be observed along with cardiomyogenic and vascular differentiation, while ventricular remodeling appeared to be significantly attenuated.

Taken together, the results of the available studies utilizing a cell based tissue patch appear to be promising with particular regard to an increased donor cell engraftment, the stimulation of cell migration,

the induction of neo-revascularization, and the improvement of cardiac performance while remodeling phenomena are reduced.

6.3. Injectable biomaterials with and without cells

Similar to patch based approaches, injectable cardiac tissue engineering has been reported to deliver stem cells and/or biomaterials as well as growth factors into the infarcted heart in order to improve cellular retention and survival. Various liquid biomaterials have been used to deliver different types of stem cells to the infarcted heart and to improve cellular survival. These liquid biomaterials include fibrin, protein-derived materials, including matrigels, synthetic materials, peptide-based nanofibers as well as polysaccharides [154,170–173].

In a recent study, Dai and colleagues assessed the effects of collagen on donor cell distribution showing that MSC remote location is significantly reduced when cell delivery is combined with collagen [174]. Next, several studies revealed a significantly increased survival of skeletal myoblasts, bone marrow stem cells, adipose tissue derived cells as well as cardiac progenitors when transplanted in fibrin glue [175–178]. In another concept, Wang et al. delivered MSC-based cell sheet fragments [179] and demonstrated enhanced cell retention and a beneficial effect of LV function along with reduced dilation. Furthermore, the authors demonstrated transdifferentiation into Nkx2.5 and α-sarcomeric actin positive cardiomyocyte-like cells, vascular cells and myofibroblasts. In another study, Martens et al. used a minimally invasive, catheter based approach to evaluate the efficacy of in situ polymerization of fibrin glue on the retention of human bone marrow derived stem cells in the infarcted myocardium of nude rats [180]. In a clinically relevant setting the authors demonstrated the principal feasibility and efficacy of this approach with particular regard to enhanced intramyocardial cell retention.

Summarizing from these studies, it appears that the increased retention when using engineered cell sheets is primarily achieved due to the preservation of endogenous ECM by the cell sheet fragments and its adhesive glycoproteins [181].

Interestingly, while injectable liquid biomaterials indeed have shown to improve cellular retention and survival, numerous materials such as alginate, collagen, decellularized tissues, matrigel, chitosan, keratin, and synthetic materials, such as self-assembling peptides and polymers [181] were also found to improve cardiac function in a similar manner when injected without cells re-emphasizing the important debate on the necessity of the cells [175]. Based on these findings, alginate was one of the first biomaterials that advanced via several preclinical animal trials [182,183] into a clinical setting addressing patients with myocardial infarction.

6.4. Scaffold-free three dimensional microtissues (3D-MTs)

The idea of scaffold-free, gravity enforced cellular self-assembly into three dimensional microtissues prior to intramyocardial delivery has been repeatedly suggested to represent a promising hybrid strategy for myocardial repair and regeneration with particular regard to an enhanced retention, integration and survival post transplantation [107,184–189]. Microtissues are 3D cell-based aggregates that can consist of somewhere between 500 and 10,000 cells with a size between 100 and 500 μm and that can be tailored to the specific application mode including direct surgical implantation or minimally invasive, catheter based transplantation. When compared to single cell suspensions they have been demonstrated to have numerous advantages comprising of (i) an overall higher organized and functional micro environment, (ii) the ability to develop endogenous extracellular matrix, (iii) enhanced adhesion properties to increase retention, (iv) the generation of pro-angiogenic factors, (v) the production of neo-vasculature and (vi) preferable anti-inflammatory characteristics [107,184–190].

Various cell sources have been utilized for the production of 3D-MTs [107,189]. In a recent report, clinically-relevant human cell sources for cardiac cell therapy including bone marrow and adipose tissue-derived mesenchymal stem cells (BMMSCs and ATMSCs), human embryonic stem cell (ESC)-derived Isl1 + cells, and induced pluripotent stem cells (iPSCs) underwent a systematic, head-to-head comparison for their capability to form 3D-MTs in vitro with a particular focus on cell-specific characteristics such as formation capacity, size and shape, proliferation, apoptotic behavior, and the potential for extracellular matrix production (Fig. 3) [191]. The results showed that all included cell types are able to undergo cellular self-assembly and to form 3D-MTs without compromising cell viability within the 3D-MTs. Furthermore, all cell-specific 3D-MTs displayed rapid formation of extracellular matrix (ECM) which may be a crucial factor in order to improve in vivo performance, retention, and importantly survival [191]. Next, a recent report has highlighted the significant benefit with regard to anti-inflammatory properties of 3D aggregation when using MSCs [184]. Moreover, the use of neonatal and adult cardiomyocytes has been demonstrated to generate myocardial neo-tissue with the ability to produce pro-angiogenic factors and mediators including VEGF [186–188,192], to integrate within the host myocardium and to connect to the host vasculature [187,193]. In this regard, Garzoni and colleagues highlighted that a sufficient vasculature represents the key aspect to ensure long-term functionality of cardiac MTs [185].

Furthermore, in a recent report, the 3D-MT technology was further advanced into a preclinical setting and combined with a transcatheter based, imaging guided delivery approach (Fig. 4). In this pilot study, the principal feasibility of transcatheter-based, intramyocardial transplantation of ATMSC derived 3D-MTs into porcine hearts could be successfully demonstrated highlighting the high clinical potential of the 3D-MT technology [194].

7. Clinical trials on cardiac cell therapy and cardiac tissue engineering

7.1. Cardiac cell therapy trials

Based on these promising, preclinical data numerous cell types have advanced into a clinical setting. While primarily focusing on the principal feasibility and safety most of the available clinical trials target patients suffering from acute myocardial infarction, chronic heart disease/refractory angina as well as ischemic cardiomyopathy.

Bone marrow derived progenitors were the first cell type to be used clinically and to date represent the most often used cell source. Several randomized trials such as the BOOST and the REPAIR-AMI trial [195,196] have demonstrated the excellent safety profile of these cells, whereas the efficacy (improvement of cardiac performance) remains controversial. While short-term efficacy has been demonstrated several other trials did not show a long-term effect after bone-marrow derived cell transplantation [197–200]. Furthermore, the recent HEBE trial as well as the SWISS AMI trial also displayed no beneficial effect at 4 months based on MRI follow-up after cell therapy [201,202]. Moreover, two further trials focused on the timing of cell application after myocardial infarction. While in the TIME trial, bone marrow mononuclear cells were applied between 3 and 7 days post MI, in the LATETIME trial the cells were only delivered 2–3 weeks after the event; however, in none of these two trials an improvement of ejection fraction could be documented [203,204]. To determine the clinical benefit of bone marrow derived progenitor cells, Jeevanantham and colleagues recently performed a meta-analysis of 50 clinical studies including cohort studies and randomized controlled trials. The results of this analysis indeed displayed a significant, but still relatively low enhancement of cardiac performance of approximately 4% after cell transplantation when compared to the controls [24]. However, although the debate on the true efficacy of bone marrow mononuclear cell based concepts is still ongoing, the largest clinical experience does exist for bone marrow derived mononuclear cells and first phase-3 studies

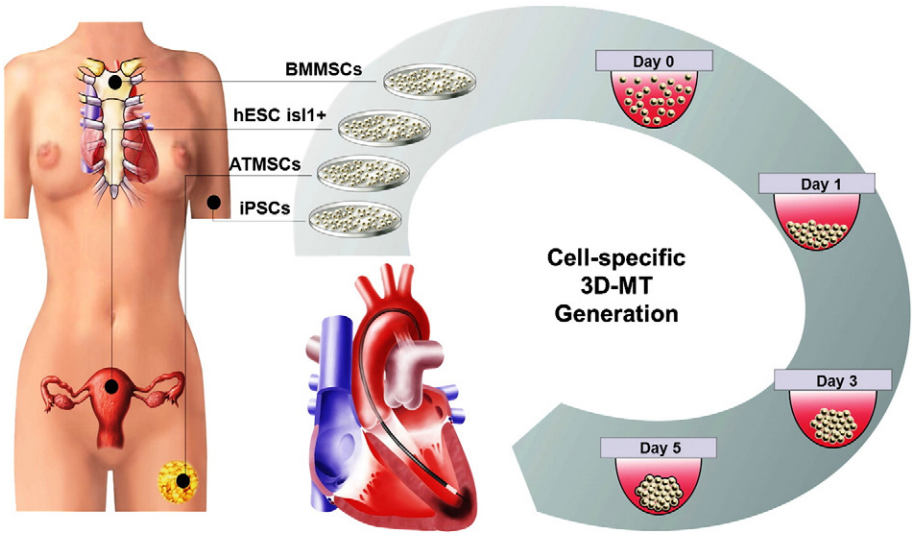

Fig. 3. Generation of human stem cell-based three-dimensional microtissues for advanced cardiac cell therapies. The concept of 3D-MT in vitro generation prior to cell transplantation may represent a promising application-format for future strategies to enhance cellular-retention and survival. Single-cell suspensions of human bone marrow- and adipose tissue-derived mesenchymal stem cells (BMMSCs and ATMSCs), Isl1 + cardiovascular progenitors derived from human embryonic stem cells (hESC− Isl1 + cells), and undifferentiated human induced pluripotent stem cells (iPSCs) were isolated and processed with clinically relevant protocols to generate three dimensional microtissues (3D-MTs) using a gravity-enforced hanging-drop culture technique.
Adapted from Emmert MY et al. Biomaterials. 2013 Sep;34(27):6339–54; with permission from Elsevier.

