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Review

Therapeutic application of adipose-derived stromal vascular fraction in myocardial infarction

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SUMMARY

The insufficiency of natural regeneration processes in higher organisms, including humans, underlies myocardial infarction (MI), which is one of the main causes of disability and mortality in the population of developed countries. The solution to this problem lies in the field of revealing the mechanisms of regeneration and creating on this basis new technologies for stimulating endogenous regenerative processes or replacing lost parts of tissues and organs with transplanted cells. Of great interest is the use of the so-called stromal vascular fraction (SVF), derived from autologous adipose tissue. It is known that the main functions of SVF are angiogenetic, antiapoptotic, antifibrotic, immune regulation, anti-inflammatory, and trophic. This study presents data on the possibility of using SVF, targeted regulation of its properties and reparative potential, as well as the results of research studies on its use for the restoration of damaged ischemic tissue after MI.

INTRODUCTION

Despite the improvement of therapeutic, endovascular, and surgical methods of treatment, cardiovascular diseases continue to lead among the causes of disability and death worldwide. Myocardial infarction (MI) is one of the most serious and dangerous conditions. MI is accompanied by necrosis of a portion of the muscular tissue of the heart, followed by the development of connective tissue (focal postinfarction fibrosis), which is formed due to the low regenerative potential of the myocardium (Figure 1).[1,2]

In recent years, the attention of researchers has been drawn to the use of cell therapy based on transplantation of stem cells and progenitor cells, which has been a promising strategy for repairing the heart after damage.[3,4] Mesenchymal stem cells (MSCs)/progenitor cells can contribute to the repair of damaged tissues by regulating the response of immunocompetent cells and the activity of structure-forming cells such as fibroblasts, which leads to a pronounced regenerative and anti-inflammatory effect. Inflammatory mediators can recruit MSCs and alter their secretory profile, a process thought to promote immune responses and wound healing.[5] It is assumed that the transforming growth factor β (TGF-β) signaling pathway, which acts through the appropriate receptors such as transforming growth factor β receptor 1/2/3 (TGF-βR1/2/3) and transcription factors of the mothers against decapentaplegic homolog family 2/3/4 (SMAD2/3/4), may be largely responsible for the development of postinfarction myocardial fibrosis and triggers the differentiation of fibroblasts into myofibroblasts, enhancing their synthetic activity.[6] In addition, TGF-β is also able to induce the synthesis of TGF-β-activated kinase 1 (TAK1) and the p38 family of mitogen-activated protein kinases 11/12/13/14 (MAPK11/12/13/14), which promote apoptosis of cardiomyocytes and the synthesis of extracellular matrix (ECM) proteins by cardiac fibroblasts (Figure 2).[6] The underlying theory is that MSCs secrete regulatory peptides that affect multiple pathways of fibrogenesis, have immunosuppressive effects, inhibit the TGF-β signal pathway, and reduce reactive oxygen species (ROS).[5] Although autologous MSCs/progenitors have been suggested to have some potential to trigger regenerative mechanisms in the postinfarction period, the invasiveness of the collection procedure (e.g., bone marrow stem cells [BMSCs]) and tenderness of the donor site, as well as the low proliferation rate (for progenitor cells), remain serious shortcomings.[7] Therefore, interest has shifted to cells that can be obtained in a less invasive way, that are highly expansive, can differentiate or transdifferentiate into all relevant phenotypes, and, importantly, can modulate regenerative processes through paracrine activity. Therefore, the possibility of using autologous stromal vascular fraction (SVF) from adipose tissue is of considerable interest to researchers. SVF transplantation, which does not require cell culture outside the human body, is a safe and effective regenerative technology with the potential to be applied in a wide range of clinical specialties.[8] In this study, we present the possibilities of using SVF in the recovery of the heart after MI (acute MI and chronic MI). In the following sections we consider the advantages and disadvantages of using this type of cell therapy.

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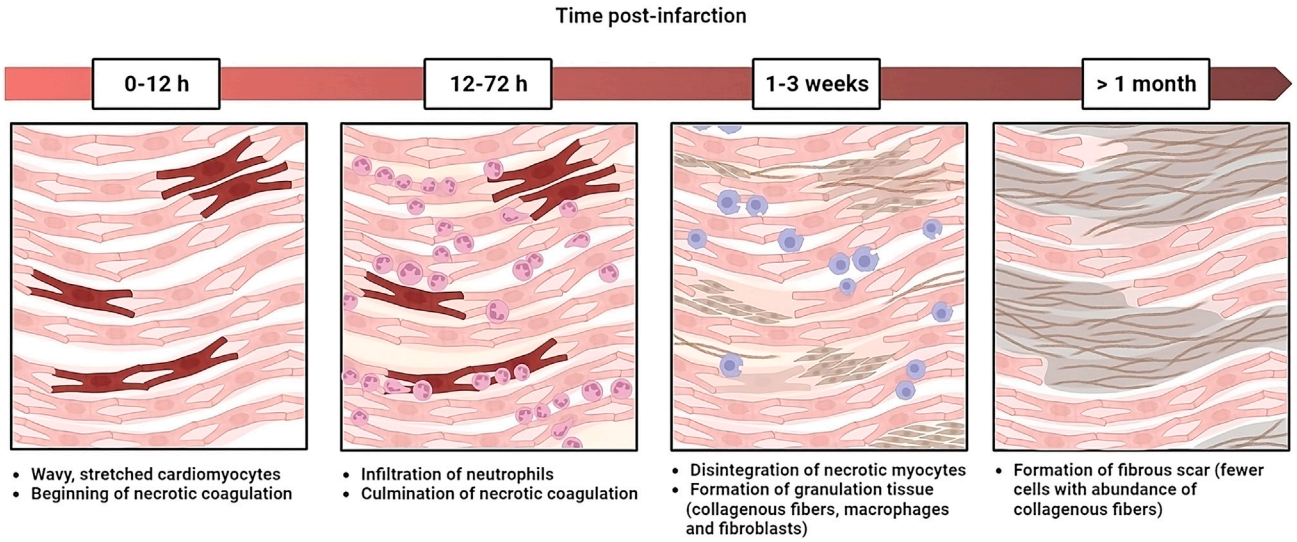

Figure 1. Schematic illustration of histological change in the myocardium after ischemic injury

In addition, primary risk factors for coronary heart disease (CHD), such as arterial hypertension (AH), diabetes mellitus (DM), and metabolic syndrome, can also lead to diffuse myocardial fibrosis. Normally, fibroblasts maintain extracellular matrix (ECM) homeostasis, which provides the structural framework for cardiomyocytes, distributes mechanical forces across cardiac tissue, and conducts electrical potential. However, compared with other organs, the heart has a low regenerative potential, and therefore the processes of repair of cardiac tissue during ischemia consist in the breakdown and absorption of cardiomyocytes that have undergone necrosis, followed by the formation of pathological fibrous tissue by cardiac fibroblasts to preserve the structure and prevent myocardial rupture [3]. At the same time, fibroblasts differentiate into myofibroblasts for accelerated secretion of ECM proteins (Figure 2) [4]. Thus, both primary and postinfarction myocardial fibrosis are characterized by excessive deposition of ECM synthesized by cardiac fibroblasts, which accelerates the progression of chronic heart failure (CHF) [1–3].

CELLULAR SUBPOPULATIONS AND CHARACTERISTICS OF SVF

Adult adipose tissue represents an alternative source of available autologous adult stem/progenitor cells. Adipose tissue can be easily obtained using a standard liposuction procedure under local anesthesia, and lipotransfer techniques are widely used in modern plastic surgery.[8,9] Of considerable interest to researchers is the possibility of using freshly isolated SVF from adipose tissue, where SVF contains a heterogeneous cell population that is interesting from a biological and clinical point of view. Currently, preclinical and clinical studies are being conducted to study the safety, cellular composition, and effectiveness of SVF in the treatment of various human diseases, including diseases of the cardiovascular system.[8] SVF is a rich source of not only stem cells, but also other cell types, such as endothelial cells, preadipocytes, fibroblasts, mast cells, macrophages, T and B lymphocytes (Figure 3).[10,11]

