Adipose derived stromal cells in cardiovascular regenerative medicine
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Download date: 29-01-2020
CHAPTER 10

GENERAL DISCUSSION AND FUTURE PERSPECTIVES
Summary of the thesis

Cardiovascular diseases are the leading cause of death globally. Myocardial Infarction (MI) occurs due to the occlusion of one of the branches of the coronary artery system. Once the coronary artery occludes, the downstream myocardium is deprived of oxygen and nutrition, which lead to myocardial ischemia. Prolonged ischemia results in the death of cardiomyocytes and surrounding vasculature. Subsequently, the death signals from apoptotic and necrotic cells activate rapid inflammatory reaction, which dominates the early post-MI murine cardiac microenvironment. Unfortunately, the proliferation capacity of adult cardiomyocytes is not sufficient to repair the extensive cardiac damage and current therapies do not alleviate the cardiac repair. Insufficient cardiomyocyte proliferation together with excessive myofibroblast proliferation leads to irreversible scar formation. This scar, however, is required to maintain the heart’s structural integrity. In the subsequent period post-MI, the cardiac tissue suffers from lack of synchronized contraction caused by the stiff scarred tissue. Moreover, to compensate for the loss of cardiomyocytes, the spared cardiomyocytes respond with hypertrophy. This is followed by adverse remodeling: left ventricular dilatation, deterioration of cardiac function and eventually heart failure. To prevent the negative cardiac remodeling phase, it is essential to provide a new source of cardiomyocytes and increase the vascularization of the damaged area. The initiation of the regenerative processes must occur in early post-MI: during the inflammatory phase that starts immediately after MI and before appearance of scar tissue, thus leaving a small window of opportunity for regeneration [1]. Despite early medical intervention and fast restoration of the blood flow to limit the damage area, still there is no cure and the number of patients who engage in heart failure post-MI increases.

A recent and promising approach for induction of cardiac repair is (stem) cell-based therapy. It is known that adult cardiac tissue has an endogenous repair capacity that leads to total replacement of cardiomyocyte every eight years during healthy life span. Moreover, the repair is even further enhanced after the incident of MI [2, 3]. Ample reports suggest that cardiac repair could be boosted by highly regenerative stem cells or stem cell-secreted factors. The application of therapeutic cells could be a tool for modulation of the inflammatory post-MI microenvironment in the damaged area and provide the healing support. The formation of scar tissue and development of heart failure could be delayed or blocked by the rapid induction of cardiomyocyte proliferation and increased revascularized area. A promising stem cell type for cardiac therapy could be of mesenchymal origin, the same embryonic origin as cardiac tissue. Mesenchyme-derived stem/progenitor cells are present in a plethora of tissues such as muscle, bone marrow or adipose tissue. Currently the most promising stromal cells are derived from adipose tissue i.e. Adipose-tissue Derived Stem/Stromal Cells. Recent clinical trials with intramyocardial injection of freshly isolated ADSC demonstrated the improvement of cardiac parameters, increased revascularization and finally the quality of life [4].

An important factor in successful post-MI stem cell therapy is the modulation of the cardiac microenvironment. The post-MI microenvironment comprises of hypoxia and inflammatory mediators among others. Inflammation should not be completely blocked but modulated because this process is essential in cardiac wound healing [5]. The inhibition of the inflammatory reactions early after the incident of myocardial infarction actually deteriorates the healing processes [6].

Thus, in this thesis, we investigated the regenerative potential of ADSC exposed to the in vitro ischemic and inflammatory conditions. We mimicked and applied the post-MI host microenvironment in vivo conditions to validate the ADSC repair potency in the...
inflammatory and hypoxic microenvironment. In the first part of this thesis, we focused on stimulation of cardiomyocyte proliferation, maturation and cardiomyocyte cell-cell organization. In chapter 2 we describe the functioning of applied therapeutic cells in the post-MI host microenvironment and the changes that influence the outcome of mesenchymal cell-based therapy. Mesenchymal Stem Cells (MSC) such as ADSC are promising because of their simple isolation method and abundant secretion of therapeutic factors. Recently, the preconditioning of “naive” stem cells gained increasing interest: previously presumed deleterious stimuli such as hypoxia and inflammation, i.e. causes of myocardial damage, have the opposite effect on mesenchymal stem cells. ADSC gain a higher therapeutic capacity under hypoxia and inflammatory conditions, which render them promising for cardiac regeneration [7].

