Adult Stem Cells and Biocompatible Scaffolds as Smart Drug Delivery Tools for Cardiac Tissue Repair

Stefania Pagliari¹, Sara Romanazzo¹, Diogo Mosqueira², Perpetua Pinto-do-Ó²,³, Takao Aoyagi¹,⁵, Giancarlo Forte¹,*,⁵

¹Biomaterials Unit, International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), Tsukuba, Japan; ²INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; ³ICBAS-Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Portugal

Abstract: The contribution of adult stem cells to cardiac repair is mostly ascribed to an indirect paracrine effect, rather than to their actual engraftment and differentiation into new contractile and vascular cells. This effect consists in a direct reduction of host cell death, promotion of neovascularization, and in a “bystander effect” on local inflammation. A number of cytokines secreted by adult stem/progenitor cells has been proposed to be responsible for the consistent beneficial effect reported in the early attempts to deliver different stem cell subsets to the injured myocardium.

Aiming to maximize their beneficial activity on the diseased myocardium, the genetic modification of adult stem cells to enhance and/or control the secretion of specific cytokines would turn them into active drug delivery vectors.

On the other hand, engineering biocompatible scaffolds as to release paracrine factors could result in multiple advantages: (1) achieve a local controlled release of the drug of interest, thus minimizing off-target effects, (2) enhance stem cell retention in the injured area and (3) boost the beneficial paracrine effects exerted by adult stem cells on the host tissue.

In the present review, a critical overview of the state-of-the-art in the modification of stem cells and the functionalization of biocompatible scaffolds to deliver beneficial soluble factors to the injured myocardium is offered.

Besides the number of concerns to be addressed before a clinical application can be foreseen for such concepts, this path could translate into the generation of active scaffolds as smart cell and drug delivery systems for cardiac repair.

Keywords: Adult stem cells, biocompatible scaffolds, cardiac repair, drug delivery, genetic modification, paracrine effect.

INTRODUCTION

Cardiac tissue repair entailing tissue engineering approaches requires an accurate selection of the cells and the scaffolds to be used [1,2]. In fact, while some reports demonstrated the possibility to attain a certain degree of direct tissue regeneration by the injection of different stem/progenitor cell types into the damaged tissue [3-7], compelling evidence that some of these adult stem cell subsets, namely hematopoietic stem cells (HSCs), are not able to produce contractile cells has also been given [8,9]. For other stem cell subsets, their transdifferentiation rate is very low (mesenchymal stem cells, MSCs), or functional coupling with host myocardium (skeletal myoblasts) is not achieved [10,11], or the graft preferentially generates vascular units (endothelial progenitor cell, EPCs) [12]. Moreover, serious doubts on the actual therapeutic efficacy of stem cell injection are raised by clinical trials in which bone marrow-derived MSCs were infused [13-17]. Among the cells proposed to regenerate damaged myocardium, resident cardiac stem/progenitor cells (CPCs) are thought to retain a higher plasticity towards tissue-specific differentiation, namely endothelial, smooth muscle and contractile cells [18,19] although contrasting results in terms of engraftment have been obtained [2,20]. So far, a number of pre-clinical and clinical studies compellingly reported a persistent, although modest beneficial effect of implanted stem cells on host tissue [21-24]. This effect appears to be independent from the poor cell engraftment detected [25,26], suggesting that the improved cardiac prognosis did not rely on the functional integration of the cells. Indeed, although stem cells transferred to the injured heart were hardly found in the host tissue within few days from the administration, a major paracrine effect - due to the secretion by such cells of molecules having a therapeutic relevance - has been demonstrated [27-29]. A similar phenomenon, dubbed “bystander effect”, has been described for neural stem cells protection of the central nervous system despite the scarce evidence of differentiation events [30].

The role of paracrine factors is now assumed to be a major determinant in cardiac function improvement [31] after adult stem cell implantation. Its relative contribution has been estimated to account for 50-80% of the total regenera-
tive effort in cardiac resident stem cells injected in the infarcted heart as single cell preparation or as cardiospheres [32].

The impact of the paracrine factors in the heart spans from anti-apoptotic, to proliferative, from neo-angiogenic to effects on cardiac contractile function [27]. Also, an enhancement in resident stem cell recruitment and differentiation has been postulated [33], although few evidences of adult- resident stem cell interplay exist [34].

In a number of animal studies, the systemic administration of growth factors and cytokines resulted in improved cardiac function and myocardial recovery, while pleiotropic (off-target) activities on other body districts could not be excluded [35-38]. Moreover, systemically delivered factors usually experience short plasma circulation times and rapid renal clearance [39]. Therefore, in the case of a systemic administration of therapeutic drugs to treat patients experiencing MI, frequent dosing regimens are required to maintain the desired therapeutic level and duration. This routine is likely to cause low patient compliance, suboptimal therapeutic outcome, side effects and toxicity.

On the other hand, cardiac continuous contractile activity together with the conditions found in the infarcted milieu could reduce drug bioavailability and/ or bioactivity. These considerations may explain the scarce results reported upon simultaneous administration of multiple soluble factors to the infarcted heart (fibroblast growth factor-2, insulin-like growth factor-1, hepatocyte growth factor, stromal cell-derived factor-1α) [40].

In this context, the development of a platform enabling sustained and targeted drug delivery, yet suitable to non-invasive procedures, is critical. Recently, the use of tissue engineering methodologies to deliver stem cells to the diseased myocardium has been proposed [41]. Such protocols guarantee the delivery to the injured site of a controlled number of cells, while allowing the tuning of the size and shape of the graft. Tissue-targeted cell delivery systems (scaffolds) would enhance stem cell retention in the damaged area, likely promoting a more controlled release of soluble regulators by the cells. In this perspective, adult stem cells can also be genetically modified and considered as vehicles for drugs or beneficial growth factors, which, in turn, can improve injected cell survival and act in a paracrine fashion on the surrounding tissue.

The scaffold itself can be conceived as a powerful drug delivery system, e.g. scaffold surface modification (by bioactive factor embedding or tethering) could improve implanted cell survival and retention in vivo, while envisaging the opportunity to design combinatorial delivery systems in which a factor is promptly secreted by the cells and another is mildly released by the degrading scaffold. Herein, a review of the state-of-the-art of the techniques so far adopted or proposed to prepare engineered adult stem cells for cardiac repair is offered. Alongside a short insight on the most relevant factors proposed to have a paracrine effect in vivo, an overview on the procedures proposed for functionalizing biocompatible, biodegradable scaffolds toward the release of therapeutic agents will be given. The combination of genetically-modified stem cells and functionalized scaffolds is likely to provide a challenging opportunity to prepare smart drug and cell delivery tools for cardiac repair.

BIOCHEMICAL FACTORS AND MOLECULAR PATHWAYS INVOLVED IN ADULT STEM CELL PARACRINE EFFECTS

A number of soluble factors have come on the spotlight as to exerting paracrine effects on cardiac tissue. Several of these molecules were since long associated to other body districts and more recently found to have an effect on heart function, while others have been identified following specific up-regulation in response to a myocardial insult. Their involvement in cardiac tissue repair has been demonstrated either by the ability to promote cardiomyocyte survival in vitro, and/or by monitoring the effect on the damaged myocardium upon delivery in vivo. Also, a number of signaling pathways has been deemed responsible of cardiac failure, thus embodying suitable targets for modulating the paracrine activity of adult stem cells. While in the present review each of these factors/ targets will be analyzed separately, interactions and synergies among them exist and will be mentioned. The existence of these interactions outlines the complexity of the paracrine regulatory network, while the possibility to trigger synergic responses advocates the potential of a multi-targeted combinatorial therapy.