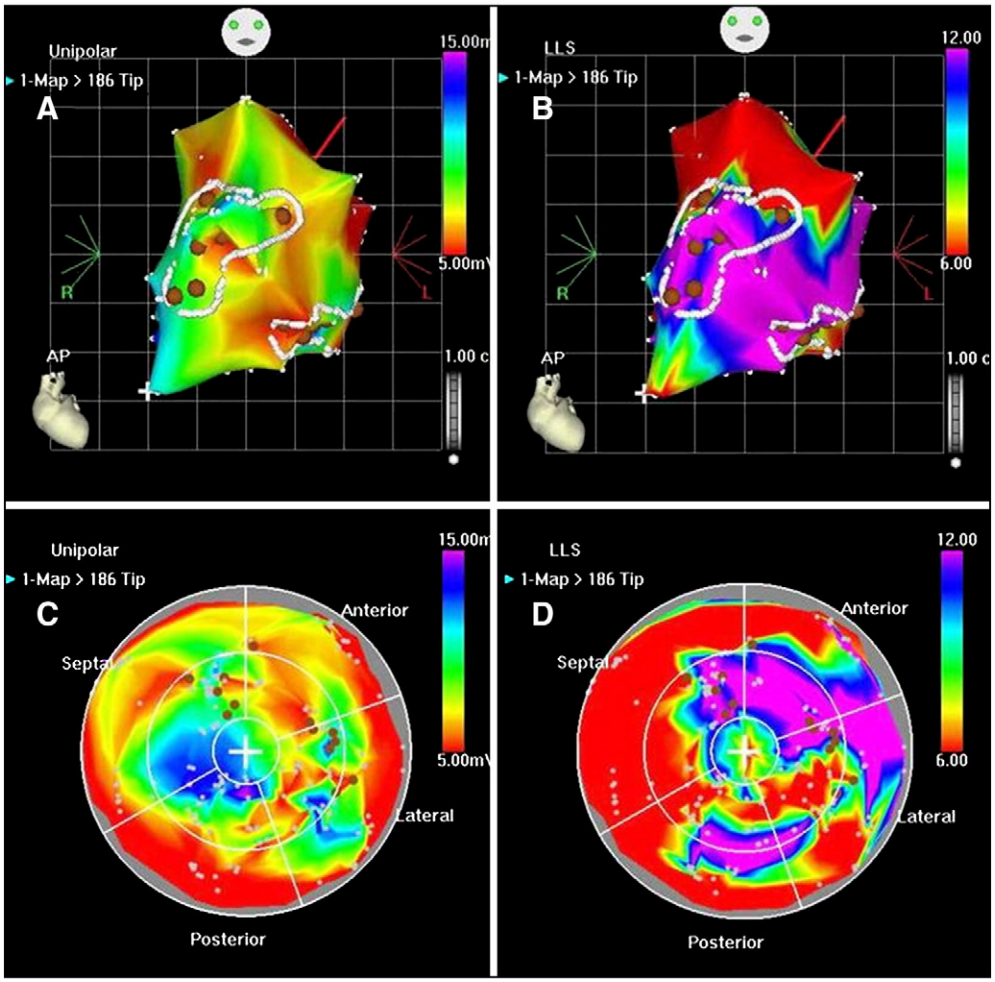

Fig. 4. Transcatheter based, NOGA mapping guided intramyocardial transplantation of human ATMSC based 3D-MTs into the porcine myocardium. Transcatheter NOGA mapping guided, intramyocardial transplantation of human adipose-tissue derived mesenchymal stem cell (ATMSC) based 3D-MTs into the left anterior wall (anterior LV wall: marked area/red dots) of a healthy porcine heart guided by unipolar voltage (A, *AP view* and C, *bull eye view*) and lineal local shortening (LLS) (B, *AP view*; D, *bull eye view*). Respective human 2D ATMSCs single cell suspensions (control) were injected into the lateral wall of the ventricle (lateral LV wall: marked area/red dots).
Adapted from Emmert M.Y. et al. Biomaterials 2013; Mar;34(10):2428–41; with permission from Elsevier.

such as the BAMI (NCT01569178) or the RENEW (NCT01508910) trial which is underway.

In addition to the use of bone marrow derived progenitors, the use of bone marrow derived mesenchymal stem cells (MSCs) has been reported as a potential strategy for cell therapy in different clinical scenarios [32,205]. In contrast to bone marrow mononuclear cells, the effect after MSC delivery appeared to be more pronounced and importantly, also the feasibility and safety of an allogenic MSC approach has been recently demonstrated [31,32,206]. The recently published *Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis* (POSEIDON; NCT01087996) trial demonstrated that both autologous and allogenic MSC treatment come with low rates of serious adverse events after transendocardial delivery in patients with ischemic cardiomyopathy [207]. Therefore, MSCs represent a promising cell source with an excellent clinical safety profile and further randomized trials are underway focusing on chronic Ischemic Heart Disease (MSC-HF; NCT00644410) and ischemic cardiomyopathy (TAC-HFT; NCT00768066). Additionally, to increase the potential of bone marrow derived MSCs, the use of a cardiopoietic cocktail was recently shown to be safe and feasible in an experimental study [33] as well as in a clinical pilot trial. In the C-Cure trial (NCT00810238) the safety and importantly beneficial effect of cardiopoietic MSCs could be shown [208] building the basis to advance into a randomized, multicenter trial (CHART trial; NCT01768702). In parallel, also the use of mesenchymal stem cells extracted from the adipose tissue has been reported to be feasible and is now under investigation in first clinical studies such as the APOLLO trial (APOLLO/NCT00442806). Next, the use of skeletal myoblasts has been reported as a potential cell type for cardiac repair. However, while initial clinical data such as from the MAGIC trial [49] and others (MYOHEART/NCT00054678) revealed promising results, a major concern with the use of these cells remains with regard to the lack of electrical coupling to the hosting myocardium which lead to severe arrhythmia in several patients after skeletal myoblast therapy [209].

Most recently, and based on exciting preclinical data cardiac resident stem and progenitor cells, CADECEUS (NCT00893360) and SCIPIO (NCT00474461), two clinical pilot trials have demonstrated a beneficial effect on cardiac function after delivery of cardiac progenitors [72,73]. Following these results, two further trials have been initiated, the AutoLogous Human CArdiac-Derived Stem Cell to Treat Ischemic cArdiomyopathy (ALCADIA; NCT00981006) study and the Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR; NCT01458405) trial which will be the first study to evaluate the safety of cardiac progenitor cell therapy in a phase 2 trial. Although the initial results for cardiac progenitors appear promising long-term results in larger patient cohorts are mandatory and importantly the exact mechanism by which cardiac progenitors contribute to the improvement of cardiac performance needs to be identified.

7.2. Cardiac tissue engineering trials

In the recent MAGNUM trial, a non-randomized, controlled phase I feasibility trial, Chachques et al. investigated the safety of a combined approach comprising of intramyocardial cell transplantation and epicardial implantation of a bone marrow cell-based collagen scaffold in patients undergoing surgical revascularization [210]. In this proof-of-concept study, ten patients received intramyocardial cell transplantation only and ten patients additionally received the bone marrow cell-derived epicardial patch. The one year results post implantation confirmed safety of this approach and no treatment related adverse events occurred. Furthermore, the combined approach improved cardiac performance, reduced remodeling and led to an increase of wall thickness in the infarcted myocardium. Although the patient numbers were low and the trial design was non-randomized, the results of this study demonstrated the principal feasibility and safety to combine intramyocardial cell therapy and an epicardial tissue engineering approach in the clinical setting [210]. In another clinical pilot trial the principal feasibility of a catheter-deliverable biomaterial (Injectable BL-1040 Implant, NCT00557531) for the treatment of myocardial infarction was demonstrated. In this study, patients with acute MI who could be successfully re-vascularized received an intracoronary alginate infusion. Being safety proven, this concept was further advanced and the PRESERVATION 1 trial (NCT01226563) a phase 2, placebo-controlled, multicenter, randomized, double-blind trial was initiated to assess the safety and efficacy of IK-5001, an alginate based biomaterial for the prevention of ventricular remodeling and congestive heart failure after acute MI.

8. Current state & challenges

One decade after the first clinical stem cell trial was performed [211] the available clinical data demonstrate that the concept of cardiac cell therapy for myocardial regeneration has continuously evolved over time. Different cell sources and delivery modes have been tested (intracoronary versus intramyocardial) in different clinical settings. While most of the available trials were designed to demonstrate principal feasibility and safety, recently initiated trials are now focusing on the efficacy of cell therapy for the heart. However, although the concept of cardiac stem cell therapy has been demonstrated to be feasible in the clinical setting numerous unanswered key aspects do remain with particular regard to the precise role and mechanism by which stem cells contribute to myocardial repair and regeneration; by (trans-)differentiation or via so called paracrine mechanisms.

Despite the abundance of preclinical animal studies directed at exploring and understanding the basic mechanisms of in-situ cardiomyoplasty, there are still conflicting data and a lack of understanding of these underlying mechanisms. Many studies investigate a paracrine mechanism as being responsible for cell and tissue changes that lead to enhanced cardiac function. Five of these proposed mechanisms are: 1) downregulation of cardiac remodeling, 2) enhanced angiogenesis, 3) immunomodulatory effect, 4) cytoprotection of surviving myocytes in and around the infarct region, and 5) recruitment of quiescent cardiac progenitor cells [212–215]. This lack of understanding of underlying mechanisms is a challenge that must be overcome and reconciled. Once a particular therapy moves from the preclinical to the clinical setting, the efforts must move past the understanding of basic mechanisms and into the realm of safety, effectiveness and outcome. In many human trials, the efficacy of cell therapy for improved recovery post-MI has been questioned.

Importantly, the too rapid translation from non-comparable small animal experiments or large animal studies (mainly porcine and ovine models) into human clinical trials has left many questions open. The so called “stem cell fate” is almost completely unclear and it remains to be elucidated what exactly happens during stem cell transplantation with regard to immunologic interaction, cellular retention, integration and importantly cell survival.

Furthermore, the optimal cell type needs to be identified for the respective indication as well as the quantity of cells, the timing and the mode of cell delivery. Furthermore, the delivery format by which cells are applied to the heart still represents a crucial aspect in current cell therapy concepts. In the clinical setting, primarily single cell suspensions have been used. However, although single cell suspensions are simple to deliver, it is well known that most of the cells are washed away with the first heart beat and the overall cardiac retention is very low. In addition, the survival of those cells that do remain in the myocardium is relatively low. Therefore, effective cell therapy requires a sufficient retention within the heart as well as optimal conditions for enhanced integration, engraftment and particularly survival. In this regard, the utilization of three dimensional bio-engineering concepts comprising of scaffold-based as well as scaffold-free approaches has been repeatedly suggested to improve retention and survival after transplantation. Finally, efficient, imaging based methods are mandatory to monitor the cells after transplantation and specific outcome

parameters to determine a potential therapeutic effect need to be defined. While most of the previous preclinical and clinical studies have been primarily focussing on the improvement of cardiac function (ejection fraction), recent reports doubt if this is the most valid parameter to evaluate the success of cell therapy. Therefore, numerous studies suggest the evaluation of cardiac remodeling and reduction of infarction size after cell transplantation.

Thus, further investigation is needed in the preclinical arena in order to develop a comprehensive understanding of basic therapeutic mechanisms and to verify that cell therapy is meeting its intended requirements. Without these studies and understanding, cell therapy will not move from clinical trial phase to the standard of care.

9. Conclusions and future outlook

There is little doubt that cell based therapies are poised to play a significant role in our battle against cardiac disease. Along with traditional devices, drugs and surgical interventions, the relative new field of cardiomyoplasty will lead to better patient outcomes and with adoption and scalability will provide better value as well. The research community was focused initially on the physiologic phenomena associated with this new field. We are now taking a more holistic look at the spectrum of technical challenges and the literature continues to grow as these challenges are addressed. Along with the technical challenges are clinical challenges, regulatory challenges and commercial challenges. Clinical challenges include patient selection and delivery strategy. Regulatory challenges are a new area where regulatory agencies are redefining the boundaries between devices, drugs and the newer field of biologics. In order for cell therapy to reach its full potential, there must be commercial opportunities. There are few examples of new technologies that play a significant role in healthcare without a commercial motivation. The future of this emerging field is promising indeed but only if each of these challenges is met and addressed as part of the overall development strategy.

Once cells and tissues are grown in vitro there must be a means for delivering them to the recipient. Suspensions of cells have been administered through local or systemic vasculature or by direct injection into the myocardium. Tracking the fate of these cells is extremely challenging and inter-subject variability makes it hard to predict clinical response.

Systems and methods for isolating, expanding and differentiating stem cells must be developed at a commercial scale before cell therapy and tissue engineering can be applied to routinely treat cardiac disease. Current bioreactors tend to be boutique devices meant to study cell and tissue behavior in a laboratory setting.

Regulatory agencies in the European Union, Japan and the United States have all been working with researchers and industry to guide the development and clinical use of cells and engineered tissues. As with any new therapeutic modality, clinical trials will be ongoing to prove safety and efficacy. In addition, regulatory oversight will be mandated for production, packaging, transport and storage as the technology becomes stable and experience is gained in this emerging field.