In chapter 3 we investigated the influence of the post-infarction cardiac microenvironment on ADSC cardiac repair mechanisms in vitro. Postnatal cardiomyocytes have a low to absent proliferation rate, while induction of proliferation seems even more rare. In metaplastic diseases, the pro-inflammatory cytokine interleukin-6 (IL-6) has been identified as potent mediator of the proliferation rate. IL-6 stimulates the proliferation of human muscle satellite cells after acute muscle damage [8]. We identified that ADSC upregulate and secrete IL-6 abundantly after exposure to pro-inflammatory cytokines. Stimulation of the cardiomyocytes with ADSC conditioned media, i.e. rich in IL-6, from hypoxia and IL-1β primed ADSC, strongly enhanced cardiomyocyte proliferation. In contrast to current data, not only hypoxia may exert a beneficial effect on ADSC [9-11]. We found that inflammation had a stronger positive effect than hypoxia on the ADSC secretion profile. Furthermore, in chapter 3, we identified previously uncharacterized function of conditioned medium of ADSC signaling in regulating cardiomyocyte proliferation. We demonstrated that stimulation of cardiomyocyte proliferation by ADSC conditioned medium was accomplished through activation of both Janus Kinase-Signal Transducer and Activator of Transcription (JAK/STAT) and Mitogen-Activated Protein (MAP) kinases (MAPK) mitogenic signaling pathways. Interestingly, reduction of STAT3 phosphorylation led to increased levels of phosphorylated Erk1/2, indicating a feedback loop between these pathways. This suggests that the stimulated proliferation rate of cardiomyocytes is a balance between STAT3 signaling and MAP-kinase signaling [12].

Induction of adult cardiomyocyte proliferation has long thought to be impossible because of the static nature of adult cardiomyocytes, although recent literature indicates that adult cardiomyocytes can proliferate and that even the induction of adult cardiomyocyte proliferation is feasible [13]. Pivotal cardiogenic factors are mitogens of the EGF family such as Neuregulin, HB-EGF, and TGF-α. Treatment using EGF ligands after murine aMI increases cardiomyocyte numbers, reduces scar formation and improves cardiac function [14, 15]. Thus, in chapter 4, we investigated the influence of ADSC and their secreted factors on the proliferation of cardiomyocytes under culture conditions that mimic the post-aMI microenvironment. We demonstrated that hypoxic and inflammatory stimuli caused increased secretion of HB-EGF by ADSC. The direct co-culture of ADSC with cardiomyocytes resulted in an increased proliferation rate of cardiomyocytes. Moreover, treatment of cardiomyocytes with the conditioned medium of ADSC resulted in increased cardiomyocyte proliferation, partially due to the presence of HB-EGF. Stimulation of HL-1 cardiomyocytes with the conditioned medium of ADSC lead to activation of EGF-MAPK-Erk1/2 signaling in HL-1 cardiomyocytes resulting in increased cardiomyocyte proliferation rate.