HEPATOCYTE GROWTH FACTOR (HGF)

Hepatocyte growth factor (HGF) signaling through its canonical receptor (c-Met) has been shown to be cardioprotective in different pre-clinical studies. This cytokine appears to feature distinct roles [42,43], namely it is a powerful angiogenic factor leading to increased capillary density [44,45] and driving endothelial cells proliferation through activation of downstream mitogenic signaling pathways [46], as well as through the induction of other endothelial cell mitogens [47]. When administered in a rat model of ischemia injury, HGF promoted the survival of cardiomyocytes under ischemic conditions, as demonstrated by smaller infarct areas and improved cardiac function [48]. This cardioprotective effect is due both to the phosphorylation and inactivation of pro-apoptotic BAD via the PI3K/Akt pathway and by the simultaneous upregulation of anti-apoptotic Bcl-xL [48,49]. A beneficial role of HGF in ventricular remodeling post-MI has been verified by the attenuation of ventricular enlargement and improved cardiac function [50]. Furthermore, the prevention of fibrotic response in hamster models of both acute ischemia and dilated cardiomyopathy [51] was reported to be likely due to HGF-mediated inhibition of TGF-β-dependent collagen deposition by myofibroblasts [52]. HGF is also credited of immunomodulatory properties, via binding to c-Met receptor on immune cells with consequent upregulation of IL-10 [53], thus impairing the onset of the fibrotic process. The ability of HGF to drive the activation and maturation of bone marrow [54] and resident cardiac stem cells in a genetic model of cardiomyopathy has been recently confirmed [55].

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

Vascular endothelial growth factor (VEGF) family comprises cell-specific mitogens that stimulate endothelial cell
proliferation, microvascular permeability, vasodilation, and angiogenesis by activating the downstream effectors of its receptors (Flk-1/KDR and Flt-1) [56,57]. VEGF secretion by cardiomyocytes plays a key role in cardiac morphogenesis and contributes to adult heart function determination [58]. The concentration of the factor increases in the infarcted myocardium as compared to healthy controls, allegedly as a mechanism to enhance local vascularization in response to hypoxia [59,60]. Remarkably, evidence has been recently given that the implantation of circulating EPCs in the infarction area correlates with an increase in VEGF local levels and microvessel density [61]. Moreover, VEGF displays cardiomyocyte anti-apoptotic activity by inducing the expression of Bcl-2 and activating the protective PI3K pathway, thus decreasing the infarction area [62,63]. As such, several clinical trials involving recombinant VEGF for cardiac repair have been designed, yet with disappointing results, due to its poor pharmacokinetic properties [64,65].

**BASIC FIBROBLAST GROWTH FACTOR (BFGF)**

Basic fibroblast growth factor (bFGF) family is involved in multiple biological functions, e.g. proliferation, survival and migration, by interacting with its cognate surface FGF receptors in endothelial cells and cardiomyocytes [66,67]. Evidence for the involvement of FGF in the response to myocardial infarction has been obtained in vivo, given that the cytokine is specifically upregulated during ischemia/reperfusion, as well as during cardiac remodeling [68]. Furthermore, FGF-2 regulates the cardiogenic differentiation of resident cardiac stem cells, as FGF-2+ CPC fail to differentiate in vitro and in vivo [69]. Moreover, cardiogenic differentiation of human cardiosphere-derived cells was shown to be bFGF-dependent, with this factor favoring the engraftment of these cells in diseased animal models [70]. When injected intramyocardially in rat infarcted heart, bFGF contributed to reduce infarct size, by lowering cardiomyocyte apoptosis [71].

**PLATELET-DERIVED GROWTH FACTORS (PDGF)**

Platelet-derived growth factor AB (PDGF-AB) heterodimer was shown to be involved in the interaction between cardiac microvascular endothelial cells (CMEC) and neighboring cardiomyocytes [72,73]. Cardiomyocyte-induced expression of PDGF-AB by CMEC favors the maintenance of vascular integrity and increases capillary density, by up-regulating angiogenic factors including VEGF and its receptor [74]. Its ability to foster bone marrow cell (BMC) transplantation into an infarcted aging rodent heart was also demonstrated together with a direct cardioprotective effect [74,75].

Finally, PDGF-BB has been proven effective in inducing bone marrow mesenchymal stem cell differentiation into beating cardiomyocytes in vitro and to foster the generation of new contractile cells in infarcted rodent heart [76].

**GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)**

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine whose pleiotropic effects include the proliferation and differentiation of myeloid progenitors, mobilization of bone marrow cells into the peripheral blood and immunomodulation [77-80]. The cardioprotective effects of G-CSF are thought to be exerted through different mechanisms and result in the attenuation of ischemic cardiomyopathy and LV dysfunction in acute MI patients [81-83]. The mobilization of bone marrow stem cells and their homing to the heart [84-86] as well as the suppression of apoptosis and the stimulation of proliferation in cardiomyocytes have been ascribed to the activation of Jak/STAT3 signaling pathways [87-89]. Similar results have been reported by using both embryonic stem cell (ESC-) and induced pluripotent stem cell (iPSC-derived cardiomyocytes [90]. Finally, anti-apoptotic effects on endothelial cells have also been described, illustrated by increased vascularization in the infarcted hearts [91,92]. Unfortunately, disappointing results have been obtained by randomized double-blind placebo-controlled clinical trials using G-CSF in human patients [93-95], thus underlining the need for more accurate basic investigations on this factor.

**STROMAL-DERIVED FACTOR 1 (SDF-1)**

Stromal-derived factor 1 (SDF-1) is regarded as the major stem cell homing factor, through its binding to chemokine receptor 4 (CXCR4), expressed in different populations of stem/progenitor cells of the bone marrow [96,97]. The activation of SDF-1-CXCR4 axis is implicated in the mobilization of stem cells to the peripheral blood, a phenomenon explored to enhance stem cell homing to the heart at the onset of ischemic diseases [98]. Importantly, myocardial SDF-1 concentration was found transiently up-regulated after ischemic cardiomyopathy, followed by increased plasma levels [99]. Thus, the sustained release of SDF-1 after MI is being sought by several approaches resulting in prolonged expression of SDF-1 in the heart, favoring the homing of different populations of stem/progenitor cells, increased cardiomyocyte survival and neoangiogenesis [100].

**INTERLEUKIN-1β (IL-1β)**

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine whose levels augment in the heart following MI; it is thought to play a role in cardiac failure, as its neutralization was shown to promote LV remodeling [101]. On the contrary, a more recent investigation demonstrated that cardiomyocyte apoptosis and cardiac remodeling and dysfunction could be attenuated by blocking IL-1β in a mouse model of acute MI [102]. Several studies performed in CMEC revealed that IL-1β inhibits angiogenesis through cell cycle arrest and by decreasing the levels of fibrillar actinin in the cytoskeleton, a prerequisite for capillary formation [103]. Moreover, IL-1β stimulation of CMEC activated different signaling pathways (e.g. ERK1/2, JNKs and protein kinase C), resulting in the downregulation of pro-angiogenic VEGF-D and the increased activity of matrix metalloproteinase-2 (MMP-2) [104,105]. However, IL-1β has also been shown to promote angiogenesis in other cell types such as EPCs and differential regulation of genes coding different families of MMPs in cardiac fibroblasts, suggesting a controversial role of this cytokine in cardiac repair [106,107].
TRANSFORMING GROWTH FACTOR BETA 1 (TGF-β1)

Transforming growth factor beta 1 (TGF-β1) is a secreted cytokine involved in a number of key processes in the healthy and diseased cardiac tissue [108,109]. In particular, the overexpression of TGF-β1 in the infarcted heart modulates angiogenesis, cardiomyocyte hypertrophy, cardiac remodeling and myofibroblast transdifferentiation, acting as a mediator of the transition from the acute inflammatory phase to the post-infarction formation of scar tissue [110-117]. Thus, when harnessed in the adequate time-frame post-infarction, the over-expression of TGF-β1 could have beneficial effects on the myocardium.