Among these cells, most attention has focused on the characteristics and functions of adipose tissue-derived stem cells (ADSCs). The number of stem cells that can be isolated from adipose tissue is 100–1000 times greater than that contained in an equivalent volume of bone marrow. In addition, ADSCs are genetically more stable over a long period of cultivation, have a lower aging coefficient and high proliferative activity.[12] To date, many studies have examined the SVF cell population, but there is still no consensus regarding the specific proportions of cell subpopulations relative to each other (Figure 3).[10,11] This is facilitated by the fact that the composition of SVF depends on many factors, such as the site of adipose tissue release, processing methods and the patient's own pathological status. Criteria for characterizing the cellular contents of the SVF using combinations of surface antigens (cluster of differentiation [CD]) are an actively developing area of research. According to research, $CD45^{-}CD235a\text{-}CD31^{-}CD34^{+}$ is a combination of markers to identify the SVF cell population.[13] ADSCs are a critical component of the SVF, accounting, according to various literature sources, from 2 to 80% of the SVF cell population. Within the SVF, ADSCs can be phenotypically identified as $CD45^{-}CD235a\text{-}CD31^{-}CD34^{+}$. Cultured ADSCs can be identified, like MSCs, as $CD13^{+}CD73{+}1CD90{+}CD105{+}CD31^{-}CD45^{-}CD235a$-, plastic adhesive cells with trilineage differentiation potential.[13,14] However, ADSCs differ phenotypically from BMSCs in that they are positive for CD36 and negative for CD106. Interestingly, CD34 expression is found on the surface of the majority of SVF cells (up to 80%), and two days after initial SVF seeding, more than 95% of adherent ADSCs express CD34.[15] But, as in MSCs, CD34 expression in ADSCs is thought to be lost during *in vitro* culture, indicating that culture conditions may influence the physiological phenotype of stem cells.[16] The differences between $CD34^{+}$ and $CD34^{-}$ populations and the importance of CD34 expression for SVF/ADSCs functionality have been widely discussed in many preclinical studies.[17,18]

FEATURES OF ADIPOSE TISSUE FOR ISOLATING THE SVF

Adipose tissue in the human body is usually divided into subcutaneous and visceral fat, which can be obtained through liposuction or surgical resection. Materials from which adipose tissue can be harvested include the abdomen, buttocks, forearms, or groin.[19] In general, methods for

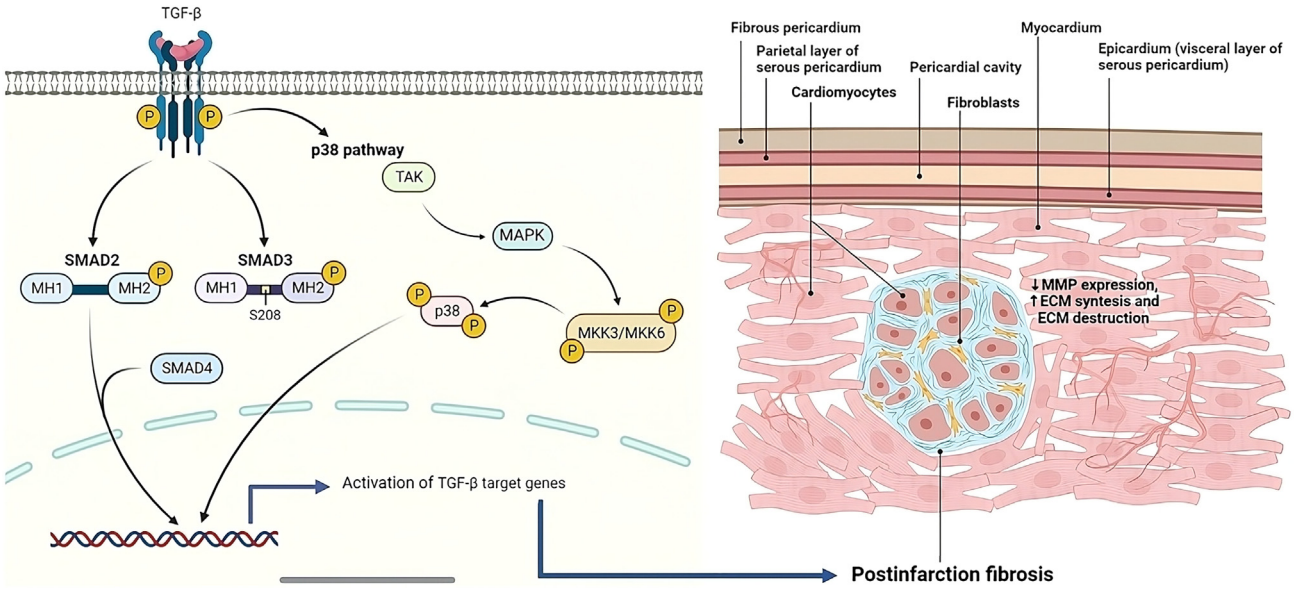

Figure 2. The role of the transforming growth factor β (TGF-β) signaling pathway in cardiac fibrosis

TGF-β regulate phenotypes of the cells through activation of mothers against decapentaplegic (SMAD)-related pathway together with p38 pathway. Direct effects on fibroblast-to-myofibroblast conversion and activation may likely be of greater importance, but TGF-β-induced fibrogenesis may be further related to its effect on macrophage phenotype, lymphocyte differentiation and function, and cardiomyocyte viability (see Figure 1) as well as activation of TGF- β targets genes like matrix metalloproteinases (MMPs). Note: ECM, extracellular matrix; TAK, transforming growth factor-β-activated kinase; MAPK, mitogen-activated protein kinase; MKK3/MKK6, mitogen-activated protein kinase kinase 3/6; MH1/MH2, DNA-binding domain 1/2.

isolating SVF can be divided into two categories: enzymatic methods, which use proteolytic enzymes to break down adipose tissue, and physical and mechanical processing methods, which do not use proteolytic enzymes.[20,21] Enzymatic methods often use type I collagenase to break down fat tissue. Physical and mechanical breakdown of adipose tissue, including methods such as serum digestion, mechanical shaking, and bolus shifting. Compared with enzymatic methods, this digestion method takes longer, and the cell yield and activity are not good enough; thus, it is not widely used.[20,21] Therefore, the most common method for isolating SVF is based on the enzymatic breakdown of adipose tissue. Although the methods for isolating SVF from adipose tissue by enzymatic digestion are quite varied, they follow a certain standard procedure. The differences lie mainly in the number of washing steps, enzyme concentrations, centrifugation parameters, erythrocyte lysis methods, and filtration and ultimately culture conditions. Good Manufacturing Practice (GMP) grade collagenases are produced by recombinant bacteria and are typically delivered in lyophilized form. Their potency varies by batch and their purity varies by manufacturer. However, enzyme concentrations are usually reported as percentage by weight per volume (w/v), which results in unevenness between different isolations, even if the same protocol is used. Concentrations reported in current literature range from 0.075% (w/v) to 0.3% (w/v).[22,23] Typically, protocols include an erythrocyte lysis step to eliminate erythrocyte contamination and reduce the number of cells of hematopoietic origin. After another optional SVF washing step, the resulting substrate is either cryopreserved or cultured in propagation medium. The fraction of plastic-attached cells, including ADSCs, can be obtained after several passaging or cryopreservation or further cultivation to increase the number of a more homogeneous population of ADSCs.[24] For instance, Haack-Sørensen et al. compared the cultivation of ASCs from stromal vascular fraction (SVF) over two passages in the automated and functionally closed Quantum Cell Expansion System (Quantum system) with traditional manual cultivation. In results, the Quantum system clearly favored cell proliferation and yield from very small initial SVF seeding densities.[26] However, although both fresh and cultured SVF cells can promote neovascularization, the vessel density of the vasculature formed by cultured SVF is lower than that of fresh SVF. In addition, fresh SVF can form more small capillary-like vessels than cultured SVF.[25,26]

However, issues to consider include differences in the sites of release as well as how the isolated adipose tissue is processed. In addition to regional anatomical differences in fat composition, for example, the molecular nature of abdominal adipose tissue is different from adipose tissue taken from the medial thigh, and there are also differences between brown adipose tissue and white adipose tissue.[19,27] Some studies have demonstrated that SVF isolated from white adipose tissue contained more hematopoietic cells, macrophages, hematopoietic progenitor cells, and immature cells, which together contributed to a higher degree of plasticity than the cell population of SVF isolated from brown adipose tissue.[28,29] White adipose tissue is not strictly limited to subcutaneous areas. White adipose tissue concentrated around internal organs, also called visceral adipose tissue, is often found in excessive amounts in obesity and metabolic disorders.[19] Increased visceral fat stores may also increase the risk of developing cardiovascular disease, digestive disorders, and some urological diseases, to name a few.[30] Given that excessive visceral fat storage is detrimental, it is reasonable to ask whether SVF isolated from visceral fat, compared with SVF from subcutaneous adipose tissue, is equally harmful or dysfunctional. Findings from O'Rourke and Benencia et al. suggest that SVF from visceral adipose tissue promotes inflammation, potentially due to a higher proportion of macrophages, natural killer cells, and T cells compared with SVF from subcutaneous fat.[31,32]