It is known that the mutual interactions of cells, soluble mediators and extracellular matrix (ECM) components in the post-MI microenvironment orchestrate the regenerative
outcome of the infarcted myocardium. Thus in chapter 5 we focused on the regenerative potential of ADSC-derived extracellular matrix components. ECM components have been recognized as key players in cardiomyogenesis and cardiac repair [16]. Injection of bone marrow-derived ECM after ischemic injury in rats resulted in improved cardiac function i.e. reduced fibrosis [17]. Still the mechanism of the interactions between ADSC and their synthesized ECM components with the cardiomyocytes is not known. We demonstrated that ADSC in addition to its activation of a paracrine driven regenerative interactions, also secrete ECM components in the intercellular space. The extracellular matrix components synthesized by ADSC directly influence the cardiomyocyte microenvironment by creation of the fibrous scaffold for proper functioning and signaling of the cells into a unified syncytium. We demonstrated that in vitro co-culture of neonatal cardiomyocytes with ADSC or with ADSC-derived ECM enhanced the cardiomyocyte proliferation, alignment, cellular interconnections and sarcomere maturation. Furthermore, cardiomyocytes cultured on ADSC-derived ECM matrices had an increased proliferation rate and decreased cell size (hypertrophy) compared to cardiomyocytes cultured on tissue culture polystyrene (TCPS) or on a Fibronectin/Gelatin coating [18].

In conclusion of Part I of this thesis, we demonstrated that ADSC have an enhanced repair potential after exposure to the hypoxia and inflammatory mediators found in post-MI microenvironment. ADSC promote the proliferation rate of cardiomyocytes by both juxtacrine and paracrine interactions and through the deposition of extracellular matrix components. One of the therapeutic options to improve the outcome of cardiac therapy would implement an ADSC-derived “of-the-shelf” product such as a serum-free ADSC conditioned medium or lyophilized ADSC-derived ECM components for the immediate treatment of post-MI. Another suggestion to further increase the therapeutic potential of ADSC would be to prime ADSC with hypoxia and pro-inflammatory cytokines prior to application of ADSC-derived therapeutic secreted factors.

Recently reported clinical trials that employ intramyocardial administration of ADSC in patients post-MI, identified that major improvement is associated with reduction of a scar tissue due to improved perfusion. Therefore, in Part II we investigated the role of ADSC in vascular network formation and its stabilization. The cells that orchestrate endothelial cells proliferation, survival, vascular tone and permeability are known as pericytes [19]. In Chapter 6, we reviewed pericyte with respect to their ontogeny, function and regenerative prospective of ADSC. In particular, the acquisition of pericyte function of ADSC in the presence of endothelial cells [20]. Pericytes are present in the vasculature of all organs including retina, brain, skeletal muscle or adipose tissue [21]. Pericytes originate from neuroectoderm or embryonic mesenchyme, thus sharing a mesenchymal phenotype with ADSC. Adipose tissue is highly vascularized with abundance of the stromal vascular fraction derived ADSC, which represent the perivascular pericytes or adventitial cells [22]. Endothelial cells directly interact with ADSC in co-cultures [23]. On confluent monolayers of cultured ADSC, endothelial cells form vascular-like networks that are reminiscent of sprouting networks formed on Matrigel [23]. However, ‘Matrigel sprouting’ is a short term angiogenesis process, while vascular network formation on ADSC monolayers requires longer time to establish (days instead of hours) and comprises of tubes build up of multiple endothelial cells as opposed to single celled capillaries. Moreover, a number of studies have shown that ADSC and bone marrow MSC engraft onto endothelial sprouting networks on matrigel [24]. In chapter 7, we show that ADSC acquired pericyte’ function in vitro and in vivo. ADSC, when in direct contact with endothelial cells, induced vascular network formation. In vitro and in vivo ADSC incorporated into the existing vasculature and
promoted vessel normalization. Additionally, in the model of oxygen induced retinopathy (OIR), ADSC replaced the lost pericytes and promoted vascular normalization by acquisition of pericyte’ function as well as by modulation of the damaged microenvironment to drive the vasculature homeostasis. This shows both that there exists a reciprocal plasticity between ADSC and pericytes and that ADSC are a promising tool to alleviate pericyte loss. Moreover, we have shown that ADSC not only support formation of endothelial tubes, but that multicellular vascular structures are formed and are maintained for several weeks through interaction with ADSC. To achieve the higher therapeutic potential of stem/progenitor cells it is possible to instruct the cells in vitro before their administration in vivo. For example, creating a pre-mature complex of vascular network associated with their stabilizing adipose tissue-derived pericytes prior the injection. The understanding of stem/progenitor plasticity in the presence or absence of post-damage microenvironmental factors helps to develop and improve therapies. Therefore, in chapter 8 we investigated the influence of pro-inflammatory and hypoxic preconditioning of ADSC on the formation of endothelial networks. The medium of hypoxic and pro-inflammatory preconditioned ADSC increased migration of EC and formation of more stable vascular networks in-vitro compared to control ADSC-conditioned medium. In direct co-culture with ADSC, EC formed vascular networks that lasted for several weeks. Other stromal cell types such as human dermal and cardiac fibroblasts or mouse 3T3 fibroblastic cells failed to induce vascular networks. We concluded that ADSC promote vascular network formation and its stabilisation in vitro through paracrine and juxtacrine interactions with EC. Priming of EC to form vascular network by direct co-culture with ADSC or culture of EC in the presence of ADSC-derived conditioned medium could offer a potent strategy to revascularize damaged myocardium. One of the potential risks in stem cell guided cardiac repair by MSC is accelerated fibrosis. The major advance in the cardiac clinical trials with ADSC application indicated reduced infarct size and scarring. Thus in Chapter 9, we focused on the impact of ADSC in tissue remodeling in vitro. We demonstrated that TGF-β-induced proliferation of primary human dermal fibroblasts (HDFa) was abolished by ADSC conditioned medium. Simultaneously, ADSC conditioned medium, reduced SM22α gene and protein expression of TGF-β-treated HDFa, while their contractility was reduced too. Furthermore, ADSC conditioned medium strongly reduced transcription of collagen I and III genes as well as their corresponding proteins. On the other hand the ADSC conditioned medium tipped the balance of matrix turnover to degradation through stimulating gene expression of MMP-1, 2 and 14, while MMP-2 activity was upregulated too. Even in the fully differentiated myofibroblasts such as from keloids (KLF), ADSC conditioned medium suppressed TGF-β-induced myofibroblast contraction, and collagen III gene expression. In this study we showed that ADSC inhibited TGF-β-induced adverse differentiation and function of HDFa and TGF-β-induced contraction in KLF, in a paracrine fashion [25]. This might have an impact the development therapies that target cardiac fibrosis and scar formation.