Cardiac disease will one day be treated with biologics such as cells, tissues and scaffolds. The advances that lead to these therapies will likely come at a steady pace as we develop a comprehensive understanding of both the diseased and therapeutic cells and tissues. Because cardiac disease is a spectrum of disease states that affect muscular, nervous, vascular and matrix structure and function so too will the therapies be many and varied. Each therapy will be unique with the therapeutic strategy developed in concert with each patient's unique disease state. These therapies will provide commercial momentum that in turn will motivate new businesses. Regulatory approval will be an arduous process at first however the cardiac market presents a tremendous business opportunity. As we see with other large potential markets and large regulatory investments, there are researchers and companies willing to take the risk and take the lead on groundbreaking new therapies.

Conflicts of interest

None.

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None.

References

[1] D. Lloyd-Jones, R.J. Adams, T.M. Brown, M. Carnethon, S. Dai, G. De Simone, T.B. Ferguson, E. Ford, K. Furie, C. Gillespie, A. Go, K. Greenlund, N. Haase, S. Hailpern, P.M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lackland, L. Lisabeth, A. Marelli, M.M. McDermott, J. Meigs, D. Mozaffarian, M. Mussolino, G. Nichol, V.L. Roger, W. Rosamond, R. Sacco, P. Sorlie, T. Thom, S. Wasserthiel-Smoller, N.D. Wong, J. Wylie-Rosett, Heart disease and stroke statistics—2010 update: a report from the American Heart Association, Circulation 121 (2010) e46–e215.
[2] M. Jessup, S. Brozena, Heart failure, N. Engl. J. Med. 348 (2003) 2007–2018.
[3] E.N. Olson, A decade of discoveries in cardiac biology, Nat. Med. 10 (2004) 467–474.
[4] G. Vunjak-Novakovic, K.O. Lui, N. Tandon, K.R. Chien, Bioengineering heart muscle: a paradigm for regenerative medicine, Annu. Rev. Biomed. Eng. 15 (2011) 245–267.
[5] P. Menasche, Cardiac cell therapy: lessons from clinical trials, J. Mol. Cell. Cardiol. 50 (2010) 258–265.
[6] A. Abdel-Latif, R. Bolli, I.M. Tleyjeh, V.M. Montori, E.C. Perin, C.A. Hornung, E.K. Zuba-Surma, M. Al-Mallah, B. Dawn, Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis, Arch. Intern. Med. 167 (2007) 989–997.
[7] P. Menasché, Cardiac cell therapy: lessons from clinical trials, J. Mol. Cell. Cardiol. 50 (2011) 258–265.
[8] M. Perez-Ilzarbe, O. Agbulut, B. Pelacho, C. Ciorba, E.S. Jose-Eneriz, M. Desnos, A.A. Hagège, P. Aranda, E.J. Andreu, P. Menasché, Characterization of the paracrine effects of human skeletal myoblasts transplanted in infarcted myocardium, Eur. J. Heart Fail. 10 (2008) 1065–1072.
[9] M. Gnecchi, H. He, N. Noiseux, O.D. Liang, L. Zhang, F. Morello, H. Mu, L.G. Melo, R.E. Pratt, J.S. Ingwall, Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement, FASEB J. 20 (2006) 661–669.
[10] L. Timmers, S.K. Lim, F. Arslan, J.S. Armstrong, I.E. Hoefer, P.A. Doevendans, J.J. Piek, R.M. El Oakley, A. Choo, C.N. Lee, Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium, Stem Cell Res. 1 (2008) 129–137.
[11] G. Vunjak-Novakovic, N. Tandon, A. Godier, R. Maidhof, A. Marsano, T.P. Martens, M. Radisic, Challenges in cardiac tissue engineering, Tissue Eng. Part B Rev. 16 (2010) 169–187.
[12] N.T. Elliott, F. Yuan, A review of three-dimensional in vitro tissue models for drug discovery and transport studies, J. Pharm. Sci. 100 (2011) 59–74.
[13] N. Bursac, M. Papadaki, R.J. Cohen, F.J. Schoen, S.R. Eisenberg, R. Carrier, G. Vunjak-Novakovic, L.E. Freed, Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies, Am. J. Physiol. 277 (1999) 433–444.
[14] R.L. Carrier, M. Rupnick, R. Langer, F.J. Schoen, L.E. Freed, G. Vunjak-Novakovic, Perfusion improves tissue architecture of engineered cardiac muscle, Tissue Eng. 8 (2002) 175–188.
[15] N. Bursac, M. Papadaki, J.A. White, S.R. Eisenberg, G. Vunjak-Novakovic, L.E. Freed, Cultivation in rotating bioreactors promotes maintenance of cardiac myocyte electrophysiology and molecular properties, Tissue Eng. 9 (2003) 1243–1253.
[16] R.L. Carrier, M. Papadaki, M. Rupnick, F.J. Schoen, N. Bursac, R. Langer, L.E. Freed, G. Vunjak-Novakovic, Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization, Biotechnol. Bioeng. 64 (1999) 580–589.
[17] M.J.A. van Luyn, R.A. Tio, X.J. Gallego y van Seijen, J.A. Plantinga, L. de Leij, M.J.L. DeJongste, P.B. van Wachem, Cardiac tissue engineering: characteristics of in unison contracting two- and three-dimensional neonatal rat ventricle cell (co)-cultures, Biomaterials 23 (2002) 4793–4801.
[18] R.E. Akins, R.A. Boyce, M.L. Madonna, N.A. Schroedl, S.R. Gonda, T.A. McLaughlin, C.R. Hartzell, Cardiac organogenesis in vitro: reestablishment of three-dimensional tissue architecture by dissociated neonatal rat ventricular cells, Tissue Eng. 5 (1999) 103–118.
[19] A. Lichtenberg, G. Dumlu, T. Walles, M. Maringka, S. Ringes-Lichtenberg, A. Ruhparwar, H. Mertsching, A. Haverich, A multifunctional bioreactor for three-dimensional cell (co)-culture, Biomaterials 26 (2005) 555–562.
[20] M. Radisic, A. Marsano, R. Maidhof, Y. Wang, G. Vunjak-Novakovic, Cardiac tissue engineering using perfusion bioreactor systems, Nat. Protoc. 3 (2008) 719–738.
[21] M. Radisic, H. Park, F. Chen, J.E. Salazar-Lazzaro, Y. Wang, R. Dennis, R. Langer, L.E. Freed, G. Vunjak-Novakovic, Biomimetic approach to cardiac tissue engineering: oxygen carriers and channeled scaffolds, Tissue Eng. 12 (2006) 2077–2091.
[22] V.F. Segers, R.T. Lee, Stem-cell therapy for cardiac disease, Nature 451 (2008) 937–942.
[23] F. Wei, T. Wang, J. Liu, Y. Du, A. Ma, The subpopulation of mesenchymal stem cells that differentiate toward cardiomyocytes is cardiac progenitor cells, Exp. Cell Res. 317 (18) (Nov. 1 2011) 2661–2670.
[24] V. Jeevanantham, M. Butler, A. Saad, A. Abdel-Latif, E.K. Zuba-Surma, B. Dawn, Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis, Circulation 126 (2012) 551–568.