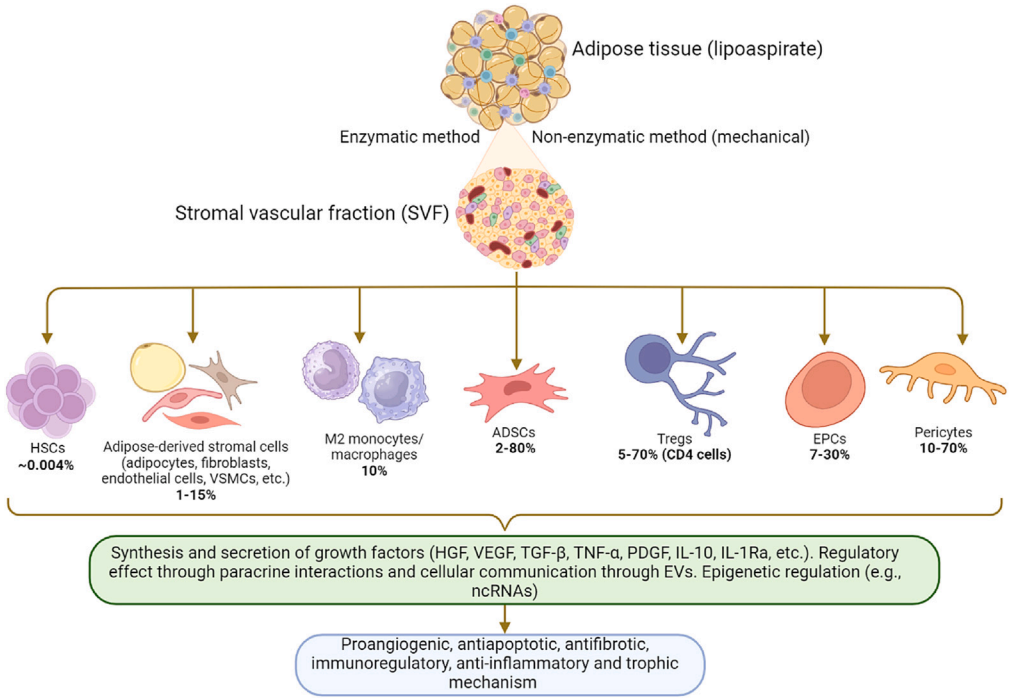

Figure 3. Cell population of the stromal-vascular fraction (SVF) and their percentage

SVF is a dynamic population of cells with potentially significant utility in clinical medicine. SVF is a heterogeneous population of cells that, interacting with each other, can affect the processes of regeneration, angiogenesis, and immunomodulation. The mechanisms of synergistic interaction of SVF cells are mainly through the paracrine effect and cellular communication through extracellular vesicles (EVs), such as exosomes, where, through the synthesis and secretion of various growth factors and epigenetic regulation (e.g., non-coding RNAs [ncRNAs]) a positive effect on tissues and organs occurs. Note: HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor alpha; PDGF, platelet-derived growth factor; IL-10, interleukin 10; IL-1R, interleukin-1 receptor; HSCs, hematopoietic stem cells; VSMCs, vascular smooth muscle cells; ADSC, adipose derived stem cells; Tregs, regulatory T cells; EPCs, human endothelial progenitor cells.

SVF OR ADSCs?

As mentioned earlier, SVF is a heterogeneous cellular mixture of different cell populations, with ADSCs occupying a special position. ADSCs have a multipotent differentiation capacity, where they can differentiate toward adipogenic, osteogenic, chondrogenic, and myogenic lineages.[33] ADSCs-based therapies are showing promising results in the field of tissue engineering and for a range of different diseases in the field of aesthetic and regenerative medicine, bone and cartilage regeneration, cardiovascular diseases, reconstructive urology, as well as in the regeneration of chronic wounds, such as Crohn's disease.[34–36] As is already known, a stem cell is characterized by the ability to self-renew and multipotency. The progenitor cell has a limited proliferation potential but is still capable of differentiating into one or more specific cell types. Although often used interchangeably, ADSCs and SVF are separate but related entities, each with different inherent attributes. Located in perivascular areas along capillaries and between adipocytes, ADSCs consist of a major cell population that contributes to the differentiation of adipocytes for grafted fat. The grafted fat at the recipient site is subject to ischemia during the first 24 h as it is transplanted without its inherent blood supply and is nourished solely by plasmatic imbibition.[37] A number of studies have shown that adipocytes are the most susceptible to ischemia among various cell subpopulations and undergo apoptosis with subsequent formation of 3 different histological regions of fat: (1) the survival zone (the most superficial layer), which is located within 300 000 μm from the periphery; (2) a regeneration zone (intermediate layer) with a thickness of 600 000 μm–1 200 000 μm, and (3) a necrosis zone (central part), where adipogenesis does not occur.[34–36] The ADSCs subpopulation, however, survives 36 h longer than the adipocyte subpopulation and provides therapeutic benefits in a variety of ways.[38]

ADSCs are known to secrete a variety of paracrine growth factors and angiogenic factors such as vascular endothelial growth factor (VEGF), which promotes vasculogenesis and endothelial tubulogenesis in an ischemic environment. These results were supported by an *in vivo* report showing that human ADSCs promote diabetic wound healing, where the treatment group demonstrated abundant new vessel formation and better tissue remodeling rather than fibrotic scarring due to increased plasma and tissue VEGF levels [36–38, 41, 42].[34–36,39,40] ADSCs also have an immune function by inhibiting T cell proliferation and proinflammatory cytokines through prostaglandin E2 blockade, thereby reducing cell damage and improving overall adipose tissue viability.[15] However, the use of SVF may be more effective than the use of ADSCs alone, where this effectiveness is achieved due to the synergy between SVF cells. Wherein, SVF-based therapy is usually performed in a single stage and intraoperatively, and paracrine signaling of various cell types contained in the SVF along with ADSCs may be more

effective than solely administering ADSCs alone.[41] SVF secretes significantly greater amounts of various soluble signaling factors, such as the angiogenic interleukin 8 (IL-8), macrophage inflammatory protein 1α and 1β, as well as reduced levels of the proinflammatory cytokines interferon gamma (IFN-γ) and interleukin 12 (IL-12) compared with single ADSCs.[42] In contrast, VEGF, interleukin 7 (IL-7), and anti-inflammatory cytokines such as interleukin 10 (IL-10) and interleukin 13 (IL-13) are released from BMSCs at higher levels.[43] In addition, providing a clinical-grade product is a critical requirement for the use of ADSCs or SVF. Unlike SVF, ADSCs depend on expansion *in vitro*, which is associated with such problems as possible risks of transformation of cells into tumor cells or loss of stemness.[43] Moreover, the use of SVF during a surgical procedure in an autologous and homologous manner is not considered an advanced therapy medicinal products (ATMPs) (European Medicines Agency, 2012). However, multiple incubation steps of collagenase and red cell lysis buffer, as well as centrifugation and filtration may negatively impact cell efficiency.[44] Unlike ADSCs, SVF is much easier to obtain, without the need for any cell separation or special culture conditions. Thus, the cellular product is obtained instantly and has minimal contact with reagents, which makes it comparatively safer. It should be noted that, while ADSCs are used in both allogeneic and autologous transplantation methods, SVF, due to the presence of various types of cells in its composition that cause immunological rejection of the graft, is suitable only for autologous transplantation methods.[45]

ROUTE OF ADMINISTRATION, DOSAGE, AND BASIC CELL CHARACTERISTICS

Numerous considerations come into play when it comes to delivering cells to the heart as a means of treating various cardiac conditions. These considerations encompass the type and severity of the cardiac injury, the optimal timing for administering the treatment, and the ability of the delivered cells to survive and function effectively. Among these factors, the choice of delivery route is a pivotal aspect that significantly impacts the distribution, retention, survival, and overall therapeutic effect of cell-based therapy. To achieve successful outcomes, it's imperative that a delivery strategy is not only effective but also straightforward to implement, ensuring both the survival of the administered cells and the acceptance of the treatment by patients. Various delivery techniques have been explored in both preliminary clinical trials and animal models.[46,47] These approaches can be broadly categorized into two main categories: systemic and local delivery. Within the realm of local delivery, researchers have investigated several specific routes for delivering SVF/ADSCs as a therapeutic intervention for MI. These routes include (Figure 4): (1) intrapericardial (IPC) delivery. This method involves the introduction of cells into the pericardial sac, either through needle injections or with the aid of specialized devices. IPC delivery offers a localized approach for treating cardiac conditions, and it comes with its own set of advantages and disadvantages; (2) epicardial (EC) delivery. A modification of IPC delivery, the EC approach entails attaching a controlled release device directly to the epicardial surface of the heart. This technique provides a more targeted delivery while mitigating some of the challenges associated with IPC delivery; (3) intramyocardial (IMC) delivery. In this method, cells are introduced into the myocardial muscle through needle injections. IMC delivery offers a direct and localized approach, but it also has its unique considerations and limitations; and (4) intracoronary (IC) delivery. This approach involves administering SVF/ADSCs directly into the coronary arteries. IC delivery offers a systemic route while still being targeted to the heart. Each of these delivery routes has its own set of advantages and disadvantages, and the choice of which one to use should be tailored to the specific clinical scenario and patient needs. The Table 1 summarizes information about the possible advantages and disadvantages of a particular method of delivery of cellular components during MI treatment.[48–58] The selection of the most suitable delivery method is crucial for optimizing the therapeutic potential of SVF-based treatments for MI and other cardiac ailments. Understanding the nuances of each approach is essential for achieving the best outcomes in cardiac cell therapy.