In line of this research, we dissected part of mechanisms that underlie the beneficial contribution of ADSC for the treatment of myocardial infarction complications trough increase of cardiomyocyte mass, improved re-vascularization and immunomodulatory balance of post-MI environmental homeostasis.

**Future perspectives**

Unhealthy, sedentary life style, excessive food availability (and intake), stress and insufficient physical activity lead to continuously expanding cardiovascular disease, obesity
and diabetes throughout the Old and the New World. The foremost cause of death globally is heart failure. Despite immediate interventions and the advances made in the treatment, still there is no cure of complications that result from acute Myocardial Infarction (MI). Moreover, the adult human heart does not have sufficient regenerative capacity to restore the damage. The occlusion of coronary arteries causes loss of cardiomyocytes and compromises perfusion. The cardiac ischemia and death signals trigger influx of pro-inflammatory cells. Subsequently the debris is cleared and the wound healing processes are initiated. Unfortunately, the heart instead of healing, form the scar tissue to maintain tissue integrity. The sustained presence of rigid tissue and inflammation lead to deterioration of cardiac function. As no conventional therapy can prevent the negative aftermath of MI, alternative treatments are warranted.

Cardiac stem cell based therapy has been put forward over the past decade. Extensive comparison of various types of therapeutics cells used in the clinical trials in post-MI patients indicated that Mesenchymal Stem Cells (MSC) are promising therapeutic agents for cardiac repair. MSC share the same mesodermal origin as of cardiomyocytes, such as muscle tissue. MSC are genuine cellular factories of therapeutic molecules such as growth factors, cytokines and extracellular matrix components among others. MSC are most frequently obtained from bone marrow or adipose tissue. Harvesting therapeutic cells by liposuction it is less painful and less invasive procedure compared to bone marrow aspiration. Moreover, isolation of therapeutic cells from adipose tissue yield higher clinically relevant cell number compared to the bone marrow [26]. The heart itself also contains mesenchymal-like stem cells, though more difficult to acquire.