[25] D.P. Francis, M. Mielewczik, D. Zargaran, G.D. Cole, Autologous bone marrow-derived stem cell therapy in heart disease: discrepancies and contradictions, Int. J. Cardiol. 168 (2013) 3381–3403.
[26] A.R. Williams, J.M. Hare, Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease, Circ. Res. 109 (2011) 923–940.
[27] M.Y. Emmert, B. Weber, P. Wolint, T. Frauenfelder, S.M. Zeisberger, L. Behr, S. Sammut, J. Scherman, C.E. Brokopp, R. Schwartlander, V. Vogel, P. Vogt, J. Grunenfelder, H. Alkadhi, V. Falk, A. Boss, S.P. Hoerstrup, Intramyocardial transplantation and tracking of human mesenchymal stem cells in a novel intra-uterine pre-immune fetal sheep myocardial infarction model: a proof of concept study, PLoS One 8 (2013) e57759.
[28] K.H. Schuleri, A.J. Boyle, M. Centola, L.C. Amado, R. Evers, J.M. Zimmet, K.S. Evers, K.M. Ostbye, D.G. Scorpio, J.M. Hare, A.C. Lardo, The adult Gottingen minipig as a model for chronic heart failure after myocardial infarction: focus on cardiovascular imaging and regenerative therapies, Comp. Med. 58 (2008) 568–579.
[29] K.H. Schuleri, M. Centola, S.H. Choi, K.S. Evers, F. Dawoud, R.T. George, J.A. Lima, A.C. Lardo, CT for evaluation of myocardial cell therapy in heart failure: a comparison with CMR imaging, JACC Cardiovasc. Imaging 4 (2011) 1284–1293.
[30] K.H. Schuleri, G.S. Feigenbaum, M. Centola, E.S. Weiss, J.M. Zimmet, J. Turney, J. Kellner, M.M. Zviman, K.E. Hatzistergos, B. Detrick, J.V. Conte, I. McNiece, C. Steenbergen, A.C. Lardo, J.M. Hare, Autologous mesenchymal stem cells produce reverse remodelling in chronic ischaemic cardiomyopathy, Eur. Heart J. 30 (2009) 2722–2732.
[31] J.M. Hare, J.H. Traverse, T.D. Henry, N. Dib, R.K. Strumpf, S.P. Schulman, G. Gerstenblith, A.N. DeMaria, A.E. Denktas, R.S. Gammon, J.B. Hermiller Jr., M.A. Reisman, G.L. Schaer, W. Sherman, A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction, J. Am. Coll. Cardiol. 54 (2009) 2277–2286.
[32] A.R. Williams, B. Trachtenberg, D.L. Velazquez, I. McNiece, P. Altman, D. Rouy, A.M. Mendizabal, P.M. Pattany, G.A. Lopera, J. Fishman, J.P. Zambrano, A.W. Heldman, J.M. Hare, Intramyocardial stem cell injection in patients with ischemic cardiomyopathy: functional recovery and reverse remodeling, Circ. Res. 108 (2011) 792–796.
[33] A. Behfar, S. Yamada, R. Crespo-Diaz, J.J. Nesbitt, L.A. Rowe, C. Perez-Terzic, V. Gaussin, C. Homsy, J. Bartunek, A. Terzic, Guided cardiopoiesis enhances therapeutic benefit of bone marrow human mesenchymal stem cells in chronic myocardial infarction, J. Am. Coll. Cardiol. 56 (2010) 721–734.
[34] Y. Zhu, T. Liu, K. Song, B. Jiang, X. Ma, Z. Cui, Collagen–chitosan polymer as a scaffold for the proliferation of human adipose tissue-derived stem cells, J. Mater. Sci. Mater. Med. 20 (2009) 799–808.
[35] C. Valina, K. Pinkernell, Y.H. Song, X. Bai, S. Sadat, R.J. Campeau, T.H. Le Jemtel, E. Alt, Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction, Eur. Heart J. 28 (2007) 2667–2677.
[36] A. Kawamoto, H. Iwasaki, K. Kusano, T. Murayama, A. Oyamada, M. Silver, C. Hulbert, M. Gavin, A. Hanley, H. Ma, M. Kearney, V. Zak, T. Asahara, D.W. Losordo, CD34-positive cells exhibit increased potency and safety for therapeutic neovascularization after myocardial infarction compared with total mononuclear cells, Circulation 114 (2006) 2163–2169.
[37] D.W. Losordo, R.A. Schatz, C.J. White, J.E. Udelson, V. Veereshwarayya, M. Durgin, K.K. Poh, R. Weinstein, M. Kearney, M. Chaudhry, A. Burg, L. Eaton, L. Heyd, T. Thorne, L. Shturman, P. Hoffmeister, K. Story, V. Zak, D. Dowling, J.H. Traverse, R.E. Olson, J. Flanagan, D. Sodano, T. Murayama, A. Kawamoto, K.F. Kusano, J. Wollins, F. Welt, P. Shah, P. Soukas, T. Asahara, T.D. Henry, Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial, Circulation 115 (2007) 3165–3172.
[38] C. Stamm, H.D. Kleine, Y.H. Choi, S. Dunkelmann, J.A. Lauffs, B. Lorenzen, A. David, A. Liebold, C. Nienaber, D. Zurakowski, M. Freund, G. Steinhoff, Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies, J. Thorac. Cardiovasc. Surg. 133 (2007) 717–725.
[39] C. Yerebakan, A. Kaminski, B. Westphal, P. Donndorf, A. Glass, A. Liebold, C. Stamm, G. Steinhoff, Impact of preoperative left ventricular function and time from infarction on the long-term benefits after intramyocardial CD133(+) bone marrow stem cell transplant, J. Thorac. Cardiovasc. Surg. 142 (2011) 1530–1539(e1533).
[40] C. Urbich, S. Dimmeler, Endothelial progenitor cells: characterization and role in vascular biology, Circ. Res. 95 (2004) 343–353.
[41] J. Hur, C.H. Yoon, H.S. Kim, J.H. Choi, H.J. Kang, K.K. Hwang, B.H. Oh, M.M. Lee, Y.B. Park, Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 288–293.
[42] N. Krankel, T.F. Luscher, U. Landmesser, "Endothelial progenitor cells" as a therapeutic strategy in cardiovascular disease, Curr. Vasc. Pharmacol. 10 (2012) 107–124.
[43] I. Gruh, J. Beilner, U. Blomer, A. Schmiedl, I. Schmidt-Richter, M.L. Kruse, A. Haverich, U. Martin, No evidence of transdifferentiation of human endothelial progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyocytes, Circulation 113 (2006) 1326–1334.
[44] G. Giannotti, C. Doerries, P.S. Mocharla, M.F. Mueller, F.H. Bahlmann, T. Horvath, H. Jiang, S.A. Sorrentino, N. Steenken, C. Manes, M. Marzilli, K.L. Rudolph, T.F. Luscher, H. Drexler, U. Landmesser, Impaired endothelial repair capacity of early endothelial progenitor cells in prehypertension: relation to endothelial dysfunction, Hypertension 55 (2010) 1389–1397.
[45] P. Menasche, Skeletal myoblasts and cardiac repair, J. Mol. Cell. Cardiol. 45 (2008) 545–553.
[46] S. Ghostine, C. Carrion, L.C. Souza, P. Richard, P. Bruneval, J.T. Vilquin, B. Pouzet, K. Schwartz, P. Menasche, A.A. Hagege, Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction, Circulation 106 (2002) I131–I136.
[47] A.A. Hagege, J.P. Marolleau, J.T. Vilquin, A. Alheritiere, S. Peyrard, D. Duboc, E. Abergel, E. Messas, E. Mousseaux, K. Schwartz, M. Desnos, P. Menasche, Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients, Circulation 114 (2006) I108–I113.
[48] W. Roell, T. Lewalter, P. Sasse, Y.N. Tallini, B.R. Choi, M. Breitbach, R. Doran, U.M. Becher, S.M. Hwang, T. Bostani, J. von Maltzahn, A. Hofmann, S. Reining, B. Eiberger, B. Gabris, A. Pfeifer, A. Welz, K. Willecke, G. Salama, J.W. Schrickel, M.I. Kotlikoff, B.K. Fleischmann, Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia, Nature 450 (2007) 819–824.
[49] P. Menasche, O. Alfieri, S. Janssens, W. McKenna, H. Reichenspurner, L. Trinquart, J.T. Vilquin, J.P. Marolleau, B. Seymour, J. Larghero, S. Lake, G. Chatellier, S. Solomon, M. Desnos, A.A. Hagege, The myoblast autologous grafting in ischemic cardiomyopathy (magic) trial: first randomized placebo-controlled study of myoblast transplantation, Circulation 117 (2008) 1189–1200.
[50] M.A. Laflamme, K.Y. Chen, A.V. Naumova, V. Muskheli, J.A. Fugate, S.K. Dupras, H. Reinecke, C. Xu, M. Hassanipour, S. Police, C. O'Sullivan, L. Collins, Y. Chen, E. Minami, E.A. Gill, S. Ueno, C. Yuan, J. Gold, C.E. Murry, Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts, Nat. Biotechnol. 25 (2007) 1015–1024.
[51] Y. Yang, J.Y. Min, J.S. Rana, Q. Ke, J. Cai, Y. Chen, J.P. Morgan, Y.F. Xiao, VEGF enhances functional improvement of postinfarcted hearts by transplantation of ESC-differentiated cells, J. Appl. Physiol. 93 (2002) 1140–1151.
[52] J. Nussbaum, E. Minami, M.A. Laflamme, J.A. Virag, C.B. Ware, A. Masino, V. Muskheli, L. Pabon, H. Reinecke, C.E. Murry, Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response, FASEB J. 21 (2007) 1345–1357.
[53] C.E. Murry, G. Keller, Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development, Cell 132 (2008) 661–680.
[54] M. Nakagawa, M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, S. Yamanaka, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nat. Biotechnol. 26 (2008) 101–106.
[55] I.H. Park, R. Zhao, J.A. West, A. Yabuuchi, H. Huo, T.A. Ince, P.H. Lerou, M.W. Lensch, G.Q. Daley, Reprogramming of human somatic cells to pluripotency with defined factors, Nature 451 (2008) 141–146.
[56] S. Yamanaka, Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors, Cell Prolif. 41 (Suppl. 1) (2008) 51–56.
[57] M. Bellin, M.C. Marchetto, F.H. Gage, C.L. Mummery, Induced pluripotent stem cells: the new patient? Nat. Rev. Mol. Cell Biol. 13 (2012) 713–726.
[58] D.K. Singla, X. Long, C. Glass, R.D. Singla, B. Yan, Induced pluripotent stem (iPS) cells repair and regenerate infarcted myocardium, Mol. Pharm. 8 (5) (Oct. 3 2011) 1573–1581.
[59] M. Kawamura, S. Miyagawa, K. Miki, A. Saito, S. Fukushima, T. Higuchi, T. Kawamura, T. Kuratani, T. Daimon, T. Shimizu, T. Okano, Y. Sawa, Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model, Circulation 126 (2012) S29–S37.
[60] D. Kim, C.H. Kim, J.I. Moon, Y.G. Chung, M.Y. Chang, B.S. Han, S. Ko, E. Yang, K.Y. Cha, R. Lanza, K.S. Kim, Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins, Cell Stem Cell 4 (2009) 472–476.
[61] R. Lister, M. Pelizzola, Y.S. Kida, R.D. Hawkins, J.R. Nery, G. Hon, J. Antosiewicz-Bourget, R. O'Malley, R. Castanon, S. Klugman, M. Downes, R. Yu, R. Stewart, B. Ren, J.A. Thomson, R.M. Evans, J.R. Ecker, Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells, Nature 471 (2011) 68–73.
[62] L. Qian, Y. Huang, C.I. Spencer, A. Foley, V. Vedantham, L. Liu, S.J. Conway, J.D. Fu, D. Srivastava, In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes, Nature 485 (2012) 593–598.
[63] M.Y. Emmert, L.S. Emmert, A. Martens, I. Ismail, I. Schmidt-Richter, A. Gawol, B. Seifert, A. Haverich, U. Martin, I. Gruh, Higher frequencies of BCRP+ cardiac resident cells in ischaemic human myocardium, Eur. Heart J. 34 (36) (Sep. 2013) 2830–2838.
[64] T. Oyama, T. Nagai, H. Wada, A.T. Naito, K. Matsuura, K. Iwanaga, T. Takahashi, M. Goto, Y. Mikami, N. Yasuda, H. Akazawa, A. Uezumi, S. Takeda, I. Komuro, Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes in vitro and in vivo, J. Cell Biol. 176 (2007) 329–341.
[65] P. Anversa, J. Kajstura, A. Leri, R. Bolli, Life and death of cardiac stem cells: a paradigm shift in cardiac biology, Circulation 113 (2006) 1451–1463.
[66] C. Bearzi, M. Rota, T. Hosoda, J. Tillmanns, A. Nascimbene, A. De Angelis, S. Yasuzawa-Amano, I. Trofimova, R.W. Siggins, N. Lecapitaine, S. Cascapera, A.P. Beltrami, D.A. D'Alessandro, E. Zias, F. Quaini, K. Urbanek, R.E. Michler, R. Bolli, J. Kajstura, A. Leri, P. Anversa, Human cardiac stem cells, Proc. Natl. Acad. Sci. U. S. A. 104 (35) (Aug. 28 2007) 14068–14073.
[67] A.P. Beltrami, L. Barlucchi, D. Torella, M. Baker, F. Limana, S. Chimenti, H. Kasahara, M. Rota, E. Musso, K. Urbanek, A. Leri, J. Kajstura, B. Nadal-Ginard, P. Anversa, Adult cardiac stem cells are multipotent and support myocardial regeneration, Cell 114 (2003) 763–776.
[68] A.P. Beltrami, K. Urbanek, J. Kajstura, S.M. Yan, N. Finato, R. Bussani, B. Nadal-Ginard, F. Silvestri, A. Leri, C.A. Beltrami, P. Anversa, Evidence that human cardiac myocytes divide after myocardial infarction, N. Engl. J. Med. 344 (2001) 1750–1757.
[69] E. Messina, L. De Angelis, G. Frati, S. Morrone, S. Chimenti, F. Fiordaliso, M. Salio, M. Battaglia, M.V. Latronico, M. Coletta, E. Vivarelli, L. Frati, G. Cossu, A. Giacomello,