INTERLEUKIN 10 (IL-10)

The suppression of the inflammation following myocardial infarction is thought to be associated to a better prognosis [118,119]. Although the levels of anti-inflammatory IL-10 were found increased following MI, in both murine and canine models [120,121], studies on the importance of IL-10 in modulating lymphocyte and neutrophil recruitment reported contrasting results [122,123]. Nonetheless, a beneficial effect on cardiac hypertrophy and remodeling has been correlated to higher levels of IL-10 following bone marrow mononuclear cell transplantation into infarcted mouse heart [124]. Finally, knockout studies revealed the importance of IL-10 for the engraftment and survival of intramyocardially transplanted EPCs following MI, hence leading to better LV functional recovery, lower infarct sizes and fibrosis [125].

TNF-ALPHA 1 RECEPTOR

Similarly to other cytokines, pro-inflammatory tumor necrosis factor-alpha (TNF-α) levels are enhanced at the onset of ischemic heart failure, both myocardial macrophages and cardiomyocytes being responsible of its secretion [126]. The increase in cardiac TNF-α has been associated with LV dysfunction due to the apoptotic activity it exerts on cardiomyocytes, thus constituting an important therapeutic target for treating congestive heart failure [127,128]. Therefore, strategies aiming at antagonizing TNF-αR1 have been proposed, such as gene transfer of the soluble form of TNF-αR1 directly to the heart [129]. The cardioprotective effects of this approach following MI have been demonstrated by the decrease of apoptosis in the cardiomyocyte compartment, as well as by the improvement of cardiac function as compared to the vehicle [130-133].

WNT/β-CATENIN SIGNALING PATHWAY

Wnt/β-catenin signaling pathway is believed to act as an important regulator of cardiac stem cell fate and required for embryonic stem cell cardiac differentiation [134]. While playing an important role in cardiac development, this pathway is silent in the adult heart and reactivated in response to cardiac injury [135,136]. Several therapeutic approaches have been designed to target this pathway and to enhance cardiac repair post MI [137]. Wnt pathway pharmacological inhibition by FDA-approved pyrvinium or by the synthetic peptide UM20672 resulted in improved cardiac function following MI, through infarct size reduction and improved angiogenesis [138,139]. Alternative strategies rely on the overproduction of the soluble forms of the receptors, thereby preventing Wnt pathway activation via ligands sequestering. In this regard, the overexpression of secreted frizzled-related protein 2 (sFRP2) following MI in mice resulted in reduced fibrosis and in enhanced engraftment of transplanted MSCs in the heart [140,141]. Likewise, sFRP1 overexpression shows similar beneficial effects in reducing infarct size following MI through the inhibition of neutrophil infiltration in the heart [142,143]. Finally, transgenic models have shown that β-catenin depletion attenuates post-infarction LV remodeling improving mouse survival, partly as a result of the differentiation of GATA4+/Sca-1+ resident cardiac progenitor cells [144].

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1)

Insulin-like Growth Factor 1 (IGF-1) is mainly produced by the liver, while it has been shown to promote cardiac growth and contractility, by enhancing cardiomyocyte survival and proliferation [145]. IGF-1 activity in cardiomyocytes is correlated to the activation of PI3K/Akt signaling, by which it protects cardiac cells from arrhythmogenesis [146], high-fat diet-induced cardiac dysfunction [147] and reduces apoptosis in a model of ischemia-reperfusion [148]. Moreover, IGF-1 ability to trigger cardiac stem cell migration to the damaged site and their differentiation in combination with HGF has been reported [33, 35].

PLACENTAL GROWTH FACTOR (PLGF)

Placental Growth Factor (PIGF) is a VEGF homolog able to promote angiogenesis [149]. PIGF myocardial concentration increases right after MI, while high plasma levels are considered predictive of a positive outcome after infarction [150]. When systemically administered for 3 days, PIGF is able to improve cardiac function by enhancing arteriogenesis and angiogenesis, while reducing ventricular remodeling [39].

GLYCOGEN SYNTHASE KINASE 3ß (GSK-3ß)

Glycogen synthase kinase 3ß(GSK-3ß) is a serine/threonine kinase that phosphorylates many intracellular substrates (e.g. β-catenin, glycogen synthase, eIF2B, GATA-4, myocardin, c-Jun, cyclin D1 and N-Myc) and correlates with the display of apoptotic and necrotic processes in cardiomyocytes [151]. GSK-3ß also regulates Wnt, Notch and hedgehog, major signaling proteins involved in cell growth/differentiation [152]. Its inactivation in end-stage heart failure as well as its role in preventing cardiomyocyte hypertrophy has been described, its activity on cardiac function being still matter of controversy [153,154].

The roadblocks preventing the transition of the aforementioned cardioprotective factors from the bench to the bedside are associated with poor pharmacokinetic properties upon administration, inadequate levels and time-frame efficiency in vivo and the possibility for undesired off-target effects to occur on other body districts. This strongly points out the need for developing smart drug delivery systems addressing these issues, including scaffolds and genetically modified stem cells.

A summary of the information conveyed for each factor/target is included in Table 1.
**Table 1. Summary of Soluble Factors and Molecular Targets Being Explored in Cardiac Regeneration and the Cardioprotective Effects they Elicit.**

MSCs: Mesenchymal Stem Cells; CPCs: Cardiac Stem/Progenitor Cells.

<table>
<thead>
<tr>
<th>Factor/ target</th>
<th>Proposed beneficial effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGF</td>
<td>Angiogenesis</td>
<td>[45-47]</td>
</tr>
<tr>
<td></td>
<td>Cardiomyocyte survival</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Ventricular remodeling, resident stem cells mobilization</td>
<td>[50, 51, 53]</td>
</tr>
<tr>
<td>VEGF</td>
<td>Angiogenesis</td>
<td>[62, 63]</td>
</tr>
<tr>
<td>bFGF</td>
<td>Angiogenesis</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Activation of resident CPCs</td>
<td>[69]</td>
</tr>
<tr>
<td>PDGF-AB</td>
<td>Angiogenesis</td>
<td>[75]</td>
</tr>
<tr>
<td></td>
<td>Cardiogenic differentiation</td>
<td>[76]</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Homing of CPCs to the heart</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Cardiomyocyte survival and proliferation</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>Cardiac remodeling</td>
<td>[87]</td>
</tr>
<tr>
<td>SDF-1</td>
<td>Homing of CPCs to the heart</td>
<td>[97]</td>
</tr>
<tr>
<td>IL-10</td>
<td>Anti-inflammatory</td>
<td>[121]</td>
</tr>
<tr>
<td></td>
<td>Ventricular remodeling</td>
<td>[122]</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Angiogenesis</td>
<td>[112]</td>
</tr>
<tr>
<td></td>
<td>Cardiac remodeling</td>
<td>[113, 117]</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Angiogenesis</td>
<td>[103, 106]</td>
</tr>
<tr>
<td></td>
<td>Ventricular remodeling</td>
<td>[105, 107]</td>
</tr>
<tr>
<td>TNF-α1 receptor</td>
<td>Cardiomyocyte survival</td>
<td>[132, 133]</td>
</tr>
<tr>
<td>Wnt pathway</td>
<td>Cardiac remodeling</td>
<td>[139]</td>
</tr>
<tr>
<td></td>
<td>Angiogenesis</td>
<td>[138]</td>
</tr>
<tr>
<td></td>
<td>Engraftment of transplanted MSCs</td>
<td>[140,141]</td>
</tr>
<tr>
<td></td>
<td>Activation of resident CPCs</td>
<td>[144]</td>
</tr>
<tr>
<td>PIGF</td>
<td>Inhibition of cardiac remodeling, neovascularization</td>
<td>[38, 150]</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Reduction in cardiomyocyte apoptosis, activation of resident stem cells</td>
<td>[33, 148]</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>Reduction of cardiomyocyte hypertrophy</td>
<td>[152]</td>
</tr>
</tbody>
</table>