Mesenchymal stem cells from adipose tissue
Adipose tissue is an abundant source of mesenchymal stem cells, known as adipose tissue-derived stem or stromal cells (ADSC) [27]. By definition, ADSC are the plastic adherent fraction of the stromal vascular fraction of adipose tissue. This high abundance of cells almost contradicts them being named as stem cells. Also their limited number of population doublings supports the suggestion to call ADSC adipose tissue-derived stromal cells, more than stem cells. Not uncommon for ‘stem’ cells, the origin of ADSC is debated. Similar to all other types of MSC, ADSC are per definition in vitro culture artifacts that acquire a phenotype that may strongly differ from their in vivo phenotype. To date, the vasculature of adipose tissue is thought to give rise of at least three types of multipotent precursor cells. Firstly, at luminal side these are endothelial precursor cells, while both other types are either supra-adventitial [28] or perivascular [29,30]. These perivascular ADSC are likely a subfraction of the pericytes, while the supra-adventitial ADSC may relate to a specific subset of advential fibroblasts [31]. Others provide evidence that ADSC originate from perivascular and adventitial vascular regions but are more similar to smooth muscle cells [31-34]. It is likely that the cells in these locations form a continuum, while the local microenvironment dictates their resident phenotype. Once disrupted from their original environment, these cells appear to show a high degree of interchangeability [30]. In comparison to BM-MSC, ADSC yields are consistently higher, while the growth rate of ADSC in a simple mesenchymal medium is higher too [35]. In vitro, ADSC have a mesenchymal phenotype and morphology.

Tuning the post-MI microenvironment/ Juxtacrine/paracrine interactions
The previous work showed that cardiac repair must be initiated immediately after post-MI. The key factor in cardiac repair is inflammation and following wound healing, van Amerongen and others demonstrated that Inhibition of the inflammatory responses, by macrophage depletion, resulted in increased cardiac adverse remodeling and deterioration of the cardiac tissue [36]. However, sustained inflammation can be deleterious by providing...
non-regenerative signals. Interestingly the cardiac repair system is activated during the early phase post-MI, still not sufficient [37]. Both inflammatory and regenerative signals need to be tuned to form balance between adverse inflammatory remodeling and regeneration.