Isolation and expansion of adult cardiac stem cells from human and murine heart, Circ. Res. 95 (2004) 911–921.
[70] H.C. Ott, T.S. Matthiesen, J. Brechtken, S. Grindle, S.K. Goh, W. Nelson, D.A. Taylor, The adult human heart as a source for stem cells: repair strategies with embryonic-like progenitor cells, Nat. Clin. Pract. Cardiovasc. Med. 4 (Suppl. 1) (2007) S27–S39.
[71] K.L. Laugwitz, A. Moretti, J. Lam, P. Gruber, Y. Chen, S. Woodard, L.Z. Lin, C.L. Cai, M.M. Lu, M. Reth, O. Platoshyn, J.X. Yuan, S. Evans, K.R. Chien, Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages, Nature 433 (2005) 647–653.
[72] R. Bolli, A.R. Chugh, D. D'Amario, J.H. Loughran, M.F. Stoddard, S. Ikram, G.M. Beache, S.G. Wagner, A. Leri, T. Hosoda, F. Sanada, J.B. Elmore, P. Goichberg, D. Cappetta, N.K. Solankhi, I. Fahsah, D.G. Rokosh, M.S. Slaughter, J. Kajstura, P. Anversa, Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial, Lancet 378 (2011) 1847–1857.
[73] R.R. Makkar, R.R. Smith, K. Cheng, K. Malliaras, L.E. Thomson, D. Berman, L.S. Czer, L. Marban, A. Mendizabal, P.V. Johnston, S.D. Russell, K.H. Schuleri, A.C. Lardo, G. Gerstenblith, E. Marban, Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (caduceus): a prospective, randomised phase 1 trial, Lancet 379 (2012) 895–904.
[74] T. Asahara, A. Kawamoto, Endothelial progenitor cells for postnatal vasculogenesis, Am. J. Physiol. Cell Physiol. 287 (2004) C572–C579.
[75] L.B. Balsam, A.J. Wagers, J.L. Christensen, T. Kofidis, I.L. Weissman, R.C. Robbins, Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium, Nature 428 (2004) 668–673.
[76] A. Haase, R. Olmer, K. Schwanke, S. Wunderlich, S. Merkert, C. Hess, R. Zweigerdt, I. Gruh, J. Meyer, S. Wagner, L.S. Maier, D.W. Han, S. Glage, K. Miller, P. Fischer, H.R. Scholer, U. Martin, Generation of induced pluripotent stem cells from human cord blood, Cell Stem Cell 5 (2009) 434–441.
[77] I. Kehat, L. Khimovich, O. Caspi, A. Gepstein, R. Shofti, G. Arbel, I. Huber, J. Satin, J. Itskovitz-Eldor, L. Gepstein, Electromechanical integration of cardiomyocytes derived from human embryonic stem cells, Nat. Biotechnol. 22 (2004) 1282–1289.
[78] S. Makino, K. Fukuda, S. Miyoshi, F. Konishi, H. Kodama, J. Pan, M. Sano, T. Takahashi, S. Hori, H. Abe, J. Hata, A. Umezawa, S. Ogawa, Cardiomyocytes can be generated from marrow stromal cells in vitro, J. Clin. Invest. 103 (1999) 697–705.
[79] C. Mauritz, K. Schwanke, M. Reppel, S. Neef, K. Katsirntaki, L.S. Maier, F. Nguemo, S. Menke, M. Haustein, J. Hescheler, G. Hasenfuss, U. Martin, Generation of functional murine cardiac myocytes from induced pluripotent stem cells, Circulation 118 (2008) 507–517.
[80] P. Menasche, Myoblast transplantation: feasibility, safety and efficacy, Ann. Med. 34 (2002) 314–315.
[81] O. Pfister, F. Mouquet, M. Jain, R. Summer, M. Helmes, A. Fine, W.S. Colucci, R. Liao, CD31− but not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation, Circ. Res. 97 (2005) 52–61.
[82] S. Zhou, J.D. Schuetz, K.D. Bunting, A.M. Colapietro, J. Sampath, J.J. Morris, I. Lagutina, G.C. Grosveld, M. Osawa, H. Nakauchi, B.P. Sorrentino, The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype, Nat. Med. 7 (2001) 1028–1034.
[83] C.W. Scharenberg, M.A. Harkey, B. Torok-Storb, The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors, Blood 99 (2002) 507–512.
[84] T.I. van der Spoel, S.J. Jansen of Lorkeers, P. Agostoni, E. van Belle, M. Gyongyosi, J.P. Sluijter, M.J. Cramer, P.A. Doevendans, S.A. Chamuleau, Human relevance of pre-clinical studies in stem cell therapy: systematic review and meta-analysis of large animal models of ischaemic heart disease, Cardiovasc. Res. 91 (2011) 649–658.
[85] C. Mias, O. Lairez, E. Trouche, J. Roncalli, D. Calise, M.H. Seguelas, C. Ordener, M.D. Piercecchi-Marti, N. Auge, A.N. Salvayre, P. Bourin, A. Parini, D. Cussac, Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction, Stem Cells 27 (2009) 2734–2743.
[86] A.F. Godier-Furnemont, T.P. Martens, M.S. Koeckert, L. Wan, J. Parks, K. Arai, G. Zhang, B. Hudson, S. Homma, G. Vunjak-Novakovic, Composite scaffold provides a cell delivery platform for cardiovascular repair, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7974–7979.
[87] W.S. Lian, W.T. Cheng, C.C. Cheng, F.S. Hsiao, J.J. Chen, C.F. Cheng, S.C. Wu, In vivo therapy of myocardial infarction with mesenchymal stem cells modified with prostaglandin I synthase gene improves cardiac performance in mice, Life Sci. 88 (2011) 455–464.
[88] M. Gnecchi, H. He, O.D. Liang, L.G. Melo, F. Morello, H. Mu, N. Noiseux, L. Zhang, R.E. Pratt, J.S. Ingwall, V.J. Dzau, Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells, Nat. Med. 11 (2005) 367–368.
[89] L.C. Amado, A.P. Saliaris, K.H. Schuleri, M. St John, J.S. Xie, S. Cattaneo, D.J. Durand, T. Fitton, J.Q. Kuang, G. Stewart, S. Lehrke, W.W. Baumgartner, B.J. Martin, A.W. Heldman, J.M. Hare, Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 11474–11479.
[90] A. Bel, E. Messas, O. Agbulut, P. Richard, J.L. Samuel, P. Bruneval, A.A. Hagege, P. Menasche, Transplantation of autologous fresh bone marrow into infarcted myocardium: a word of caution, Circulation 108 (Suppl. 1) (2003) II247–II252.
[91] R. de Silva, A.N. Raval, M. Hadi, K.M. Gildea, A.C. Bonifacino, Z.X. Yu, Y.Y. Yau, S.F. Leitman, S.L. Bacharach, R.E. Donahue, E.J. Read, R.J. Lederman, Intracoronary infusion of autologous mononuclear cells from bone marrow or granulocyte colony-stimulating factor-mobilized apheresis product may not improve remodelling, contractile function, perfusion, or infarct size in a swine model of large myocardial infarction, Eur. Heart J. 29 (2008) 1772–1782.
[92] C. Brasselet, M.C. Morichetti, E. Messas, C. Carrion, A. Bissery, P. Bruneval, J.T. Vilquin, A. Lafont, A.A. Hagege, P. Menasche, M. Desnos, Skeletal myoblast transplantation through a catheter-based coronary sinus approach: an effective means of improving function of infarcted myocardium, Eur. Heart J. 26 (2005) 1551–1556.
[93] E. Messas, A. Bel, M.C. Morichetti, C. Carrion, M.D. Handschumacher, S. Peyrard, J.T. Vilquin, M. Desnos, P. Bruneval, A. Carpentier, P. Menasche, R.A. Levine, A.A. Hagege, Autologous myoblast transplantation for chronic ischemic mitral regurgitation, J. Am. Coll. Cardiol. 47 (2006) 2086–2093.
[94] P.V. Johnston, T. Sasano, K. Mills, R. Evers, S.T. Lee, R.R. Smith, A.C. Lardo, S. Lai, C. Steenbergen, G. Gerstenblith, R. Lange, E. Marban, Engraftment, differentiation, and functional benefits of autologous cardiosphere-derived cells in porcine ischemic cardiomyopathy, Circulation 120 (2009) 1075–1083(1077 p. following 1083).
[95] C. Menard, A.A. Hagege, O. Agbulut, M. Barro, M.C. Morichetti, C. Brasselet, A. Bel, E. Messas, A. Bissery, P. Bruneval, M. Desnos, M. Puceat, P. Menasche, Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study, Lancet 366 (2005) 1005–1012.
[96] C. Templin, R. Zweigerdt, K. Schwanke, R. Olmer, J.R. Ghadri, M.Y. Emmert, E. Müller, S.M. Küest, S. Cohrs, R. Schibli, P. Kronen, M. Hilbe, D. Strunk, A. Haverich, S.P. Hoerstrup, T.F. Lüscher, P.A. Kaufmann, U. Landmesser, U. Martin, Transplantation and tracking of human induced pluripotent stem cells in a pig model of myocardial infarction: assessment of cell survival, engraftment and distribution by hybrid spect-ct imaging of sodium iodide symporter trangene expression, Circulation 126 (4) (Jul. 24 2012) 430–439.
[97] M. Gyongyosi, J. Blanco, T. Marian, L. Tron, O. Petnehazy, Z. Petrasi, R. Hemetsberger, J. Rodriguez, G. Font, I.J. Pavo, I. Kertesz, L. Balkay, N. Pavo, A. Posa, M. Emri, L. Galuska, D.L. Kraitchman, J. Wojta, K. Huber, D. Glogar, Serial noninvasive in vivo positron emission tomographic tracking of percutaneously intramyocardially injected autologous porcine mesenchymal stem cells modified for transgene reporter gene expression, Circ. Cardiovasc. Imaging 1 (2008) 94–103.
[98] V. Barron, E. Lyons, C. Stenson-Cox, P.E. McHugh, A. Pandit, Bioreactors for cardiovascular cell and tissue growth: a review, Ann. Biomed. Eng. 31 (2003) 1017–1030.
[99] M. Govoni, C. Muscari, C. Guarnieri, E. Giordano, Mechanostimulation protocols for cardiac tissue engineering, BioMed. Res. Int. 2013 (2013) 918640.
[100] I. Martin, D. Wendt, M. Heberer, The role of bioreactors in tissue engineering, Trends Biotechnol. 22 (2004) 80–86.
[101] D. Massai, G. Cerino, D. Gallo, F. Pennella, M.A. Deriu, A. Rodriguez, F.M. Montevecchi, C. Bignardi, A. Audenino, U. Morbiducci, Bioreactors as engineering support to treat cardiac muscle and vascular disease, J. Healthc. Eng. 4 (2013) 329–370.
[102] P.A. Sotiropoulou, S.A. Perez, M. Salagianni, C.N. Baxevanis, M. Papamichail, Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells, Stem Cells 24 (2006) 462–471.
[103] I. Sekiya, B.L. Larson, J.R. Smith, R. Pochampally, J.G. Cui, D.J. Prockop, Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality, Stem Cells 20 (2002) 530–541.
[104] K. Ksiazek, A comprehensive review on mesenchymal stem cell growth and senescence, Rejuvenation Res. 12 (2009) 105–116.
[105] F. Dos Santos, P.Z. Andrade, J.S. Boura, M.M. Abecasis, C.L. da Silva, J.M. Cabral, Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia, J. Cell. Physiol. 223 (2010) 27–35.
[106] P. Godara, C.D. McFarland, R.E. Nordon, Design of bioreactors for mesenchymal stem cell tissue engineering, J. Chem. Technol. Biotechnol. 83 (2008) 408–420.
[107] J.E. Frith, B. Thomson, P.G. Genever, Dynamic three-dimensional culture methods enhance mesenchymal stem cell properties and increase therapeutic potential, Tissue Eng. Part C Methods 16 (2010) 735–749.
[108] S. Hollister, Y. Wang, G. Wang, L. Chen, H. Li, T. Yin, B. Wang, J. Lee, Q. Yu, J. Kim, Scaffold Engineering: A Bridge to Where? 2009.
[109] T. Eschenhagen, W.H. Zimmermann, Engineering myocardial tissue, Circ. Res. 97 (2005) 1220–1231.
[110] H. Naito, I. Melnychenko, M. Didie, K. Schneiderbanger, P. Schubert, S. Rosenkranz, T. Eschenhagen, W.H. Zimmermann, Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle, Circulation 114 (2006) I72–I78.
[111] W.H. Zimmermann, R. Cesnjevar, Cardiac tissue engineering: implications for pediatric heart surgery, Pediatr. Cardiol. 30 (2009) 716–723.
[112] S. Masuda, T. Shimizu, M. Yamato, T. Okano, Cell sheet engineering for heart tissue repair, Adv. Drug Deliv. Rev. 60 (2008) 277–285.
[113] J. Doshi, D. Reneker, Electrospinning process and applications of electrospun fibers, J. Electrost. 35 (1995) 151–160.
[114] D. Reneker, A. Yarin, Electrospinning jets and polymer nanofibers, Polymer 49 (2008) 2387–2425.
[115] Z. Huang, Y. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Compos. Sci. Technol. 63 (2003) 2223–2253.
[116] B.M. Baker, A.O. Gee, R.B. Metter, A.S. Nathan, R.A. Marklein, J.A. Burdick, R.L. Mauck, The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers, Biomaterials 29 (2008) 2348–2358.
[117] A.S. Rowlands, S.A. Lim, D. Martin, J.J. Cooper-White, Polyurethane/poly(lactic-co-glycolic) acid composite scaffolds fabricated by thermally induced phase separation, Biomaterials 28 (2007) 2109–2121.
[118] J.D. Fromstein, P.W. Zandstra, C. Alperin, D. Rockwood, J.F. Rabolt, K.A. Woodhouse, Seeding bioreactor-produced embryonic stem cell-derived cardiomyocytes on