**TURNING ADULT STEM CELLS INTO ACTIVE DELIVERY SYSTEMS FOR CARDIOPROTECTIVE FACTORS**

Gene therapy has emerged as a challenging opportunity to treat cardiac diseases. To this end, a number of different vectors has been tested to increase gene expression efficiency and reduce the potential toxicity and immunogenicity. The vectors proposed for cardiac gene therapy can be grouped into two major classes: 1) viral vectors, which include lentivirus, adenovirus, and adeno-associated viruses; 2) non-viral vectors, represented by naked plasmids, oligonucleotides, and lipid/polymer complexes. Viral vectors guarantee very high gene delivery efficiency (40-90%), whereas non-viral based-therapies, although yielding lower success rate, overcome the hazards of increased immunogenicity and insertional mutagenesis associated to viral particles [155]. While the usefulness of such vectors in cardiac therapy has been assessed by intravenous or intramyocardial delivery, this review deals specifically with the use of vectors to selectively express molecules of interest in adult stem cells in vitro or ex vivo. For a detailed description of heart gene delivery vectors the readers are recommended to refer to [156].

The use of gene therapy to drive the overexpression of paracrine factors in adult stem cells entails the concept of converting the cells in active drug delivery systems. A number of attempts with different adult stem cell types, in which the MSC outstands as the favorite target, have been proposed so far.

Given the acknowledged ability of IGF-1 to increase cardiomyocyte survival and promote cardiac repair, MSCs were transduced with adenoviral vectors coding for IGF-1 transgene and injected in a rat model of acute myocardial infarction [157]. In *in vitro* assays, IGF-1-transduced MSCs (IGF-1-MSCs) showed to be protected from apoptosis while displaying increased release of other growth factors, e.g. HGF, bFGF, VEGF, SDF-1 alpha. Likewise, *in vivo* analysis revealed that IGF-1-MSCs survival upon implantation was significantly higher as compared to non-transduced MSCs, while vessel density and myogenesis were increased. Moreover, the release of SDF-1 alpha from IGF-1-MSCs contributed to massive resident stem cell mobilization, with enhanced numbers of c-kit-, MDR1-, CD31- and CD34-expressing cells. Remarkably, a reduction in the infarcted area was reported. Mesenchymal stem cells have also been transduced with FGF-2, thus showing increased resistance to hypoxic conditions *in vitro*. Additionally, following *in vivo* implantation in the myocardial infarcted heart, FGF-2-MSC displayed higher viability and performance in terms of cardiac repair [158].

In an attempt to assess the effect of glycogen synthase kinase 3β (GSK-3β) on cell engraftment, MSCs isolated from TET-OFF-GSK-3β transgenic mice were injected in a murine MI model obtained by permanent ligation of the left anterior descending (LAD) coronary artery. GSK-3β overexpression increased the efficiency of cell-based therapy after MI, and modified MSCs were seen for up to 12 weeks in the peri-infarction area. Moreover, GSK-3β-MSCs improved cardiac regeneration by directly affecting host precursor cell recruitment, as inferred from both a higher number of Ki67 positive myocytes and of c-kit positive cells at the peri-implant site. This effect was ascribed to the up-regulation of VEGF-A in the transduced cells [159].

MSCs infected with adenoviruses carrying HGF cDNA and delivered to the infarcted myocardium reduced the ischemic area by increasing capillary density and reducing
ASC implantation, increased angiogenesis and reduced fibrosis were observed [161].

As repeatedly confirmed, VEGF has been deemed to be a crucial factor in cell survival in ischemic areas. The implantation of retrovirally transduced VEGF-ASCs led to increased vessel density [162], while that of VEGF-transduced MSCs was seen to enhance angiogenesis [163]. SDF-1 up-regulation in VEGF-expressing cells also promoted massive homing of co-transplanted cardiac stem cells observed to directly participate in tissue regeneration at the infarcted area and at the border zone [164]. Likewise, the direct transduction of SDF-1 in MSCs resulted in a pro-survival and neo-angiogenetic effect on damaged myocardium [165] while similar results were obtained when ASCs were transduced by a means of recombinant baculovirus encoding for Angiopoietin-1 (Ang-1), a factor acting together with VEGF in promoting neo-vascularization. When injected into a rat model of myocardial infarction, genetically modified ASCs showed higher cell retention as compared to the controls [166].

ASCs secrete significant amounts of angiogenic and anti-apoptotic factors [167] and, when engineered to express heme oxygenase-1 (HO-1) and transplanted in infarcted rabbit hearts, showed the capacity to overcome MI-induced oxidative injury and apoptosis [168]. HO-1 transduced BM MSCs have also been found to exert anti-inflammatory and anti-fibrotic effects on the infarcted myocardium [169].

Furthermore, the expression of VEGF is known to be up-regulated by the activation of Hypoxia-response elements contained in its promoter operated by Hypoxia Inducible Factors (HIF). Remarkably, higher capillary density and improved cardiac function were achieved on an experimental setting comprising the expression of p6HRE-CMV-VEGF165 or pCMV-VEGF165 in rat BM-derived endothelial progenitor cells (BM-EPCs) [170].

Although the established inability of HSCs to directly contribute to damaged myocardium regeneration, implantation of VEGF- and PDGF-expressing HSCs displayed an increase in capillary and arteriole density in infarcted rats. Moreover, a reduction in myocardial fibrosis accompanied by an improvement in heart function was also reported in this model [171].

Importantly, VEGF release was also ectopically triggered in skeletal myoblasts to be injected in infarcted rat heart, thus confirming the ability of the molecule to promote neo-vascularogenesis and cardiac repair independently of the cell type used [172].

Skeletal muscle progenitors were transplanted with a plasmid encoding for PIGF and transplanted into a rat model of myocardial infarction. As a result, left ventricular cardiac function and fractional shortening were significantly improved, while cardiac remodeling was attenuated [173]. Also, these cells were transduced with feline leukemia virus-based lentiviral vector encoding for Wnt11 gene, a factor involved in cell proliferation and differentiation. Wnt11 over-expression led to Nkx-2.5, GATA-4 and Cx43 enhancement in skeletal myoblasts, driving their cardiac differentiation [174].

The first clinical trials employing skeletal myoblasts for cardiac repair reported the arising of arrhythmia events, likely due to the lack of electromechanical coupling between the graft and the host cells [175]. To favor implanted cell electromechanical coupling with host myocardium, skeletal myoblasts overexpressing Cx43 were obtained with different methods (retroviral, adenoviral and plasmid-mediated gene transfer). The genetically modified myoblasts were able to form viable cardiac grafts and establish electromechanical bridges with host cardiomyocytes [176].