Determining the most effective dosage of SVF for therapeutic benefits is a persistent challenge. MI, which results in the loss of roughly 1 billion cardiomyocytes, highlights the need for substantial cell therapy doses. However, studies exhibit a wide range of SVF dosages administered to patients, varying from as low as 1×10^6 to as high as 2×10^8 cells.[59] This variation is notably lower than the number of cells lost due to MI. Adding to the complexity, the engraftment and survival rates of transplanted cells are often suboptimal, suggesting that higher cell doses might be necessary to effectively regenerate the damaged myocardium. Nevertheless, concerns arise when considering administering high cell quantities, as this can lead to the formation of cell aggregates, which in turn increase the risk of arrhythmias, a potentially dangerous heart rhythm disorder.[60] Remarkably, clinical trials have presented a paradoxical observation: lower cell doses may be more effective than higher doses.[61] This counterintuitive outcome is potentially linked to the heightened paracrine effects of the administered cells, the release of protective factors, the induction of angiogenesis, and the stimulation of cardiomyocyte hypertrophy (an increase in the size of heart muscle cells).[62] This phenomenon underscores the intricate nature of SVF-based therapies and emphasizes that therapeutic success depends on factors beyond just the quantity of cells. As SVF cell-based therapies progress into human clinical trials, establishing the optimal cell dosage for clinical effectiveness becomes imperative. For instance, Carstens et al., focused on chronic, nonhealing diabetic foot ulcer (DFU) (>3 cm2) in a population of patients with type 2 diabetes mellitus (T2DM) and underlying microangiopathy.[63] Based on the observed clinical responses to even the lowest dose of SVF and considering the smaller injection area in the current study, the authors used a fixed cell dose of 30×10^6 cells for this study. This dose was shown to be safe and demonstrated clear efficacy with closure response rates among the patients evaluated between 86% and 93% at the 6- and 12-month endpoints. However, the exact dose-response relationship for these therapies remains inconclusive, underscoring the need for extensive research to determine the most effective cell dosages for cardiac regeneration. This ongoing quest for the right cell dosage is vital for ensuring the success and safety of SVF-based therapies in the treatment of heart-related conditions.

Maijub et al. conducted a study that introduced the intriguing hypothesis that the development of blood vessels from SVF is intricately linked to the concentration of these cells.[64] Their research involved a comprehensive array of *in vitro* and *in vivo* experiments, with the primary objective of establishing a clear connection between the dosage of SVF cells and their efficacy in fostering the formation of blood vessels. Their findings brought to light that a maximum concentration of 4×10^6 cells per milliliter appeared to support vasculogenesis without any untoward effects, such as the emergence of necrotic zones or the formation of undifferentiated cell masses. However, while the overall

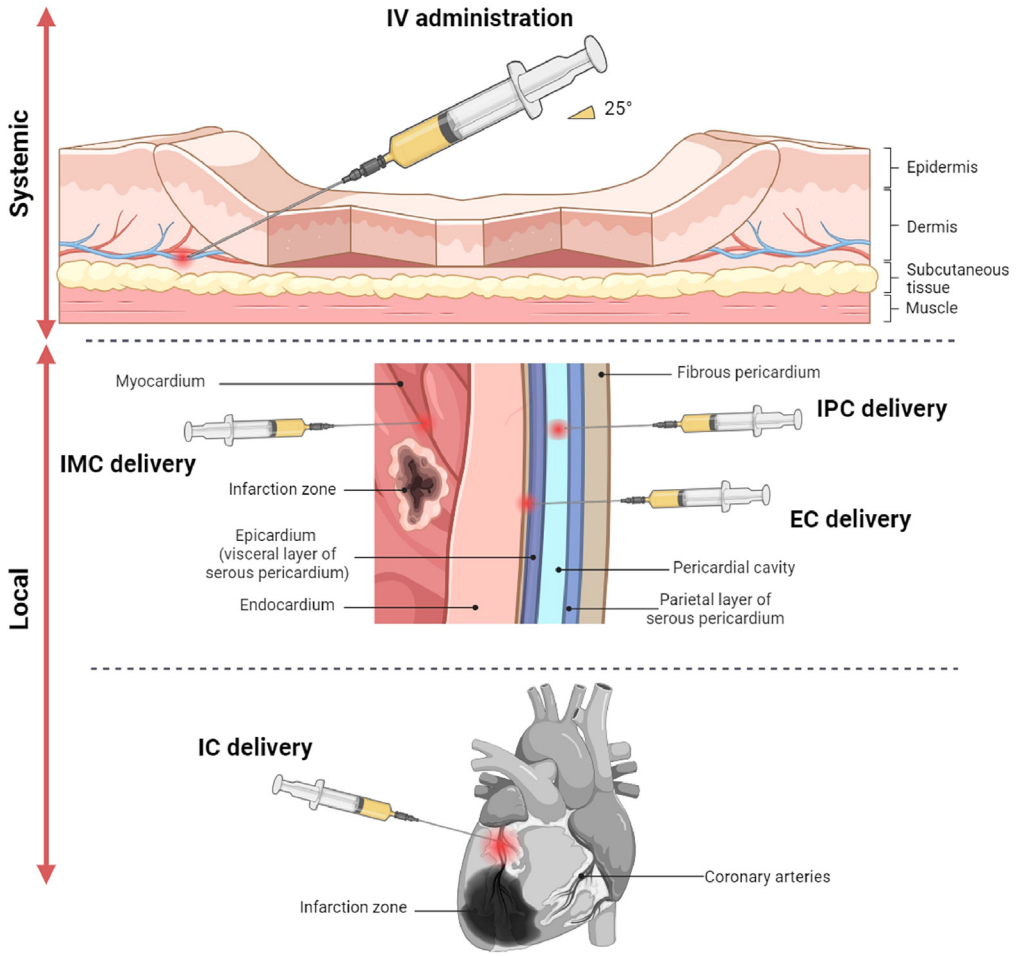

Figure 4. Systemic and local delivery of stromal vascular fraction (SVF)/adipose-derived stem cells (ADSCs) for repairing injured myocardium, promoting myocardium regeneration, revascularization, and improving cardiac function

Major techniques for SVF/ADSCs administration, such as intravenous (IV), intracoronary (IC), intramyocardial (IMC), epicardial (EC), and intrapericardial (IPC) administration. The IC delivery technique has been widely used mainly in the following ways: antegrade intracoronary infusion (AICI) and retrograde coronary venous infusion (RCVI).

length of the vascular network did not exhibit a significant statistical difference compared to lower concentrations, there was an indication of a declining trend in vasculogenic effectiveness. Of note, the study observed a fascinating temporal discrepancy when it came to the process of forming new blood vessels following the implantation of human SVF. This process displayed a delay of roughly two weeks when juxtaposed with the outcomes seen with rat-derived SVF. Even with the utilization of the highest cell dosage, namely 4×10^6 cells/ml, the human SVF-driven vasculogenesis was characterized by a slower pace in contrast to previous investigations involving rat SVF.[65] While the precise mechanisms underpinning this species-specific variance in the rate of vasculogenesis remain elusive, it is conceivable that the relative cellular composition of SVF varies between rats and humans. Furthermore, the specific growth factors and cytokines needed to support vasculogenesis likely differ between these two species. However, what's unequivocal is that in both rat and human scenarios, the study unequivocally demonstrated that SVF cell populations play a pivotal role in dose-dependent vasculogenesis *in vivo*. This research strongly endorses the notion that achieving the reconstruction of a fully functional microcirculation through cell transplantation is undeniably reliant on the cell dosage employed. This understanding of the factors dependent on dosage is pivotal for the advancement of therapeutic approaches involving SVF in the realm of vascular tissue regeneration.