different porous, degradable, polyurethane scaffolds reveals the effect of scaffold architecture on cell morphology, Tissue Eng. Part A 14 (2008) 369–378.
[119] M.T. Khorasani, S. Shorgashti, Fabrication of microporous polyurethane by spray phase inversion method as small diameter vascular grafts material, J. Biomed. Mater. Res. A 77 (2006) 253–260.
[120] B.J. Papenburg, L. Vogelaar, L.A.M. Bolhuis-Versteeg, R.G.H. Lammertink, D. Stamatialis, M. Wessling, One-step fabrication of porous micropatterned scaffolds to control cell behavior, Biomaterials 28 (2007) 1998–2009.
[121] B.J. Papenburg, J. Liu, G.A. Higuera, A.M. Barradas, J. de Boer, C.A. van Blitterswijk, M. Wessling, D. Stamatialis, Development and analysis of multi-layer scaffolds for tissue engineering, Biomaterials 30 (2009) 6228–6239.
[122] J.P. Kennedy, S.P. McCandless, A. Rauf, L.M. Williams, J. Hillam, R.W. Hitchcock, Engineered channels enhance cellular density in perfused scaffolds, Acta Biomater. 7 (2011) 3896–3904.
[123] N. Bursac, K.K. Parker, S. Iravanian, L. Tung, Cardiomyocyte cultures with controlled macroscopic anisotropy: a model for functional electrophysiological studies of cardiac muscle, Circ. Res. 91 (2002) e45–e54.
[124] T. Pong, W.J. Adams, M.A. Bray, A.W. Feinberg, S.P. Sheehy, A.A. Werdich, K.K. Parker, Hierarchical architecture influences calcium dynamics in engineered cardiac muscle, Exp. Biol. Med. (Maywood) 236 (2011) 366–373.
[125] P.A. Galie, M.V. Westfall, J.P. Stegemann, Reduced serum content and increased matrix stiffness promote the cardiac myofibroblast transition in 3D collagen matrices, Cardiovasc. Pathol. 20 (6) (Nov-Dec 2011) 325–333.
[126] A. Marsano, R. Maidhof, L.Q. Wan, Y. Wang, J. Gao, N. Tandon, G. Vunjak-Novakovic, Scaffold stiffness affects the contractile function of three-dimensional engineered cardiac constructs, Biotechnol. Prog. 26 (2010) 1382–1390.
[127] L. Moroni, J.R. de Wijn, C.A. van Blitterswijk, 3D fiber-deposited scaffolds for tissue engineering: influence of pores geometry and architecture on dynamic mechanical properties, Biomaterials 27 (2006) 974–985.
[128] J.P. Kennedy, S.P. McCandless, R.A. Lasher, R.W. Hitchcock, The mechanically enhanced phase separation of sprayed polyurethane scaffolds and their effect on the alignment of fibroblasts, Biomaterials 31 (2010) 1126–1132.
[129] C. Liu, R. Abedian, R. Meister, C. Haasper, C. Hurschler, C. Krettek, G. von Lewinski, M. Jagodzinski, Influence of perfusion and compression on the proliferation and differentiation of bone mesenchymal stromal cells seeded on polyurethane scaffolds, Biomaterials 33 (2011) 1052–1064.
[130] R. Maidhof, N. Tandon, E.J. Lee, J. Luo, Y. Duan, K. Yeager, E. Konofagou, G. Vunjak-Novakovic, Biomimetic perfusion and electrical stimulation applied in concert improved the assembly of engineered cardiac tissue, J. Tissue Eng. Regen. Med. 6 (10) (Nov. 2012) e12–e23.
[131] T. Zhang, L.Q. Wan, Z. Xiong, A. Marsano, R. Maidhof, M. Park, Y. Yan, G. Vunjak-Novakovic, Channelled scaffolds for engineering myocardium with mechanical stimulation, J. Tissue Eng. Regen. Med. (Nov. 14 2011), http://dx.doi.org/10.1002/term.481, [Epub ahead of print].
[132] R. Maidhof, A. Marsano, E.J. Lee, G. Vunjak-Novakovic, Perfusion seeding of channeled elastomeric scaffolds with myocytes and endothelial cells for cardiac tissue engineering, Biotechnol. Prog. 26 (2010) 565–572.
[133] S. Kaihara, J. Borenstein, R. Koka, S. Lalan, E. Ochoa, M. Ravens, H. Pien, B. Cunningham, J. Vacanti, Silicon micromachining to tissue engineer branched vascular channels for liver fabrication, Tissue Eng. 6 (2000) 105–117.
[134] B.J. Papenburg, L.A. Bolhuis-Versteeg, D.W. Grijpma, J. Feijen, M. Wessling, D. Stamatialis, A facile method to fabricate poly(L-lactide) nano-fibrous morphologies by phase inversion, Acta Biomater. 6 (7) (Jul. 2010) 2477–2483.
[135] C. Fink, S. Ergun, D. Kralisch, U. Remmers, J. Weil, T. Eschenhagen, Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement, FASEB J. 14 (2000) 669–679.
[136] W.H. Zimmermann, K. Schneiderbanger, P. Schubert, M. Didie, F. Munzel, J.F. Heubach, S. Kostin, W.L. Neuhuber, T. Eschenhagen, Tissue engineering of a differentiated cardiac muscle construct, Circ. Res. 90 (2002) 223–230.
[137] Z. Feng, T. Matsumoto, Y. Nomura, T. Nakamura, An electro-tensile bioreactor for 3D culturing of cardiomyocytes, IEEE Eng. Med. Biol. Mag. 24 (2005) 73–79.
[138] M. Radisic, H. Park, H. Shing, T. Consi, F.J. Schoen, R. Langer, L.E. Freed, G. Vunjak-Novakovic, Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 18129–18134.
[139] N. Tandon, C. Cannizzaro, P.H.G. Chao, R. Maidhof, A. Marsano, H.T.H. Au, M. Radisic, G. Vunjak-Novakovic, Electrical stimulation systems for cardiac tissue engineering, Nat. Protoc. 4 (2009) 155–173.
[140] C. Cannizzaro, N. Tandon, E. Figallo, H. Park, S. Gerecht, M. Radisic, N. Elvassore, G. Vunjak-Novakovic, Practical aspects of cardiac tissue engineering with electrical stimulation, Methods Mol. Med. 140 (2007) 291–307.
[141] P. Akhyari, P.W.M. Fedak, R.D. Weisel, T.Y.J. Lee, S. Verma, D.A.G. Mickle, R.K. Li, Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts, 106 (2002) 137–142.
[142] R.K. Birla, Y.C. Huang, R.G. Dennis, Development of a novel bioreactor for the mechanical loading of tissue-engineered heart muscle, Tissue Eng. 13 (2007) 2239–2248.
[143] J. Zhuang, K.A. Yamada, J.E. Saffitz, A.G. Kleber, Pulsatile stretch remodels cell-to-cell communication in cultured myocytes, Circ. Res. 87 (2000) 316–322.
[144] M. Gonen-Wadmany, L. Gepstein, D. Seliktar, Controlling the cellular organization of tissue-engineered cardiac constructs, Ann. N. Y. Acad. Sci. 1015 (2004) 299–311.
[145] M. Radisic, V.G. Fast, O.F. Sharifov, R.K. Iyer, H. Park, G. Vunjak-Novakovic, Optical mapping of impulse propagation in engineered cardiac tissue, Tissue Eng. Part A 15 (2009) 851–860.
[146] M. Radisic, H. Park, S. Gerecht, C. Cannizzaro, R. Langer, G. Vunjak-Novakovic, Biomimetic approach to cardiac tissue engineering, Philos. Trans. R. Soc. Lond. B Biol. Sci. 362 (2007) 1357–1368.
[147] L.G. Griffith, G. Naughton, Tissue engineering—current challenges and expanding opportunities, Science 295 (2002) 1009–1014.
[148] T. Eschenhagen, C. Fink, U. Remmers, H. Scholz, J. Wattchow, J. Weil, W. Zimmermann, H.H. Dohmen, H. Schafer, N. Bishopric, T. Wakatsuki, E.L. Elson, Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system, FASEB J. 11 (1997) 683–694.
[149] K.L. Christman, R.J. Lee, Biomaterials for the treatment of myocardial infarction, J. Am. Coll. Cardiol. 48 (2006) 907–913.
[150] T. Dvir, A. Kedem, E. Ruvinov, O. Levy, I. Freeman, N. Landa, R. Holbova, M.S. Feinberg, S. Dror, Y. Etzion, J. Leor, S. Cohen, Prevascularization of cardiac patch on the omentum improves its therapeutic outcome, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14990–14995.
[151] R.K. Iyer, L.L. Chiu, L.A. Reis, M. Radisic, Engineered cardiac tissues, Curr. Opin. Biotechnol. 22 (2011) 706–714.
[152] J.C. Nawroth, H. Lee, A.W. Feinberg, C.M. Ripplinger, M.L. McCain, A. Grosberg, J.O. Dabiri, K.K. Parker, A tissue-engineered jellyfish with biomimetic propulsion, Nat. Biotechnol. 30 (2012) 792–797.
[153] O. Caspi, A. Lesman, Y. Basevitch, A. Gepstein, G. Arbel, I.H. Habib, L. Gepstein, S. Levenberg, Tissue engineering of vascularized cardiac muscle from human embryonic stem cells, Circ. Res. 100 (2007) 263–272.
[154] B. Bhana, R.K. Iyer, W.L. Chen, R. Zhao, K.L. Sider, M. Likhitpanichkul, C.A. Simmons, M. Radisic, Influence of substrate stiffness on the phenotype of heart cells, Biotechnol. Bioeng. 105 (2010) 1148–1160.
[155] J.G. Jacot, A.D. McCulloch, J.H. Omens, Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes, Biophys. J. 95 (2008) 3479–3487.
[156] S. Miyagawa, Y. Sawa, S. Sakakida, S. Taketani, H. Kondoh, I.A. Memon, Y. Imanishi, T. Shimizu, T. Okano, H. Matsuda, Tissue cardiomyoplasty using bioengineered contractile cardiomyocyte sheets to repair damaged myocardium: their integration with recipient myocardium, Transplantation 80 (2005) 1586–1595.
[157] W.H. Zimmermann, I. Melnychenko, G. Wasmeier, M. Didie, H. Naito, U. Nixdorff, A. Hess, L. Budinsky, K. Brune, B. Michaelis, S. Dhein, A. Schwoerer, H. Ehmke, T. Eschenhagen, Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts, Nat. Med. 12 (2006) 452–458.
[158] T. Xue, H.C. Cho, F.G. Akar, S.Y. Tsang, S.P. Jones, E. Marban, G.F. Tomaselli, R.A. Li, Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers, Circulation 111 (2005) 11–20.
[159] T.P. Kraehenbuehl, L.S. Ferreira, A.M. Hayward, M. Nahrendorf, A.J. van der Vlies, E. Vasile, R. Weissleder, R. Langer, J.A. Hubbell, Human embryonic stem cell-derived microvascular grafts for cardiac tissue preservation after myocardial infarction, Biomaterials 32 (2011) 1102–1109.
[160] B.M. Leung, M.V. Sefton, A modular approach to cardiac tissue engineering, Tissue Eng. Part A 16 (2010) 3207–3218.
[161] Y. Miyagi, L.L. Chiu, M. Cimini, R.D. Weisel, M. Radisic, R.K. Li, Biodegradable collagen patch with covalently immobilized VEGF for myocardial repair, Biomaterials 32 (2011) 1280–1290.
[162] L. Barandon, T. Couffinhal, P. Dufourcq, P. Alzieu, D. Daret, C. Deville, C. Duplaa, Repair of myocardial infarction by epicardial deposition of bone-marrow-cell-coated muscle patch in a murine model, Ann. Thorac. Surg. 78 (2004) 1409–1417.
[163] C.H. Chen, H.J. Wei, W.W. Lin, I. Chiu, S.M. Hwang, C.C. Wang, W.Y. Lee, Y. Chang, H.W. Sung, Porous tissue grafts sandwiched with multilayered mesenchymal stromal cell sheets induce tissue regeneration for cardiac repair, Cardiovasc. Res. 80 (2008) 88–95.
[164] H.J. Wei, S.C. Chen, Y. Chang, S.M. Hwang, W.W. Lin, P.H. Lai, H.K. Chiang, L.F. Hsu, H.H. Yang, H.W. Sung, Porous acellular bovine pericardia seeded with mesenchymal stem cells as a patch to repair a myocardial defect in a syngeneic rat model, Biomaterials 27 (2006) 5409–5419.
[165] J. Jin, S.I. Jeong, Y.M. Shin, K.S. Lim, H. Shin, Y.M. Lee, H.C. Koh, K.S. Kim, Transplantation of mesenchymal stem cells within a poly(lactide-co-epsilon-caprolactone) scaffold improves cardiac function in a rat myocardial infarction model, Eur. J. Heart Fail. 11 (2009) 147–153.
[166] D. Simpson, H. Liu, T.H. Fan, R. Nerem, S.C. Dudley Jr., A tissue engineering approach to progenitor cell delivery results in significant cell engraftment and improved myocardial remodeling, Stem Cells 25 (2007) 2350–2357.
[167] M.Y. Tan, W. Zhi, R.Q. Wei, Y.C. Huang, K.P. Zhou, B. Tan, L. Deng, J.C. Luo, X.Q. Li, H.Q. Xie, Z.M. Yang, Repair of infarcted myocardium using mesenchymal stem cell seeded small intestinal submucosa in rabbits, Biomaterials 30 (2009) 3234–3240.
[168] Y. Miyahara, N. Nagaya, M. Kataoka, B. Yanagawa, K. Tanaka, H. Hao, K. Ishino, H. Ishida, T. Shimizu, K. Kangawa, S. Sano, T. Okano, S. Kitamura, H. Mori, Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction, Nat. Med. 12 (2006) 459–465.
[169] L. Zakharova, D. Mastroeni, N. Mutlu, M. Molina, S. Goldman, E. Diethrich, M.A. Gaballa, Transplantation of cardiac progenitor cell sheet onto infarcted heart promotes cardiogenesis and improves function, Cardiovasc. Res. 87 (2010) 40–49.
[170] T. Kofidis, D.R. Lebl, E.C. Martinez, G. Hoyt, M. Tanaka, R.C. Robbins, Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury, Circulation 112 (2005) I173–I177.
[171] Y.D. Lin, M.L. Yeh, Y.J. Yang, D.C. Tsai, T.Y. Chu, Y.Y. Shih, M.Y. Chang, Y.W. Liu, A.C. Tang, T.Y. Chen, C.Y. Luo, K.C. Chang, J.H. Chen, H.L. Wu, T.K. Hung, P.C. Hsieh, Intramyocardial peptide nanofiber injection improves postinfarction ventricular