The post-MI hostile microenvironment is characterized by lack of perfusion and massive cell death and thus is a non-attractive playground for therapeutic stem cells. The understanding of the working mechanism of MSC has greatly increased and led to the discovery that these cells can be primed to a higher level a therapeutic capacity by potentially deleterious stimuli such as hypoxia and inflammation. The stem cell mediated cardiac therapy might be hampered by the post-MI microenvironment. Interestingly, the preconditioning of stem cells is gaining more interest: previously presumed deleterious stimuli such as hypoxia and inflammation, i.e. causes of myocardial damage, have the opposite effect on mesenchymal stem cells. MSC gain a higher therapeutic capacity under hypoxia and inflammatory conditions. We identified that ADSC activate their regenerative potential under in-vitro mimicked post-MI pro-inflammatory microenvironment. Adipose stromal cells primed with hypoxia and inflammation (2% O$_2$ and IL-1β) enhance cardiomyocyte proliferation rate in vitro. ADSC under pro-inflammatory conditions assessed paracrine-driven and juxtacrine driven enhancement of cardiomyocyte proliferation rate and induction of myogenic signaling pathways such as JAK-STAT and MAPK by induction of STAT3 and Erk1/2 phosphorylation. ADSC despite the activation of the mitogenic signaling pathways also increase the proangiogenic potency. ADSC induce simultaneous growth of cardiomyocytes and vasculature. ADSC can modulate the post-MI microenvironment through secretion of the broad range of growth factors and cytokines. These trophic factors include mitogens such as hepatocyte growth factor (HGF), insulin growth factor (IGF), and fibroblast growth factors (FGF), vascular endothelial growth factor (VEGF), but also immunological factors such interleukin-1β, -6 (IL-1β, IL-6) and transforming growth factor-beta (TGF-beta) as well as matrix remodeling enzymes such as matrix metalloproteases (MMPs) [38-40]. ADSC are strongly proangiogenic through secretion of e.g. VEGF, FGFs, and angiopoietins. Along with paracrine interaction through the secreted mediators, ADSC also promote vessel formation and cardiomyocyte proliferation in vitro through juxtacrine interaction with endothelial cells and cardiomyocytes. Part of this juxtacrine interaction that promotes stable vessels, is governed by secretion of extracellular matrix components by ADSC [23]. Moreover, the ‘pericytic’ ADSC fraction from adipose tissue stabilize vascular structures in vitro, which depended on cell-cell contacts and on mutual signaling with paracrine factors [41]. We have shown that ADSC not only support formation of the multi-cellular vascular endothelial tubes but also ameliorate its stability for several weeks in-vitro. Furthermore, we have shown in a murine model for retinal pericyte loss that ADSC can normalize pathologic capillaries. The intra-retinal injected ADSC acquired a typical pericytic position in the vasculature. This shows high reciprocal plasticity, functioning and retention of ADSC.

Despite the known paracrine influence of ADSC in the cardiac repair, the juxtacrine signaling i.e. cell-cell and cell-ECM role of ADSC in post-MI repair process is not fully understood. The post-MI myocardial repair requires remodeling the ECM for adequate de novo cardiomyogenesis and vasculogenesis. The ECM is instrumental in both processes. ADSC-derived ECM components provide a suitable substrate to support cardiomyocyte homeostasis i.e. cardiomyocyte alignment, maturation and synchronized contraction in vitro. This finding supports the use of human, autologous, ADSC as a promising modality to treat MI. Moreover, i.e. lyophilized ADSC-derived ECM or in a form of injectable biomaterial i.e. ECM patches or in combination with ADSC [42] could offer an off-the shelf product for the
treatment of post-MI complications. Alternatively, ADSC-secreted ECM components could augment scaffolds used to tissue-engineer myocardial replacement patches that are based on e.g. iPSC-derived autologous cardiomyocytes. The ECM components would improve the organization and compaction of the cardiomyocytes on scaffolds. Moreover, the ADSC-derived ECM could act as a sponge for capture, trafficking and delivery of the secreted paracrine factors, which remains to be investigated.

Taking together, ADSC are a most promising cell type to augment and orchestrate cardiac repair through increase of cardiomyocyte proliferation, angiogenesis and modulation of the post-MI microenvironment by tuning the pro-inflammatory reactions. This renders ADSC the strong clinical pre-requisite for post-MI cardiac repair.