Adverse matrix remodeling is a hallmark of myocardial infarction. To limit the impact of negative remodeling, tissue inhibitor of matrix metalloproteinase-3 (TIMP3) and VEGF were transduced in MSCs. A significant enhancement in cardiac function was reported in the follow-up study [177].

A major goal in cardiac tissue repair would be the activation and recruitment of resident stem/progenitor cells. With the aim to attract resident stem cells to the damaged site, MSCs were transfected with stem cell factor (SCF) by lipid-based technique before cardiac implantation. An improvement in cardiac function was reported, although the formation of intra-cardiac tumors was described, probably due to the uncontrolled activation of resident stem cells [178].

An interesting approach to solve the issue of implanted cells vanishing few days after the intervention was proposed by overexpressing Bcl-2 in MSCs. The ectopic expression of the anti-apoptotic gene in MSCs was induced through pcDNA3.1 plasmid transfection. In vitro, Bcl-2high-MSCs showed a reduced susceptibility to apoptosis and enhanced VEGF secretion under hypoxic conditions, as compared to their controls. MI models injected with Bcl-2high MSCs showed 15% higher capillary density and 17% smaller infarct size, as compared to hearts receiving non-manipulated MSCs injection [179].

On the same line, the overexpression of Akt survival pathway in MSCs transplanted intramyocardially correlated with a drastic increase of cardiac recovery post-infarction, characterized by the inhibition of cardiac remodeling, recovery of myocardial volume and improved systolic and diastolic function in a rat experimental model of coronary occlusion [180,181].

In addition, Akt-MSCs displayed protective effects on adult rat ventricular cardiomyocytes subjected to hypoxia in vitro [182]. When overexpressed in MSCs implanted in infarcted pig heart, the same factor prevented infarcted area expansion and improved cardiac contractility [183].

Furthermore, cardiac progenitor cells transplanted with Pim-1 kinase, a downstream effector of nuclear Akt, displayed cardioprotective activity indicating a role for Pim-1 in the enhancement of cell survival and proliferation [184]. Follow-up studies have shown that several genes coding for cardioprotective factors (such as VEGF, FGF-2 and HGF) are upregulated in Akt-overexpressing MSCs [183], rendering this effect as a potential mediator of the positive outcome reported in both rat [181] and porcine [183] MI animal models.
MSCs have also been recently infected with a lentiviral vector coding for Periostin before the implantation in the infarcted heart. Periostin is a secreted extracellular matrix (ECM) protein expressed at very early stages of embryogenesis and upregulated in adult tissues when damage is triggered. Overexpression of this ECM-associated protein in MSCs was demonstrated to enhance the resistance of MSCs and cardiomyocytes to hypoxia in vitro. Strikingly, MSCs expressing periostin could significantly improve cardiac function through a direct effect on cardiomyocyte survival [185].

An indirect method to induce adult stem cell production of beneficial factors consisted in transducing MSCs with GATA-4 using murine stem cell virus retroviral expression system [186]. In this setting, IGF-1, VEGF-A, bone morphogenetic protein, beta-nerve growth factor and basic transcription factor 3 were found overexpressed, hence increasing regional blood flow, capillary density, preventing fibrosis and increasing cell resistance to oxidative stress [187].

Finally, the overexpression of stem cell chemo-attractant SDF-1α in resident cardiac progenitor cells was shown to trigger the secretion of angiogenic factors and stem cell recruitment to the heart [188].

An overview of the adult stem/progenitor cell subsets so far engineered to ectopically express molecules considered beneficial to cardiac tissue or to stem cell engraftment and recruitment is given in Table 2.

### Table 2. Adult Stem Cells have been Genetically Modified to Overexpress Putative Cardiac Beneficial Factors.

<table>
<thead>
<tr>
<th>Adult stem cell</th>
<th>Transfected gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchymal stem cells (MSCs)</td>
<td>IGF-1</td>
<td>[157]</td>
</tr>
<tr>
<td></td>
<td>FGF2</td>
<td>[158]</td>
</tr>
<tr>
<td></td>
<td>GSK-3β</td>
<td>[159]</td>
</tr>
<tr>
<td></td>
<td>HGF</td>
<td>[160]</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>[163,164]</td>
</tr>
<tr>
<td></td>
<td>SDF-1</td>
<td>[165]</td>
</tr>
<tr>
<td></td>
<td>TIMP3</td>
<td>[177]</td>
</tr>
<tr>
<td></td>
<td>SCF</td>
<td>[178]</td>
</tr>
<tr>
<td></td>
<td>Bcl2</td>
<td>[179]</td>
</tr>
<tr>
<td></td>
<td>AKT</td>
<td>[180-183]</td>
</tr>
<tr>
<td></td>
<td>Periostin</td>
<td>[185]</td>
</tr>
<tr>
<td>Adipose tissue-derived mesenchymal stem cells (ASCs)</td>
<td>HGF</td>
<td>[161]</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>[162]</td>
</tr>
<tr>
<td></td>
<td>Ang1</td>
<td>[166]</td>
</tr>
<tr>
<td></td>
<td>HO-1</td>
<td>[168,169]</td>
</tr>
<tr>
<td>Endothelial progenitor cells (EPCs)</td>
<td>VEGF</td>
<td>[170]</td>
</tr>
<tr>
<td>Haematopoietic stem cells (HSCs)</td>
<td>VEGF, PDGF</td>
<td>[171]</td>
</tr>
<tr>
<td>Cardiac progenitor cells (CPCs)</td>
<td>Pim-1 kinase</td>
<td>[184]</td>
</tr>
<tr>
<td></td>
<td>SDF-1α</td>
<td>[188]</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>[172]</td>
</tr>
<tr>
<td></td>
<td>PlGF</td>
<td>[173]</td>
</tr>
<tr>
<td></td>
<td>Wnt11</td>
<td>[174]</td>
</tr>
<tr>
<td>Skeletal myoblasts (skMyo)</td>
<td>Connexin43</td>
<td>[176]</td>
</tr>
</tbody>
</table>

Biomaterials as Cardiac-Specific Drug Delivery Systems

Preclinical studies showed that, when bioactive molecules are embedded into polymeric delivery systems, their therapeutic effects on the heart are maximized, as compared to the local or systemic administration of the same factors [189-191].

A number of different biodegradable polymers, both synthetic (Poly(ethylene glycol) or PEG, poly(lactic-co-glycolic acid) or PLGA, poly(lactic acid) or PLA) and natural (collagen, gelatin, fibrin) has been widely used to fabricate systems for the controlled release of defined factors. Compared with natural composites, synthetic polymers display reproducible and tunable physico-chemical properties and controlled degradation. Moreover, while the release of bioactive molecules from natural derivatives can be relatively fast, in biodegradable synthetic polymers this process can be controlled by modulating polymer characteristics over a longer period of time (from days to weeks) to attain optimal therapeutic efficacy. Additionally, synthetic polymers can be manufactured as to degrade or change their conformation in response to stimuli arising specifically in the delivery site (change in pH, salt concentration or temperature) [192].

The “release on demand” can also be tuned as to respond to the activity exerted by enzymes, such as MMPs in the local environment and enable the cleavage of substrates with consequent release of bioactive moieties. The drugs to be delivered can be covalently immobilized, physically encapsulated or associated via secondary bonding to the delivery system. While choosing the appropriate method to conjugate the factor with the delivery system, the preservation of the bioactivity of the molecule has to be taken into account [193]. In this regard, covalent immobilization offers the advantage to locally present the factor of interest for a prolonged period (i.e.: the time of degradation of the delivery system).