The research conducted by Karina et al. has made a substantial contribution to our understanding of SVF-based therapy, affirming its feasibility, effectiveness, and overall safety as a therapeutic approach.[66] This autologous method, which harnesses SVFs derived from a patient's own adipose tissue, demonstrated exceptional tolerability, with no instances of patient complaints or discomfort reported. Their study involved a thorough analysis of 421 patient records, offering a comprehensive overview of the safety profile of SVF-based therapy. Notably, the study provided intriguing insights into the administration of SVF therapy, particularly with respect to IV injections. It was revealed that SVF-based therapy could be effectively delivered with a concentration of fewer than 10 billion SVFs in 250 mL of normal saline. Similarly,

Table 1. Routes of administration of the stromal-vascular fraction (SVF)/adipose-derived stem cells (ADSCs) as regenerative therapy for myocardial infarction (MI)

Route of administration	Advantages	Disadvantages	Reference
1) Intravenous	- Minimally invasive; - Ease of implementation; - No need for recovery after the procedure; - Applicable in the acute MI	- Donor cells can be caught up in the liver, lungs, and spleen; - Requires several infusions, which could lead to tremendous cell loss; - Needs a high-dose that is more likely to induce a systemic immune response; - Not applicable for chronic period MI (e.g., HF);	Sim et al., Kahlon Tang et al., Yamaguchi et al.,[48,49,50,51]
2) Intracoronary	- Minimally invasive; - Relatively safe; - Homogeneous distributions of injected cells in the target area	- Larger cells compositions and higher doses will induce significant microvascular occlusion; - Difficulty to perform in small animals; - Inefficient remuscularization; - There is a need for specialized gear like OTW angioplasty balloon catheters	Kahlon et al., Li et al., Bartunek et al.,[49,52,53]
AICI	- Homogeneous distribution of cells at the target zone;	- Not applicable for chronic period MI; - Have the risk of occlusion or emboli; - Donor cells have poor myocardium attachment due to high blood flow rate in the coronary artery	
RCVI	- Venous system is generally fully open, whereas the inflow artery is generally occluded in most MI patients; - Can be used in any hospital	- May not be suitable for patients with vulnerable coronary sinus and having a high risk of sinus rupture; - Have the risk of occlusion or emboli; - Low evidence base for the effectiveness of the method	
3) Intrapericardial	- High retention rate and high target cell distribution pattern in ischemic area; - Availability 3D MRI-electromagnetic fusion mapping; - Small risk of perforation and arrhythmias	- Invasive; - Availability of specialized equipment and conditions; - Low evidence base for the effectiveness of the method	Kahlon et al., Blázquez et al.,[49,54]
4) Epicardial	- Most reliable delivery method; - Possibility of use on small animals	- Invasive; - Possibility of leakage; - Cells can only be delivered once a time during surgery; - Lack of dosage control; - Reduce cell engraftment (in the tissue with hostile immunoreactive, ischemic or necrotic environment)	Kahlon et al., Araña et al., Hamdi et al.,[49,55,56]
5) Intramyocardial	- High cell concentration in the targeted area with negligible washout - Possibility of use on small animals; - Less or no risk of embolization	- Invasive; - Difficulty in distinguishing between the infarcted and normal myocardium; - Not applicable for chronic period MI (postinfarction fibrosis); - Poor mechano-electrical coupling of donor cells after injection; - Higher incidence of arrhythmias	Kahlon et al., Otto Beitnes et al., Park et al.,[49,57,58]

HF, Heart failure; OTW, Over-the-wire; MRI, Magnetic resonance imaging; AICI, Antegrade intracoronary infusion; RCVI, Retrograde coronary venous infusion.

for intra-articular and spinal injections, the recommended SVF concentration was less than 1 billion SVF. This careful dosing approach emphasizes the importance of aligning the volume of saline solution used for IV injections and the infusion rate with the SVF cell count. This meticulous calibration is pivotal in ensuring the well-being of the patient. Conversely, the research also shed light on potential risks associated with SVF concentrations exceeding 20 billion cells, underscoring the need for cautious dosing to avert potential harm. Furthermore, the investigation unearthed crucial insights regarding the safety of SVF-based therapy. It revealed that, in the rare instances where adverse events occurred, they were typically low-risk, transient, and manageable, falling well within the range of anticipated outcomes. This reassuring discovery solidifies the safety profile of SVF therapy as a viable clinical intervention. In summary, Karina et al.'s research has advanced our knowledge of SVF therapy, highlighting its practicality and safety. It underscores the importance of precise dosing and careful consideration of SVF concentrations to ensure patient well-being, while also reassuring the medical community about the therapy's overall safety and efficacy.

In a distinct clinical investigation led by Simunec et al. the study focused on patients grappling with grade 3 and 4 osteoarthritis.[67] These patients, on average, received approximately 7.5×10^6 SVF, and the injected volumes ranged from 7 to 37 mL. What made the results particularly intriguing was that regardless of the volume of SVF cells injected, there was a consistent pattern indicating that fewer of these cells were associated with greater improvements in the Knee Injury and Osteoarthritis Outcome Score (KOOS). This curious relationship between cell quantity and the degree of clinical enhancement raises intriguing questions and underscores the intricacies inherent in SVF-based therapy. Looking ahead, it's imperative to stress that while the safety and effectiveness of SVF-based therapy have been affirmed in specific medical contexts, there remains a wealth of uncharted territory concerning the implications of SVF concentration and the total volume of injection in post-MI scenarios. As the field of SVF therapies continues to evolve and expand, it becomes increasingly critical to further refine our comprehension of these dosage-dependent factors. This ongoing exploration is vital for optimizing the safety and efficacy of SVF-based therapy across a wide spectrum of clinical situations, ultimately contributing to advancements in the realm of regenerative medicine.

The challenges of cell retention, engraftment, and survival represent significant obstacles in the domain of cardiac stem cell therapy. Both in preclinical and clinical trials, it has become evident that in the initial 24 h post-administration, cell retention in the heart rarely surpasses the 10% mark.[68,69] This diminished retention rate can be ascribed to the swift clearance of injected cells and their limited capacity to effectively integrate into the cardiac tissue. Multiple contributing factors exacerbate this challenge, including the inflammatory milieu in the heart following an MI, the phagocytosis of cellular remnants, and the struggles encountered by donor cells in withstanding the high mechanical forces within the recipient heart. These factors collectively contribute to substantial cell loss during stem cell therapy. Acknowledging the gravity of these challenges, efforts have intensified to devise strategies aimed at enhancing cell retention. Various innovative approaches have come to the forefront to tackle this issue. Some of these methods involve sealing the injection site with fibrin compounds to prevent the retrograde flow of injected cells, the transplantation of engineered cell sheets, and the use of natural or synthetic polymers to enhance the retention, engraftment, and overall survival of the administered cells.[70] Furthermore, the issue of cell rejection has gained prominence, particularly when the cell source is allogeneic, originating from a different individual. Mitigating the risks associated with rejection has led to a shift toward utilizing cells that necessitate minimal or no immunosuppressive therapy. SVF-derived cells, often autologous and derived from the patient's own adipose tissue, have garnered attention as they appear to offer a reduced risk of immunological rejection. As the field of cardiac stem cell therapy advances, novel strategies continue to emerge, each aimed at elevating acute cell retention, diminishing the likelihood of rejection, and ultimately improving the long-term efficacy of this therapeutic approach. Addressing these challenges remains pivotal for fully realizing the potential of cell-based therapies in the treatment of cardiac conditions, a development that holds great promise for the future of cardiovascular medicine.