remodeling and efficacy of bone marrow cell therapy in pigs, Circulation 122 (2010) S132–S141.
[172] S.T. Wall, C.C. Yeh, R.Y. Tu, M.J. Mann, K.E. Healy, Biomimetic matrices for myocardial stabilization and stem cell transplantation, J. Biomed. Mater. Res. A 95 (2010) 1055–1066.
[173] T. Wang, X.J. Jiang, Q.Z. Tang, X.Y. Li, T. Lin, D.Q. Wu, X.Z. Zhang, E. Okello, Bone marrow stem cells implantation with alpha-cyclodextrin/MPEG-PCL-MPEG hydrogel improves cardiac function after myocardial infarction, Acta Biomater. 5 (2009) 2939–2944.
[174] W. Dai, S.L. Hale, G.L. Kay, A.J. Jyrala, R.A. Kloner, Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology, Regen. Med. 4 (2009) 387–395.
[175] K.L. Christman, H.H. Fok, R.E. Sievers, Q. Fang, R.J. Lee, Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction, Tissue Eng. 10 (2004) 403–409.
[176] M.E. Danoviz, J.S. Nakamuta, F.L. Marques, L. dos Santos, E.C. Alvarenga, A.A. dos Santos, E.L. Antonio, I.T. Schettert, P.J. Tucci, J.E. Krieger, Rat adipose tissue-derived stem cells transplantation attenuates cardiac dysfunction post infarction and biopolymers enhance cell retention, PLoS One 5 (2010) e12077.
[177] H.D. Guo, H.J. Wang, Y.Z. Tan, J.H. Wu, Transplantation of marrow-derived cardiac stem cells carried in fibrin improves cardiac function after myocardial infarction, Tissue Eng. Part A 17 (2011) 45–58.
[178] J.S. Nakamuta, M.E. Danoviz, F.L. Marques, L. dos Santos, C. Becker, G.A. Goncalves, P.F. Vassallo, I.T. Schettert, P.J. Tucci, J.E. Krieger, Cell therapy attenuates cardiac dysfunction post myocardial infarction: effect of timing, routes of injection and a fibrin scaffold, PLoS One 4 (2009) e6005.
[179] C.C. Wang, C.H. Chen, W.W. Lin, S.M. Hwang, P.C. Hsieh, P.H. Lai, Y.C. Yeh, Y. Chang, H.W. Sung, Direct intramyocardial injection of mesenchymal stem cell sheet fragments improves cardiac functions after infarction, Cardiovasc. Res. 77 (2008) 515–524.
[180] T.P. Martens, A.F. Godier, J.J. Parks, L.Q. Wan, M.S. Koeckert, G.M. Eng, B.I. Hudson, W. Sherman, G. Vunjak-Novakovic, Percutaneous cell delivery into the heart using hydrogels polymerizing in situ, Cell Transplant. 18 (2009) 297–304.
[181] M. Radisic, K.L. Christman, Materials science and tissue engineering: repairing the heart, Mayo Clin. Proc. 88 (2013) 884–898.
[182] N. Landa, L. Miller, M.S. Feinberg, R. Holbova, M. Shachar, I. Freeman, S. Cohen, J. Leor, Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat, Circulation 117 (2008) 1388–1396.
[183] J. Leor, S. Tuvia, V. Guetta, F. Manczur, D. Castel, U. Willenz, O. Petnehazy, N. Landa, M.S. Feinberg, E. Konen, O. Goitein, O. Tsur-Gang, M. Shaul, L. Klapper, S. Cohen, Intracoronary injection of in situ forming alginate hydrogel reverses left ventricular remodeling after myocardial infarction in swine, J. Am. Coll. Cardiol. 54 (2009) 1014–1023.
[184] T.J. Bartosh, J.H. Ylostalo, A. Mohammadipoor, N. Bazhanov, K. Coble, K. Claypool, R.H. Lee, H. Choi, D.J. Prockop, Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13724–13729.
[185] L.R. Garzoni, M.I. Rossi, A.P. de Barros, V. Guarani, M. Keramidas, L.B. Balottin, D. Adesse, C.M. Takiya, P.P. Manso, I.B. Otazu, N. Meirelles Mde, R. Borojevic, Dissecting coronary angiogenesis: 3D co-culture of cardiomyocytes with endothelial or mesenchymal cells, Exp. Cell Res. 315 (2009) 3406–3418.
[186] J.M. Kelm, C. Diaz Sanchez-Bustamante, E. Ehler, S.P. Hoerstrup, V. Djonov, L. Ittner, M. Fussenegger, VEGF profiling and angiogenesis in human microtissues, J. Biotechnol. 118 (2005) 213–229.
[187] J.M. Kelm, V. Djonov, L.M. Ittner, D. Fluri, W. Born, S.P. Hoerstrup, M. Fussenegger, Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units, Tissue Eng. 12 (2006) 2151–2160.
[188] J.M. Kelm, M. Fussenegger, Microscale tissue engineering using gravity-enforced cell assembly, Trends Biotechnol. 22 (2004) 195–202.
[189] W. Wang, K. Itaka, S. Ohba, N. Nishiyama, U.I. Chung, Y. Yamasaki, K. Kataoka, 3D spheroid culture system on micropatterned substrates for improved differentiation efficiency of multipotent mesenchymal stem cells, Biomaterials 30 (2009) 2705–2715.
[190] J.M. Kelm, V. Djonov, S.P. Hoerstrup, C.I. Guenter, L.M. Ittner, F. Greve, A. Hierlemann, C.D. Sanchez-Bustamante, J.C. Perriard, E. Ehler, M. Fussenegger, Tissue-transplant fusion and vascularization of myocardial microtissues and macrotissues implanted into chicken embryos and rats, Tissue Eng. 12 (2006) 2541–2553.
[191] M.Y. Emmert, P. Wolint, N. Wickboldt, G. Gemayel, B. Weber, C.E. Brokopp, A. Boni, V. Falk, A. Bosman, M.E. Jaconi, S.P. Hoerstrup, Human stem cell-based three-dimensional microtissues for advanced cardiac cell therapies, Biomaterials 34 (2013) 6339–6354.
[192] G. Kensah, A. Roa Lara, J. Dahlmann, R. Zweigerdt, K. Schwanke, J. Hegermann, D. Skvorc, A. Gawol, A. Azizian, S. Wagner, L.S. Maier, A. Krause, G. Drager, M. Ochs, A. Haverich, I. Gruh, U. Martin, Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro, Eur. Heart J. 34 (15) (Apr. 2013) 1134–1146.
[193] U. Testa, G. Pannitteri, G.L. Condorelli, Vascular endothelial growth factors in cardiovascular medicine, J. Cardiovasc. Med. (Hagerstown) 9 (2008) 1190–1221.
[194] M.Y. Emmert, P. Wolint, S. Winklhofer, P. Stolzmann, N. Cesarovic, T. Fleischmann, T.D. Nguyen, T. Frauenfelder, R. Boni, J. Scherman, D. Bettex, J. Grunenfelder, R. Schwartlander, V. Vogel, M. Gyongyosi, H. Alkadhi, V. Falk, S.P. Hoerstrup, Transcatheter based electromechanical mapping guided intramyocardial transplantation and in vivo tracking of human stem cell based three dimensional microtissues in the porcine heart, Biomaterials 34 (2013) 2428–2441.
[195] V. Schachinger, S. Erbs, A. Elsasser, W. Haberbosch, R. Hambrecht, H. Holschermann, J. Yu, R. Corti, D.G. Mathey, C.W. Hamm, T. Suselbeck, B. Assmus, T. Tonn, S. Dimmeler, A.M. Zeiher, Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction, N. Engl. J. Med. 355 (2006) 1210–1221.
[196] K.C. Wollert, G.P. Meyer, J. Lotz, S. Ringes-Lichtenberg, P. Lippolt, C. Breidenbach, S. Fichtner, T. Korte, B. Hornig, D. Messinger, L. Arseniev, B. Hertenstein, A. Ganser, H. Drexler, Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial, Lancet 364 (2004) 141–148.
[197] S. Janssens, C. Dubois, J. Bogaert, K. Theunissen, C. Deroose, W. Desmet, M. Kalantzi, L. Herbots, P. Sinnaeve, J. Dens, J. Maertens, F. Rademakers, S. Dymarkowski, O. Gheysens, J. Van Cleemput, G. Bormans, J. Nuyts, A. Belmans, L. Mortelmans, M. Boogaerts, F. Van de Werf, Autologous bone marrow-derived stem-cell transfer in patients with st-segment elevation myocardial infarction: double-blind, randomised controlled trial, Lancet 367 (2006) 113–121.
[198] K. Lunde, S. Solheim, S. Aakhus, H. Arnesen, M. Abdelnoor, T. Egeland, K. Endresen, A. Ilebekk, A. Mangschau, J.G. Fjeld, H.J. Smith, E. Taraldsrud, H.K. Grogaard, R. Bjornerheim, M. Brekke, C. Muller, E. Hopp, A. Ragnarsson, J.E. Brinchmann, K. Forfang, Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction, N. Engl. J. Med. 355 (2006) 1199–1209.
[199] M. Tendera, W. Wojakowski, W. Ruzyllo, L. Chojnowska, C. Kepka, W. Tracz, P. Musialek, W. Piwowarska, J. Nessler, P. Buszman, S. Grajek, P. Breborowicz, M. Majka, M.Z. Ratajczak, Intracoronary infusion of bone marrow-derived selected CD34+ CXCR4+ cells and non-selected mononuclear cells in patients with acute stemi and reduced left ventricular ejection fraction: results of randomized, multicentre myocardial regeneration by intracoronary infusion of selected population of stem cells in acute myocardial infarction (regent) trial, Eur. Heart J. 30 (2009) 1313–1321.
[200] E.C. Perin, J.T. Willerson, C.J. Pepine, T.D. Henry, S.G. Ellis, D.X. Zhao, G.V. Silva, D. Lai, J.D. Thomas, M.W. Kronenberg, A.D. Martin, R.D. Anderson, J.H. Traverse, M.S. Penn, S. Anwaruddin, A.K. Hatzopoulos, A.P. Gee, D.A. Taylor, C.R. Cogle, D. Smith, L. Westbrook, J. Chen, E. Handberg, R.E. Olson, C. Geither, S. Bowman, J. Francescon, S. Baraniuk, L.B. Piller, L.M. Simpson, C. Loghin, D. Aguilar, S. Richman, C. Zierold, J. Bettencourt, S.L. Sayre, R.W. Vojvodic, S.I. Skarlatos, D.J. Gordon, R.F. Ebert, M. Kwak, L.A. Moye, R.D. Simari, Cardiovascular Cell Therapy Research N, Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial, JAMA 307 (2012) 1717–1726.
[201] A. Hirsch, R. Nijveldt, P.A. van der Vleuten, J.G. Tijssen, W.J. van der Giessen, R.A. Tio, J. Waltenberger, J.M. ten Berg, P.A. Doevendans, W.R. Aengevaeren, J.J. Zwaginga, B.J. Biemond, A.C. van Rossum, J.J. Piek, F. Zijlstra, Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial, Eur. Heart J. 32 (2011) 1736–1747.
[202] D. Surder, R. Manka, V. Lo Cicero, T. Moccetti, K. Rufibach, S. Soncin, L. Turchetto, M. Radrizzani, G. Astori, J. Schwitter, P. Erne, M. Zuber, C. Auf der Maur, P. Jamshidi, O. Gaemperli, S. Windecker, A. Moschovitis, A. Wahl, I. Buhler, C. Wyss, S. Kozerke, U. Landmesser, T.F. Luscher, R. Corti, Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: effects on global left ventricular function, Circulation 127 (2013) 1968–1979.
[203] J.H. Traverse, T.D. Henry, S.G. Ellis, C.J. Pepine, J.T. Willerson, D.X. Zhao, J.R. Forder, B.J. Byrne, A.K. Hatzopoulos, M.S. Penn, E.C. Perin, K.W. Baran, J. Chambers, C. Lambert, G. Raveendran, D.I. Simon, D.E. Vaughan, L.M. Simpson, A.P. Gee, D.A. Taylor, C.R. Cogle, J.D. Thomas, G.V. Silva, B.C. Jorgenson, R.E. Olson, S. Bowman, J. Francescon, C. Geither, E. Handberg, D.X. Smith, S. Baraniuk, L.B. Piller, C. Loghin, D. Aguilar, S. Richman, C. Zierold, J. Bettencourt, S.L. Sayre, R.W. Vojvodic, S.I. Skarlatos, D.J. Gordon, R.F. Ebert, M. Kwak, L.A. Moye, R.D. Simari, Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: the LATETIME randomized trial, JAMA 306 (2011) 2110–2119.
[204] J.H. Traverse, T.D. Henry, C.J. Pepine, J.T. Willerson, D.X. Zhao, S.G. Ellis, J.R. Forder, R.D. Anderson, A.K. Hatzopoulos, M.S. Penn, E.C. Perin, J. Chambers, K.W. Baran, G. Raveendran, C. Lambert, A. Lerman, D.I. Simon, D.E. Vaughan, D. Lai, A.P. Gee, D.A. Taylor, C.R. Cogle, J.D. Thomas, R.E. Olson, S. Bowman, J. Francescon, C. Geither, E. Handberg, C. Kappenman, L. Westbrook, L.B. Piller, L.M. Simpson, S. Baraniuk, C. Loghin, D. Aguilar, S. Richman, C. Zierold, D.B. Spoon, J. Bettencourt, S.L. Sayre, R.W. Vojvodic, S.I. Skarlatos, D.J. Gordon, R.F. Ebert, M. Kwak, L.A. Moye, R.D. Simari, Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: the time randomized trial, JAMA 308 (2012) 2380–2389.
[205] P.J. Psaltis, A.C. Zannettino, S.G. Worthley, S. Gronthos, Concise review: mesenchymal stromal cells: potential for cardiovascular repair, Stem Cells 26 (2008) 2201–2210.
[206] S.L. Chen, W. Fang, F. Ye, Y.H. Liu, J. Qian, S. Shan, J. Zhang, R.C.H. Zhao, L.M. Liao, S. Lin, J.P. Sun, Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction, Am. J. Cardiol. 94 (2004) 92–95.
[207] J.M. Hare, J.E. Fishman, G. Gerstenblith, D.L.D. Velazquez, J.P. Zambrano, V.Y. Suncion, M. Tracy, E. Ghersin, P.V. Johnston, J.A. Brinker, E. Breton, J. Davis-Sproul, I.H. Schulman, J. Byrnes, A.M. Mendizabal, M.H. Lowery, D. Rouy, P. Altman, C.W.P. Foo, P. Ruiz, A. Amador, J. Da Silva, I.K. McNiece, A.W. Heldman, Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial, JAMA 308 (2012) 2369–2379.
[208] J. Bartunek, A. Behfar, D. Dolatabadi, M. Vanderheyden, M. Ostojic, J. Dens, B. El Nakadi, M. Banovic, B. Beleslin, M. Vrolix, V. Legrand, C. Vrints, J.L. Vanoverschelde, R. Crespo-Diaz, C. Homsy, M. Tendera, S. Waldman, W. Wijns, A. Terzic, Cardiopoietic