The encapsulation of bioactive molecules into polymeric carriers can be highly beneficial to deliver and protect them against degradation in vitro and in vivo, thus improving their therapeutic activity [194]. However, the use of organic solvents during the preparation of the scaffold as well as the potentially harsh microenvironment conditions generated by polymer degradation can negatively affect the therapeutic efficacy of the factor [195]. Notably, controlling the release of the pharmacologically active agent at the therapeutically
optimal rate and site is a critical parameter both to decrease systemic dosage and side effects as well as to maintain drug concentration within the therapeutic range for a given period of time [196]. The drugs may be released with high local concentrations at the site of delivery and low systemic exposure through different mechanisms such as: 1) diffusion, 2) desorption of the drug bound to the surface, 3) polymer erosion and 4) a combined erosion–diffusion process [197].

Currently, to improve the delivery efficiency and local retention of infused agents, different routes of drug administration are being employed. In general, drug delivery systems for cardiac applications can be grouped into two categories: a) solid scaffold-based delivery systems to be sutured onto the epicardial surface; b) injectable systems including hydrogels and nano/microparticles for localized and minimally invasive catheter-based approaches.

Regardless of the nature of the scaffold used, the in vivo implantation of biomaterials has been associated to the arising of an inflammatory reaction.

This reaction usually starts with an acute phase, which is driven by neutrophil recruitment and activation, and is followed by a chronic inflammation mediated by the infiltration of monocytes and their differentiation to macrophages. The subsequent formation of a granulation tissue by macrophage and fibroblast matrix secretion is usually paralleled by the appearance of multinucleated giant cells, driving the so-called “foreign body reaction”. This reaction can last for months or years [198] and is thought to be a function of the chemical and physical properties of the biomaterial [199].

Thus, the possibility that biomaterials to be used in cardiac patch generation could lead to a severe reaction per se has to be considered before the successful implementation of cardiac tissue engineering devices is achieved [200, 201]. Nonetheless, the possibility to exploit the inflammatory response as a method to control scaffold degradation and thus drug release can be hypothesized.

SOLID SCAFFOLDS AS DRUG DELIVERY SYSTEMS IN THE TREATMENT OF CARDIAC PATHOLOGIES

In order to be used in cardiac tissue engineering applications, biomaterials are required to have reduced immunogenicity and toxicity, allow for their safe and timely removal and be compliant enough to assist cardiac contractile activity [2]. The fabrication of biocompatible and bio-inspired scaffolds for cardiac tissue engineering is aimed at providing a sophisticated and bio-inspired 3D environment for implanted or recruited cells, while serving for the targeted presentation of individual or multiple cytokines and growth factors [202-204]. An attractive approach to generate functional cardiac tissue constructs relies on the development of a system for sustained spatio-temporal delivery of multiple factors, with distinct kinetics, over multiple time scales, as to obtain a controlled, combinatorial release of different factors and recapitulate their natural orchestrated signaling cascade [205-207]. To this end, the carrier can be modified as to exhibit slow degradation allowing for a more controlled and prolonged release of bioactive factors by diffusion or scaffold degradation [193, 208, 209]. The mechanical properties of the scaffold can be tailored in order to prevent scar expansion, ventricular remodeling and encourage tissue healing, hence leading to the formation of a functionally organized tissue [210, 211]. A prerequisite for the successful application of engineered cardiac tissue is its ability to support vascular infiltration to locally promote cell survival and help the neovascularization of the surrounding ischemic tissue. For this purpose, major efforts have been made to deliver pro-angiogenic factors in order to improve neovascularization of the peri-infarcted area. It is the case of synthetic (tubular PLGA) and natural polymeric scaffolds (collagen sponges) being used as tools to deliver factors such as bFGF and VEGF, involved in vasculogenesis in ischemic tissues [212,213]. In particular, a cardiac patch composed of a porous collagen scaffold with covalently immobilized VEGF has proven capable to promote MSCs recruitment and engraftment and enhance the formation of myogenic tissue and blood vessels within the graft. The importance of angiogenic factors in healing mechanisms of the infarcted heart has also been confirmed in another study in which hydrogels mixed with BM-derived MSCs and angiogenic cytokines (SCF, SDF-1) were implanted in MI border zone. Hydrogel injection occurred immediately after the implantation of a biodegradable gelatin sponge (GELFOAM) superficially modified with poly-e-caprolactone (PCL) to strengthen the scaffold. The combination of cells, scaffold and cytokines promoted tissue and vasculature regeneration and ameliorated functional cardiac outcome [214].

Another interesting advantage of 3D scaffolds in cardiac tissue engineering strategies is the possibility to theoretically prepare vascularized heart muscle patches ex vivo before their implantation. Recently, a porous alginate scaffold loaded with neonatal rat heart cells was transplanted onto the omentum in an attempt to promote its vascularization. After 7 days, it was grafted onto the infarcted myocardium. The formation of new blood vessels and enhanced cardiomyocyte survival was reported when the matrix was supplemented with factors promoting stem cell homing (SDF-1), neoangiogenesis (Matrigel, VEGF), and cardiomyocyte survival (IGF-1). Moreover, the vascularized cardiac patch showed structural and electrical integration while preventing ventricular dysfunction 28 days after being implanted onto the infarcted myocardium [215]. These results confirmed the importance of a proper mature vascular network to support the growth and functional development of a bioengineered cardiac patch.

To counteract the negative effects of inflammation in myocardial infarction, scaffolds can be functionalized with anti-inflammatory factors that can also contribute to minimize the rejection of the cardiac patch and improve transplanted cell survival, when locally released. A collagen scaffold functionalized with a plasmid carrying the gene encoding for the anti-inflammatory IL-10 has been successfully used as a device to deliver MSCs to the infarcted myocardium. Four weeks post-transplant, cell retention and survival were found significantly enhanced. As a result, reduced inflammatory response as well as improved cardiac function were observed [216]. On the contrary, host response to scaffold implantation can be exploited to control bio-active factor release from scaffolds. In fact, host fibrin deposition on Periostin-loaded scaffolds has been proven able to retard the
release of the pro-survival and mitogenic factor. An improvement in cardiomyocyte proliferation and angiogenesis was reported [217].

**INJECTABLE DRUG DELIVERY SYSTEMS FOR CARDIAC TISSUE REPAIR**

**Hydrogels as Cardiac-Targeted Drug Delivery Tools**

Hydrogels are biocompatible materials with attractive properties making them ideal candidates for biomedical applications. In fact, given their high water content and 3D network structure, their mechanical properties are more similar to those of soft biological tissues and thus they are more suitable to comply with beating myocardium. Moreover, their chemo-physical structure can be easily modified and functionalized to mimic the properties of natural ECM [218].

In situ gelling hydrogels can be tailored to respond to a number of stimuli (pH, temperature) enabling sustained and controlled drug delivery and allowing minimal invasive implantation as cell and drug carriers [219,220]. Hydrogels can be classified as natural, synthetic and copolymer hydrogels. ECM proteins can be purified and used to prepare hydrogels with unique 3D structure and biological properties and able to release tissue-specific signaling factors. Biological drug-eluting hydrogels have been successfully fabricated from collagen, gelatin, fibrin, hyaluronic acid, or their combinations (e.g. MATRIGEL), but also from chitosan and alginate. They display rapid biodegradability and easy in vitro or in vivo gelation in the presence of cells and soluble factors.