MECHANISM OF THERAPEUTIC ACTION OF SVF

As is already known, the cellular and molecular restructuring of the myocardium, from the acute to the chronic period, which occurs in response to ischemia, is undoubtedly the basis for the development of complications such as chronic heart failure (CHF) and cardiac remodeling. At the same time, there is a complexity and many mechanisms with direct and feedback that determine the inflammatory response in response to ischemia, the transition of inflammation to healing, changes in the intercellular matrix and scar formation, the processes of regeneration and angiogenesis. Many attempts have been made to determine the most effective treatment method that could have a positive effect on important links in the post-infarction period, namely angiogenesis, inflammation, and fibrosis. And this is where cell therapy comes in, namely the possibilities of SVF therapy. SVF may well act in a variety of ways, and its biological activity may be determined by the microenvironment of the host tissue. In general, SVF is considered to have a proangiogenic, antiapoptotic, antifibrotic, immunoregulatory, anti-inflammatory, and trophic mechanism of action (Figure 5).[10,11] In this chapter, we will consider the possibilities of SVF cell population therapy for the main pathogenetic mechanisms of MI, both in acute and chronic conditions. Table 2 summarizes the results of all studies that assessed the therapeutic application of SVF in post-MI condition and MI-related diseases.[71–85]

STIMULATION OF ANGIOGENESIS IN POST-MI CONDITION

In cases in which therapy to activate angiogenic pathways is critical, such as MI, the SVF cell population may serve as a platform for angiogenesis, since they are an important source of cellular phenotypes associated with blood vessels, including pericytes (adventitial location), endothelial progenitor cells (EPCs) (luminal location), ADSCs, supra-adventitial adipose stromal cells (surround the vessel as a membrane), and endothelial cells.[10,11] In the SVF cell population, ADSCs not only occupy a relatively large percentage of cells, but also have a high differentiation capacity. ADSCs are pluripotent stem cells that can differentiate directly into endothelial cells, smooth muscle cells (SMCs), and pericytes.[19] ADSCs regulate vascular growth, stabilization, and maturation by activating the expression of TGF-β, angiopoietin-2,

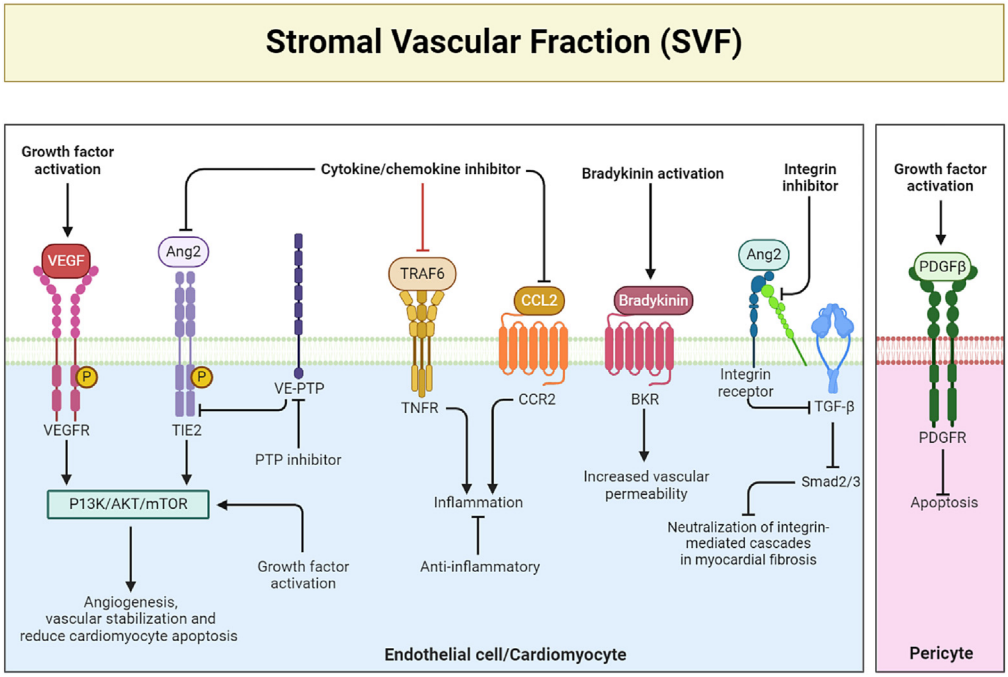

Figure 5. Schematic illustration of the mechanism of action of the stromal vascular fraction (SVF) through certain receptors and signaling pathways in post-myocardial infarction (post-IM) period

This illustration demonstrates only some points in the therapeutic effect of SVF on cardiomyocytes, coronary artery endothelial cells and their pericytes, demonstrating the potential regenerative effect, such as activation of angiogenesis, reduction of cell apoptosis, and inflammation. Note: VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Ang2, angiotensin 2; P13K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; VE-PTP, vascular endothelial-protein tyrosine phosphatase; TRAF6, TNF receptor associated factor 6; CCL2, C-C motif chemokine ligand 2; CCR2, C-C motif chemokine receptor 2; TNFR, tumor necrosis factor receptor; BKR, β-keto acyl carrier protein (ACP) reductase; TGF-β, transforming growth factor β; PDGFβ, platelet-derived growth factor β; PDGFR, platelet-derived growth factor receptor A.

platelet-derived growth factor (PDGF), neurogenic locus homolog protein (Notch), and sphingosine 1-phosphate (S1P)/G-linked protein (EDG) signaling pathway.[19] In addition, pericytes not only promote the emergence of EPCs but also maintain vascular integrity to form the vasculature.[11] Several studies have shown that during SVF transplantation, new microvessels are formed with the participation of host endothelial cells to form a stable vascular network system.[86,87] The SVF cell population can effectively secrete a large number of proangiogenic and antiapoptotic factors, such as hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), VEGF, and TGF-β.[88,89] In addition, ADSCs treated with anti-VEGF antibodies lose their proangiogenic ability in ischemic tissues *in vivo*.[73] The SVF cell population can secrete a large number of cytokines (tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), etc.) through a paracrine effect, and these active substances can accelerate the healing of the damaged area and the formation of new blood vessels.[86] This indicates that cytokines secreted by the SVF cell population may promote angiogenesis.

VEGF and TNF-α produced by SVF induce host EPCs and endothelial cells to secrete various cathepsins, which can inhibit EC apoptosis and stimulate their proliferation and migration.[89] On the other hand, there is evidence that the formation of a new vascular network under the influence of SVF can not only be a consequence of a direct stimulating effect on existing cells (host cells), but also develop due to the so-called disassembly and reassembly of the vascular network.[90,91] It is known that adipose tissue contains a huge number of blood vessels, the destruction of which occurs during the preparation of SVF (disassembly of the vasculature). Then, when SVF is introduced into the damaged area, the disconnected cells of the vascular wall are able, together with the existing blood vessels of the host, to create a hybrid vascular network (assembly). This is further evidence that the relationship between the SVF cell population and host cells provides relevant therapeutic potential.

It is known from preclinical and clinical studies that therapies using BMSCs, or amniotic/placenta stem cells have already been used in acute MI; however, culture and expansion of these stem cells takes too long for patients with acute MI, for whom rapid treatment is critical.[92] Accordingly, an innovative method that could quickly provide an adequate number of "working cells" without cell culture and be effective in the treatment of acute MI would be of great clinical significance. As stated previously, SVF can be used immediately and, without the need for cell culture, accelerates wound healing through angiogenesis. Indeed, adipose tissue-derived fresh heterogeneous populations of undifferentiated mononuclear elements enriched in SVF based on CD antigens in these multipotent are emerging as a simple and safe method for the treatment of acute MI or chronic MI. For instance, Sheu et al. demonstrated the potential therapeutic effect of SVF on improving left ventricle (LV) function and inhibiting LV remodeling in rat after acute MI.[71] The authors demonstrated that angiogenesis was one of the most important factors in preserving cardiac function and LV myocardial architecture after the use of SVF. In addition, their results showed that the

Table 2. List of preclinical and clinical experiments on the study of the regenerative effect of the stromal-vascular fraction (SVF) during myocardial infarction (MI) and related heart pathologies