stem cell therapy in heart failure: the C-CURE (cardiopoietic stem cell therapy in heart failure) multicenter randomized trial with lineage-specified biologics, J. Am. Coll. Cardiol. 61 (2013) 2329–2338.
[209] P. Menasche, Stem cell therapy for heart failure: are arrhythmias a real safety concern? Circulation 119 (2009) 2735–2740.
[210] J.C. Chachques, J.C. Trainini, N. Lago, M. Cortes-Morichetti, O. Schussler, A. Carpentier, Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM trial): clinical feasibility study, Ann. Thorac. Surg. 85 (2008) 901–908.
[211] B.E. Strauer, G. Steinhoff, 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice, J. Am. Coll. Cardiol. 58 (2011) 1095–1104.
[212] A. Moreno-Gonzalez, F.S. Korte, J. Dai, K. Chen, B. Ho, H. Reinecke, C.E. Murry, M. Regnier, Cell therapy enhances function of remote non-infarcted myocardium, J. Mol. Cell. Cardiol. 47 (2009) 603–613.
[213] I. Chimenti, R.R. Smith, T.S. Li, G. Gerstenblith, E. Messina, A. Giacomello, E. Marban, Relative roles of direct regeneration versus paracrine effects of human cardiosphere-derived cells transplanted into infarcted mice, Circ. Res. 106 (2010) 971–980.
[214] H. Hamdi, V. Planat-Benard, A. Bel, E. Puymirat, R. Geha, L. Pidial, H. Nematalla, V. Bellamy, P. Bouaziz, S. Peyrard, L. Casteilla, P. Bruneval, A.A. Hagege, O. Agbulut, P. Menasche, Epicardial adipose stem cell sheets results in greater post-infarction survival than intramyocardial injections, Cardiovasc. Res. 91 (3) (Aug. 1 2011) 483–491.
[215] A. Bel, V. Planat-Bernard, A. Saito, L. Bonnevie, V. Bellamy, L. Sabbah, L. Bellabas, B. Brinon, V. Vanneaux, P. Pradeau, S. Peyrard, J. Larghero, J. Pouly, P. Binder, S. Garcia, T. Shimizu, Y. Sawa, T. Okano, P. Bruneval, M. Desnos, A.A. Hagege, L. Casteilla, M. Puceat, P. Menasche, Composite cell sheets: a further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells, Circulation 122 (2010) S118–S123.