The delivery of Thymosin β4 (Tβ4), a cardioprotective and angiogenic peptide [221], by injection of hydrogels composed of collagen-chitosan was found to foster angiogenesis and cardiomyocyte survival, while preserving left ventricular wall thickness after MI in rats [222]. Similar results were achieved 4 weeks after implantation of FGF-2-eluting chitosan hydrogels onto ischemic myocardial surface of a rabbit model of chronic myocardial infarction [190].

Despite providing target tissue with more appropriate biological cues capable to instruct cells towards specific phenotypes, the use of natural hydrogels in clinical practice is still debated due to their immunogenic potential and lack of control over the physical properties and degradation rate.

Synthetic hydrogels offer an alternative strategy as injectable drug delivery systems because of their high processability, appropriate biodegradability and long-term stability. Drug release and degradation rate can be easily controlled by changing the density of crosslinking and swelling [223,224].

Several synthetic polymers used to fabricate hydrogels, such as poly(ethylene glycol) (PEG), 2-hydroxyethyl methacrylate (HEMA) and Poly(N-isopropylacrylamide) (PNIPAAm), are non-biodegradable and display higher mechanical strength, these properties negatively affecting the release of biomolecules into the target tissue. Thus, hybrid bio-erodible hydrogels have been prepared by the incorporation of synthetic polymers (PLA, PLGA) and gelatin [225], peptides [226], collagen [227] or fibrin [228]. These materials have been reported to have beneficial effects on cardiac function and remodeling.

For example, PEGylated fibrin gels carrying covalently bound SDF-1α was implanted in a mouse infarction model: 28 days after gel implantation, c-kit+ stem cells were recruited at the damaged site, thus contributing to limit scar expansion and improve cardiac function [229]. A significant advancement is represented by the use of the same hydrogel formulation to deliver HGF and BM-derived stem cells to the infarcted heart. By these means, a 15-fold increase in stem cell engraftment, but no evidence of direct tissue regeneration was reported. The beneficial effect on heart function lasted for up to 4 weeks only in groups treated with HGF- and cell-loaded Biomatrix, thus confirming that combined strategies with controlled release of growth factor and cells can boost the effects of stem cell therapy by increasing its therapeutical potential [230].

Recently, hydrogels composed of self-assembly peptides have been proposed as promising tools for regenerative medicine. They consist of short peptides able to rapidly self-assemble into stable nanofibers (~10nm diameter) upon exposure to physiological pH and osmolarity [231].

Controlled delivery of PDGF-BB by injection of self-assembling peptide nanofibrous gels to the infarcted myocardium was reported for up to 14 days, hence providing cardiomyocyte protection against apoptotic signals, improving systolic function and reducing infarct size after ischemia/reperfusion [232].

The myocardial delivery of IGF-1-tethered self-assembling peptides in combination with neonatal cardiomyocytes improved implanted cell survival, while positively affecting cardiac performance in an experimental model of myocardial infarction [233]. Moreover, the simultaneous infusion of this drug-eluting material and cardiac progenitor cells in the border zone of infarction could increase cardiomyocyte survival, vessel formation and reduce infarct size, although cardiac performance could be only partially recovered [234]. Self-assembling peptides were also used to deliver SDF-1 intramyocardially in order to protect it from proteolytic degradation. The observed chemotactant and angiogenic effects of SDF-1 on CXCR4+ endothelial stem cells accounted, at least partially, for the improvement in rat cardiac function 4 weeks after experimental MI [235].

Hydrogels are generally more appropriate systems for the rapid delivery of drugs, with burst release being observed within the initial few hours. The inclusion of micro/nanoparticles carrying growth factors into hydrogel is one of the strategies being used to reduce the rate of drug release while protecting them from degradation [219]. Recently, a platform for sequential delivery of factors has been developed: injectable affinity-binding alginate hydrogel containing microbeads loaded with IGF-1 and HGF protected such factors from enzymatic proteolysis. Moreover, by modulating their equilibrium binding constants and concentrations, a sequential delivery of IGF-1 followed by HGF was successfully achieved. As a result, the sequential release of IGF-1 factor in the first phase post-infarction was shown to promote healthy tissue survival protecting it against cell death, whereas the late release of HGF prevented adverse cardiac remodeling while improving cardiac function [189].
NANO- AND MICROPARTICLES AS DRUG DELIVERY SYSTEMS FOR CARDIAC TISSUE REPAIR

Injectable biodegradable and biocompatible particles with different sizes, within micro- and nano- range, are currently being investigated as systems allowing the protection and prolonged release of beneficial moieties.

Over the past decade the emergence of nanotechnology has driven the development of new tools for efficient delivery of therapeutic agents to target tissue. By definition, pharmaceutical nanoparticles are submicron-sized (from 10 nm to a few hundred nanometers) drug delivery vehicles, also known as nanocarriers, used for the delivery of drugs with low molecular weight as well as proteins, peptides or genes [236-238].

The high surface-to-volume ratio of nanoparticles allows for improved bioavailability, sensitivity and retention of bioactive molecules at the targeted site. Among the other advantages of the small particle size are the ability of nanoparticles to cross biological barriers and penetrate deep into tissues where they can be more efficiently up-taken by cells [239]. These properties guarantee a higher therapeutic index by lowering systemic toxicity and increasing its local concentration.

The diffusion rate of the drug can be significantly modified by modulating particle size [240], while the functionalization of their surface with antibodies, receptor binding molecules, peptides and proteins allows for the delivery of specific drugs directly to the area of interest [241].

Although nanomedicine has been mainly applied to cancer research, its suitability to prepare diagnostic tools to detect specific pathological targets in cardiovascular medicine has been shown [242]. Additionally, silica nanoparticles were successfully delivered intramyocardially without causing significant changes in the hemodynamic parameters [243], and, when loaded with cardioprotective agent adenosine, they limited infarct size in a rat model of ischemia/reperfusion injury [244].

Liposome-based particles have been also proposed to encapsulate bioactive molecules. These biocompatible nontoxic, non-immunogenic carrier systems can have great flexibility in particle size (from few nanometers to >1μm) and lipid composition allows for the incorporation of both hydrophobic and hydrophilic molecules [245]. Targeted delivery of VEGF-loaded anti-P-selectin-conjugated liposomes to infarcted rat heart resulted in an increase in capillary density within the infarct area up to 4 weeks after infarction. Also, significant improvements in left ventricular cardiac function were reported, as compared to sham controls and systemic administered VEGF [246].

However, liposomes suffer from poor stability in the bloodstream, reduced control-over-drug-release and low encapsulation efficiency. These drawbacks can be surpassed by the preparation of polymer/liposome composites, allowing the tuning of drug release and enhancing its stability in the bloodstream.

Capryol 90-based gelation of VEGF-loaded liposomal nanoparticles contained in Pluronic F-127 shell was successfully applied to prepare a hydrogel system able to sustain VEGF delivery, thus promoting neovascularization and significantly increasing cardiac function [247].

Although nanoparticle-based drug delivery holds the promise to reduce the off-target effects associated to systemic treatments, major concerns still remain regarding nanoparticle removal; in fact, they were shown to accumulate in remote organs (mainly lungs and kidney) and persist for a long time. The risk of clot formation in small blood vessels has also been suggested [248].