Direction	Study model	Isolation methods	Route of administration	Dosage, cells	Therapeutic factors	Biological effect	Reference
AMI	Male SD rats	Enzymatic	IMC	1.2×10^6	CXCR4, VEGF and SDF-1α,	Angiogenesis, anti-apoptotic, fibrotic anti-fibrotic effects	Sheu et al.,[71]
AMI	Male SD rats	Enzymatic	EC	1×10^6	VEGFR2, HGF and VEGF	Sustains coronary microvascular function, angiogenesis	Leblanc et al.,[72]
CMI	SD eGFP rats, rat SMCs and murine ECs	Enzymatic	IMC	1.15×10^6	VEGF, HGF, MCP-1 and TIMP1/4	Promotes a persistent benefit in cardiac function and metabolism by inducing tissue revascularization and protection against deleterious tissue remodeling	Mazo et al.,[73]
CMI	Male syngeneic Lewis rats	Enzymatic	IMC	1×10^6	VEGF, MMP1, TIMP1, and IL-6	Angiogenesis, anti-inflammation, fibrotic anti-fibrotic effects	Premaratne et al.,[74]
AMI	Male SD rats	Enzymatic	IMC	1×10^5	PGC-1α, MMP-9, TNF-α, IL-1β, NF-κB, NOX-1/2, Bax, caspase 3, PARP, Smad3 TGF-β, eNOS, CD31, VEGF, CXCR4, and SDF-1α	Preserves LVEF and inhibits LV remodeling. inhibitions of inflammatory reaction, fibrosis, apoptosis, DNA damage, generation of oxidative stress, and promote angiogenesis	Sung et al.,[75]
AMI	Human fat and neonatal rat ventricular cardiomyocyte	Enzymatic	Cultured in a 3D perfusion bioreactor system	–	HGF and IGF	Recover hypoxia-induced loss of cardiomyocyte function	Mytsyk et al.,[76]
HF	SD rat's fat cardiomyocytes	Enzymatic	gelatin/mTG hydrogel culture	–	GATA-4, MEF2C, alpha-actinin-2, TNNI3, and GJA1	Demonstrated spontaneous beating behavior, cellular calcium transient activity, and pharmacological response like native cardiomyocytes	Yang et al.,[77]
AMI	GFP-tagged adult male Fischer-344 rats	Enzymatic	EC	1×10^6	VEGF	Prevents or halts the worsening of cardiac function and targeted coronary vascular perfusion in the infarct area, allowing sustained coronary viability	Leblanc et al.,[78]
MVD	Female Fischer-344 rats	Enzymatic	IV	1×10^6	DRP-1, MFN-1 and ETC	Prevention acute MI and cardiac remodeling. Ameliorates aging-induced mitochondrial ROS coinciding with recovered microvascular function	Tracy et al.,[79]
IC	Clinical study, phase II/III	Enzymatic	IMC	3×10^6 -5×10^6	ADSCs and growth factors	All procedures were well tolerated, safe, and feasible. In addition, patients injected with SVF may have preserved ventricular function, myocardial perfusion, and exercise capacity	Comella et al.,[80]

(*Continued on next page*)

Table 2. *Continued*

Direction	Study model	Isolation methods	Route of administration	Dosage, cells	Therapeutic factors	Biological effect	Reference
RA	Case report	Enzymatic	IMC and IV	1×10^6 and 2×10^6	ADSCs, DKK-1 and ID proteins	Prevention acute MI and cardiac remodeling. LVEF was improved to 35% with recovery in the contractility.	Khalpey et al.,[81]
AMI	Male Wistar rats	Enzymatic	IV	1×10^5	ADSCs and growth factors	Reduces infarct size and improves cardiac function. Differentiation occurs in relation to cardiomyocytes, and activation of angiogenesis	van Dijk et al.,[82]
MVD	Female Fischer-344 rats	Enzymatic	IV	1×10^7	β-adrenergic receptor and growth factors	Prevention acute MI. improves coronary microvascular function (vasodilatation)	Rowe et al.,[83]
CR	Human fat and HUVECs	Enzymatic	Co-culture of SVF and HUVECs	–	CASP1	Prevention cardiac remodeling. SVF transplantation shows differential effects according to gender in inflammatory and angiogenetic properties	Lim et al.,[84]
CMI	Male Lewis rats	Enzymatic	IMC	5×10^6	VEGF, Ang-1 and HGF	Prevents LV remodeling post MI, induces new capillary and arteriole formation	Schenke-Layland et al.,[85]

AIM, Acute myocardial infarction; CMI, Chronic myocardial infarction; RA, Recurrent angina; CR, Cardiac remodeling; IC, Ischemic cardiomyopathy; MVD, Coronary microvascular disease; SD rats, Sprague-Dawley rats; IV, Intravenous; EC, Epicardial; IM, Intramyocardial; HF, Heart failure; GFP, Green fluorescent protein; HUVECs, Human umbilical vein endothelial cells; LV, Left ventricle; LVEF, Left ventricular ejection fraction; ADSCs, Adipose-derived stem cells; ROS, Reactive oxygen species; SMCs, Smooth muscle cells; EC, Endothelial cells;CXCR4, C-X-C motif chemokine receptor 4; VEGF, Vascular endothelial growth factor; SDF-1α, Stromal cell-derived factor alpha; VEGFR2, Vascular endothelial growth factor receptor 2; HGF, Hepatocyte-growth factor; MCP-1, Monocyte chemoattractant protein-1; TIMP1/4, Tissue inhibitor metalloproteinase 1/4; MMP1, Matrix metalloproteinase 1; IL-6, Interleukin 6; PGC-1α, Peroxisome proliferator-activated receptor-gamma coactivator-1alpha; MMP9, Matrix metalloproteinase 9; TNF-α, Tumor necrosis factor alpha; IL-1β, Interleukin-1β; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NOX-1/2, NADPH1/2 oxidase; Bax, Bcl-2-like protein 4; PARP, Poly(ADP-ribose) polymerase; TGF-β, Transforming growth factor- β; eNOS, Endothelial nitric oxide synthase; IGF, Insulin-like growth factor; GATA4, GATA binding protein 4; MEF2C, MADS box transcription enhancer factor 2; TNNI3, Troponin I3, Cardiac Type; GJA1, Gap junction alpha-1 protein;DRP-1, Dynamin-related protein 1; MFN-1, Mitofusin 1; DKK-1, Dickkopf WNT signaling pathway inhibitor 1; ID proteins, Inhibitor of DNA; CASP1, Caspase 1; Ang-1, Angiopoietin 1; -, Not detected.

angiogenesis capacity was higher in SVFs than in ADSCs. Another important finding was that protein and cellular expression of angiogenesis biomarkers (endothelial nitric oxide synthase (eNOS), CD31, von Willebrand factor (vWF), C-X-C motif chemokine receptor 4 (CXCR4), VEGF, and stromal cell-derived factor (SDF)-1α) and the number of small vessels in cardiac samples were markedly increased in ADSC-treated rats and further increased in SVF-treated rats compared with acute MI rats only. In another study, LeBlanc et al. developed a cardiac construct that can be used for the treatment of acute MI by targeting the coronary microcirculation.[72] In particular, the authors created a three-dimensional adipose SVF cell construct where implanted on the epicardium after acute MI *in vivo*. In results, only acute MI hearts treated with the SVF construct exhibited a sustained coronary microvascular blood flow (BF) reserve 4 weeks after the ischemia and clinical indexes of overall heart function, such as ejection fraction (EF), stroke volume, end-systolic volume (ESV), end-diastolic volume (EDV), were significantly improved in the MI SVF animal group. Layland et al. have suggested that transplanted SVF may secrete autocrine or paracrine factors that could have beneficial effects independent of myocardial regeneration in chronic MI, for instance, by promoting angiogenesis *in vivo*.[85] The authors have examined arteriole density in the healthy, infarct and border zones. SVF-treated animals showed significantly more arterioles per mm^2 within the border zones and infarcted myocardium than the control animals. The clinical potential of adipose tissue-derived SVF is high, as this platform may not only serve as an excellent therapy to promote microvascular survival and/or new vessel growth after MI but can also be used to restore microcirculation in ischemic tissue in various cardiomyopathies (see Table 2).

ANTIFIBROTIC AND ANTI-INFLAMMATION EFFECTS

It is known that morphological changes in organs or tissues, such as fibrosis, develop because of impaired microcirculation and the development of hypoxia. In addition, the aseptic and chronic inflammatory process, accompanied by hypoxia, leads to active fibrosis of the injury site, which suggests possibilities the use of SVF in the treatment of chronic MI, accompanied by an inflammatory process, impaired trophism and blood circulation.[1,2]

SVF, isolated from connective tissue associated with subcutaneous fat and blood vessels, is known to contain ADSCs, T regulatory cells (Tregs), anti-inflammatory M2 macrophages, and numerous growth factor cytokines.[93] The process of myocardial recovery after ischemia is a complex cascade of molecular and cellular events that results in the replacement of damaged or dead cells and remodeling of the ECM to restore normal tissue.[1,2,94] In this regard, the acute wound healing response can be divided into hemostasis, inflammation, activation and proliferation of collagen-producing cells, tissue remodeling and resolution. This process is fundamental to survival; however, it can become pathological if the resolution phase is not completed. In this case, remodeling progresses to exaggerated and uncontrolled ECM deposition. This leads to the formation of permanent scar tissue or fibrosis, in which inflammation, tissue destruction and repair processes simultaneously occur. Excessive accumulation of ECM gradually alters the architecture of normal myocardium, impairing cardiac function and ultimately leading to CHF and cardiac remodeling.[1,2,94]

There are no effective treatments to stop and reverse fibrosis, and organ transplantation is the only treatment for many fibrotic conditions. Numerous experimental studies support the concept of anti-inflammatory, immunomodulatory, and potential antifibrotic properties of SVF.[10,11] Despite the aforementioned promising mechanistic potential concerns regarding functionality, the fate and role of SVFs in a pro-fibrotic microenvironment remains to be resolved, which hinders their widespread clinical application. However, anti-inflammatory cytokines, immune system cells, and certain growth factors in the SVF may speed up healing and reduce the excess collagen deposition activity associated with chronic MI. One study showed that SVF produced a statistically significant long-term (3 months) improvement in cardiac function and tissue metabolism, which was associated with increased revascularization and positive cardiac remodeling, with a significant reduction in infarct size, inhibition of fibrosis, and less cardiac hypertrophy *in vivo*.[73] In addition, the SVF cell population has been demonstrated to release angiogenic (VEGF and HGF) and proinflammatory (monocyte chemoattractant protein-1 [MCP-1]) cytokines, as well as tissue inhibitor metalloproteinase 1/4 (TIMP1/4), *in vitro* and *in vivo*, strongly suggesting that they have a trophic effect. These results demonstrate the potential of SVF to promote regeneration of ischemic tissue and provide long-term functional benefit in an animal model of chronic MI through both direct and indirect mechanisms.