**ADSC in clinical trials**

Over the past decade, mesenchymal stem cells emerged as a most promising type of stem cell for cardiovascular regeneration. Besides bone marrow and cardiac cells, scientists focused on other tissue sources for mesenchymal stem cells with a high regenerative potential [43]. For example, adipose tissue contains 500-fold more MSC than adult bone marrow, while it is easier accessible than bone marrow. ADSC can be isolated from liposuction aspirates and prepared as fresh cells for immediate administration in cell therapy. In our experience, up to one hundred million ADSC can be easily isolated from as little as one liter of lipoaspirate. ADSC have rapidly reached the clinic. The APOLLO double-blind randomized clinical trial for treatment of acute myocardial infarction employed autologous ADSC. These ADSC are harvested from lipoaspirates and enriched through processing with the Celution system of Cytori. This closed system procedure takes only a few hours which allows for administration of the ADSC during the early post-MI, while conventional treatment of the acute MI is maintained. The clinical trial involved ten patients that received ADSC and four placebo group with 36 h after the infarction occurred. APOLLO trial showed two important benefits for the intraoperative use of ADSC. Firstly, an up to 60% reduction of infarct size was observed and secondly an improved revascularization of the ischemic tissue was observed which ameliorated blood flow. In addition, contractility was improved, while no arrhythmia was found [44]. According to the investigators not only the short term follow up (six months) but also the long term follow up (eighteen months) of the APOLLO clinical trial showed a persistent benefit for the patient that had been treated with ADSC. Meanwhile, ADVANCE has started which is a follow-up clinical trial focusing to prevent heart failure. It includes a near 375 patients that will be treated with ADSC within twenty-four hours after acute myocardial infarction. Whereas several trials focus on early intervention, other clinical trials, like PRECISE and ATHENA, focus on the aftermath of myocardial infarction i.e. treatment of chronic myocardial ischemia with ADSC [45]. Recently published data of the PRECISE trial anticipated for the long-term efficacy (up to 36 months) of ADSC-based cardiac stem cell therapy. Patients with ischemic cardiomyopathy after trans-endocardially injected ADSC showed significant improvements in metabolic equivalents and maximal oxygen consumption, increase in total left ventricular mass, reduction in ischemia and no malignant arrhythmia compared to the placebo controls patients [46]. These results furthermore confirm that ADSC-based stem cell therapy is safe, feasible and may preserve ventricular function, myocardial perfusion and exercise capacity of patients with ischemic cardiomyopathy.
**Novel approaches for ADSC-stem cell based therapy**

The results described in this thesis can be implemented to adapt and improve ADSC-based therapy for cardiovascular diseases such as myocardial infarction. The best results in the treatment of post-MI complications were found after the immediate revascularization of the damaged area. Cytori system used in the current clinical trials show that early application of ADSC procedure resulted in improved cardiac function. Here we suggest that not only ADSC but the frozen or lyophilized ADSC product could offer of the shelf product for early application. Furthermore, as described, the increased occurrence of myocardial infarction coincides with diabetes, obesity and aging. Indeed, recent reports have been shown that ADSC decrease their secretion of pro-angiogenic factors in patients with coronary artery diseases or type 2 diabetes [47]. One possibility to overcome metabolic related attenuation of ADSC’s repair potential is to prime the cells with the pro-inflammatory and/or hypoxic conditions. We identified that ADSC increase their mitogenic and regenerative secretion of growth factors and cytokines after stimulation with pro-inflammatory cytokines and hypoxia. This led to the “re-juvenation” of the cells used in case of autologous application of ADSC from the aged or T2D patients. Moreover, we also showed that there is no need to isolation and injection of therapeutic cells but instead could use e.g. conditioned medium or extracellular matrix components. As there is no risk of the rejection or foreign body reaction, these therapeutic agents could be implemented intramyocardial right after the primary revascularization procedure to modulate acute pro-inflammatory microenvironment, boost cardiomyocyte proliferation and increased revascularization of the damaged area to obtain enhanced cardiac repair.

**Concluding remarks**

In this thesis we have acquired better understanding of use of Adipose-tissue Derived Stem/Stromal Cells in post-myocardial infarction therapy. We have shown that in contrast to the previous paradigm, therapeutic cells of mesenchymal origin such as ADSC gain higher regenerative potential under hypoxic and pro-inflammatory conditions, which render them the most promising cells for cardiac therapy. We have identified that not only ADSC but their secreted factors in the conditioned medium or deposited extracellular matrix enhance cardiomyocyte proliferation, organization and maturation as well as lead to increased vascular network formation. This could help to overcome the costs and the limitation of stem/stromal cell therapy as well as offer of the shelf product for immediate application. We propose that use of ADSC or ADSC-derived conditioned medium or extracellular matrix components based therapy is feasible and clinically relevant option for treatment of post-MI complications.

**References**


[41] Traktuev D O, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and