As compared to nano-sized particles, systems in the micron range may offer the advantage to be retained longer at the injection site. Moreover they may sustain higher doses of therapeutic agent and allow for concomitant cell delivery [249]. In this context, the sustained, dual delivery of IGF-1 and VEGF was achieved over a 4 week period by incorporating the factors into gelatin microspheres, hence reducing infarcted area and promoting blood vessel maturation in rats [250]. Moreover, the sequential release of FGF and HGF encapsulated into albumin-alginate microcapsules synergistically increased local blood vessel density and perfusion, while reducing cardiac fibrosis and hypertrophy 3 months after injection into the border zone of rat infarcted myocardium. The combined therapy improved cardiac function by reducing LV dilation and dysfunction as compared to untreated animals or monotherapies [251].

A new drug delivery system has been developed based on microspheres formulated by polyketal polymer, showing the great advantage of using materials degrading into neutral compounds. The injection of polyketal-based microparticles loaded with an inhibitor of p38 MAPK into rat infarcted myocardium resulted in anti-inflammatory and anti-fibrotic effects and an improvement of fractional shortening 2 and 3 weeks after LAD coronary occlusion [252]. Consequently, N-acetyl-glucosamine (GlcNAc)-functionalized particles were developed to enhance the uptake of the anti-apoptotic factor by cardiomyocytes and improve significantly short-term cardiac function during the acute phase following ischemia/reperfusion [253]. Also, the sustained and controlled administration of angiogenic factor VEGF entrapped into PLGA microparticles was accomplished for more than a month after transplant into rat infarcted myocardium. During this timeframe, increased vascularization and reduced left ventricular remodeling were observed, although the local accumulation of macrophages could reduce the therapeutic efficacy of the treatment [254].

CONCLUSIONS

The use of biocompatible scaffolds as cell delivery systems holds the potential to improve the retention of stem cells to the target site and potentially their efficacy in cardiac disease treatment.

The paracrine effects of adult stem cells account for the majority of the beneficial results reported in the pre-clinical studies performed so far. Hence, the identification of the molecules – or, more likely, a combination of them – responsible for the described effects, could lead to the implementation of modified scaffolds able to enhance the pharmacological properties and the release kinetics of the beneficial factors to be used in the treatment of cardiac diseases. Scaffolds themselves could be manufactured as to attain a timely release of different cytokines in a physiological fashion. In this respect, the body reaction to biomaterial implantation could...
be exploited as a favorable event, allowing the controlled release of the factors tethered or embedded in the delivery system. Alternatively, a plus could be represented by the use of genetically modified stem cells releasing paracrine factors once implanted. Although the use of genetically modified cells is still hindered by safety considerations, the combination of tissue engineering technologies to prepare combinatorial cell and drug delivery platforms could result in the generation of smart systems to treat cardiac diseases.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENT

The present work was supported by the Japan Society for the Promotion of Science (JSPS) through the “Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)”, the World Premier International (WPI) Research Center Initiative. PPO was supported by Ciência2007 from FCT-Portuguese Foundation for Science and Technology.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCs</td>
<td>Hematopoietic stem cells</td>
</tr>
<tr>
<td>MSCs</td>
<td>Mesenchymal stem cells</td>
</tr>
<tr>
<td>EPCs</td>
<td>Endothelial progenitor cells</td>
</tr>
<tr>
<td>CPCs</td>
<td>Cardiac stem/progenitor cells</td>
</tr>
<tr>
<td>skMyo</td>
<td>Skeletal myoblast</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>HGF</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma protein 2</td>
</tr>
<tr>
<td>Bcl-xL</td>
<td>B-cell lymphoma protein extra large</td>
</tr>
<tr>
<td>BAD</td>
<td>Bcl-2-associated death promoter protein</td>
</tr>
<tr>
<td>PI3k</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>c-Met</td>
<td>Hepatocyte growth factor receptor</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>Flk-1</td>
<td>Fetal liver kinase</td>
</tr>
<tr>
<td>KDR</td>
<td>Kinase domain receptor</td>
</tr>
<tr>
<td>Flt-1</td>
<td>FMS-like tyrosine kinase 1</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>CMEC</td>
<td>Cardiac microvascular endothelial cell</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone marrow cell</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factor</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>Janus kinase/signal transducer and activator of transcription</td>
</tr>
<tr>
<td>ESC</td>
<td>Embryonic stem cell</td>
</tr>
<tr>
<td>iPSC</td>
<td>Induced pluripotent stem cell</td>
</tr>
<tr>
<td>SDF-1</td>
<td>Stromal derived factor 1</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Chemokine receptor 4</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin 1 beta</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>Extacellular signal regulated kinase 1/2</td>
</tr>
<tr>
<td>JNK</td>
<td>Jun N-terminal kinase</td>
</tr>
<tr>
<td>MMP</td>
<td>Metalloproteinase</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TNF-α R1</td>
<td>Tumor necrosis factor alpha receptor 1</td>
</tr>
<tr>
<td>sFRP2</td>
<td>Secreted frizzled-related protein 2</td>
</tr>
<tr>
<td>Sca-1</td>
<td>Stem cell antigen 1</td>
</tr>
<tr>
<td>GATA-4</td>
<td>GATA binding factor 4</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>PlGF</td>
<td>Placental growth factor</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>Glycogen synthase kinase 3 beta</td>
</tr>
<tr>
<td>eIF2B</td>
<td>Eukaryotic translation initiator factor 2b</td>
</tr>
<tr>
<td>N-myec</td>
<td>Avian myelocytomatosis virus oncogene neuroblastoma homolog</td>
</tr>
<tr>
<td>MDR-1</td>
<td>Multi drug resistance 1</td>
</tr>
<tr>
<td>CD31</td>
<td>Cluster of differentiation 31</td>
</tr>
<tr>
<td>CD34</td>
<td>Cluster of differentiation 34</td>
</tr>
<tr>
<td>c-kit</td>
<td>C kit receptor</td>
</tr>
<tr>
<td>TET-OFF</td>
<td>Tetracycline off</td>
</tr>
<tr>
<td>ASC</td>
<td>Adipose-derived mesenchymal stem cells</td>
</tr>
<tr>
<td>HIF</td>
<td>Hypoxia inducible factor</td>
</tr>
<tr>
<td>Ang-1</td>
<td>Angiopetin 1</td>
</tr>
<tr>
<td>HO-1</td>
<td>Heme oxygenase 1</td>
</tr>
<tr>
<td>Nkx-2.5</td>
<td>Cardiac-specific homeobox 2.5</td>
</tr>
<tr>
<td>Cx43</td>
<td>Connexin 43</td>
</tr>
<tr>
<td>SCF</td>
<td>Stem cell factor</td>
</tr>
<tr>
<td>PIM-1</td>
<td>Pim 1 kinase</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly-ε-caprolactone</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>Tβ4</td>
<td>Thymosin β4</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-hydroxil methacrylate</td>
</tr>
<tr>
<td>PNIPAAm</td>
<td>Poly(N-isopropylacrylamide)</td>
</tr>
<tr>
<td>LAD</td>
<td>Left anterior descending coronary artery</td>
</tr>
</tbody>
</table>
tion correlates with the release of cardioactive cytokines. Stem Cells, 2007, 25(1), 236-244.


on stem-cell homing and tissue regeneration in ischemic cardiomyopathy. Lancet, 2003, 362(9385), 697-703.


Zhu, J.; Marchant, R.E. Design properties of hydrogel tissue-engineering scaffolds.


Chiu, L.L.; Reis, L.A.; Momen, A.; Radisic, M. Controlled release of basic fibroblast growth factor: experimental studies in myocardial revascularization.