As mentioned previously, the biological effect of SVF is also associated with its immunological and anti-inflammatory properties. The monocyte/macrophage compartment (approximately 10% of cells based on $CD14^+$ expression) expresses IL-10 and interleukin 1Ra (IL-1Ra), which is consistent with M2 polarization and exhibits anti-inflammatory activity. In addition, interbreeding of different cell phenotypes can modulate inflammation; for example, SCATs inhibit dendritic cell activation, suppressing subsequent adaptive immunity through the generation of Tregs involved in maintaining macrophages in the M2 phenotype. In the SVF cell population, more than 90% of macrophages are of the M2 type. M2 type macrophage can secrete anti-inflammatory factors such as interleukin-4 (IL-4), IL-10, TGF-β, thereby suppressing the inflammatory response. Premaratne et al. demonstrated the protective effects of SVF transplantation and the anti-inflammatory role of transplanted SVF after implanted into a rat chronic MI.[74] They find that IMC injection of SVF was effective in enhancing neovascularization, inhibiting collagen deposition, and reducing gene expression of inflammatory cytokines such as TNF-α, IL-6, TIMP-1, and pro-brain natriuretic peptide (BNP) as well as inflammatory cells CD3, in rat chronic IM. In results, SVF transplantation improved cardiac function, attenuated LV dilation, and thus prevented further myocardial remodeling. These data suggest that transplantation of SVF might be a useful therapeutic option for antifibrotic and anti-inflammation direction in MI (see Table 2).

SVF/ADSCs-DERIVED EXOSOMES

Among the variety of substances secreted by SVF cells into the external environment (intercellular space), extracellular vesicles (EVs) (mainly exosomes) play a special role. Being enclosed in a membrane like the membrane of the cell itself, they can carry both small portions of

ordinary cytoplasmic contents and completely defined sets of biologically active molecules.[10,11] Exosomes measuring 30–100 nm are formed from early endosomes, from which they receive several membrane proteins, such as proteins of the major histocompatibility complex, receptors, tetraspanins, etc. Proteins, RNA and DNA, enter exosomes from the cytoplasm of the mother cell, with the help of adenosine triphosphate (ATP)-dependent transport. The main function of exosomes is the ability to transport information from donor cells to recipient cells (target cells).[95,96] One of the compensatory reactions of the myocardium, developing in response to its ischemic and reperfusion injury, is the synthesis of exosomes by cardiomyocytes, EC and SMCs, heart stem cells and fibroblasts.[97–99] Exosomal synthesis has a paracrine function and is aimed at ensuring intercellular interaction within the ischemic zone, causing cardioprotection and triggering regenerative processes. The paracrine effects of synthesized exosomes include increased proliferation and migration of endothelial cells and a decrease in apoptosis in the hypoxic zone. An increase in the permeability of the endothelial layer, which precedes the migration of endothelial cells, is ensured by exosome-mediated inhibition of the synthesis of the tight intercellular junction proteins zonula occludens-1 (ZO-1) and claudin-5 in them.[100,101] At the same time, the proteins (e.g., HSP70) and nucleic acids (e.g., non-coding RNAs) contained in exosomes work as an additional modulator of intercellular interaction in the process of myocardial repair after MI.[96,98]

In recent years, a large volume of research has been devoted to exosomes of various stem cells, such as MSCs, heart stem cells, and embryonic stem cells, and their effect on the myocardium during ischemic damage.[102] However, the results of using SVF-derived exosomes in the treatment of MI are rarely reported in the scientific literature. However, an important role for ADSCs-derived exosomes in myocardial recovery after ischemia *in vitro* and *in vivo* has already been demonstrated (Table 3); exosomes and their contents help regulate this process and promote myocardial regeneration after MI (Figure 6).[103–114] Of particular importance are microRNAs, the transport of which from exosomes to target cells can change the biological functions of the latter by regulating the expression of target genes in them. Exosomal miRNAs derived from ADSCs have been repeatedly shown in *in vitro* and *in vivo* studies to demonstrate a cardioprotective role after myocardial ischemic injury.[105,106,108,109,112,113]

As for other cells from the general SVF cell population, endothelial cells also secrete exosomes with a certain set of factors (for example, VEGF, and TGF-β), and neighboring endothelial cells (coronary arteries) can act as target cells for binding to these exosomes, which as a result, promotes growth, migration, and neovascularization. Endothelial cells can activate the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway by increasing the expression of chemokine ligand 1 (CXCL-1), inducing epidermal growth factor (EGF) secretion, and promoting angiogenesis.[115,116] Therefore, the effectiveness of SVF application is achieved due to the synergy between SVF cell populations. However, further research is needed to clarify the therapeutic effect of SVF in general through the mechanisms of transfer of secreted molecules by exosomes between cell populations of transplanted SVF and host cells in MI.

PERSPECTIVES AND LIMITATIONS

Recently, in basic experiments using animal models, it was found that SVF transplantation suppresses myocardial infarction through regeneration of cardiac muscle through direct differentiation of transplanted cells into cardiac muscle cells, as well as through stimulation of angiogenesis and suppression of fibrosis and inflammation caused by the paracrine effect of the produced growth factors and cytokines. Currently, only preclinical trials are being conducted on SVF transplantation for IF through various routes of administration. However, many issues remain to be addressed, such as, for example, issues related to transplantation, such as the physical costs associated with collection, the cost and human effort involved in culturing cell cultures (when isolating individual cells from SVF, such as ADSCs), and the required time until cell transplantation.

Clinicians/researchers should be aware of the regulatory requirements and quality control measures that must be implemented to obtain reliable data and ensure safety when used in a clinical setting. Important factors to consider are the composition of SVF, the dosing schedule, the route of administration appropriate for each specific treatment, and adequate sterility control protocols. One of the major challenges in bringing SVF-based therapeutics into the clinic is obtaining clinically acceptable levels of SVF cells with minimal manipulation. It is important to identify and control all possible factors that may affect the safety and quality of SVF, which should be done in accordance with current GMP guidelines.[117] Isolation of SVF requires that adipose tissue be harvested by liposuction and transported to a GMP-compliant laboratory for further processing.[117] The various steps of SVF isolation generally include washing of adipose tissue to remove blood cells, enzymatic digestion, and centrifugation to extract SVF.[44] Therefore, manual processing requires expensive infrastructure and qualified specialists, which are not available in most clinics. These challenges have been largely overcome by efforts to develop fully automated point-of-care devices that can separate SVF from adipose tissue in a highly quality controlled and consistent manner.

Cell counting and cell viability assessment are critical for the correct use of SVF for therapeutic purposes. For a "working sample" of SVF, it is necessary to isolate a minimum number of viable cells whose viability is above a certain level (usually $\geq$70%). If SVF isolation does not meet predefined sample batch release criteria, then therapy should not be initiated or continued. The number of cells and their viability are indicators of the efficiency of the isolation process by one or another method. It is important to know the average cell yield (number of cells per gram of tissue) as different methods and/or systems may produce different results.[118]

For the therapeutic use of SVF in clinical settings, one of the most important quality control and patient safety measures is infection control. Elevated endotoxin levels may be an indicator of severe bacterial contamination of the sample during the isolation process. In addition, endotoxins may be present in SVF samples because of the choice of method using proteolytic enzymes, since the enzymes are usually of bacterial origin. If an enzymatic method of SVF isolation is used, where collagenase is used, there is also a possible risk of residual proteolytic enzymes in the final product. The toxicity of residual enzymes to the SVF cell population is not fully understood, but there is a potential for an allergic reaction or unwanted tissue degradation if not properly removed.[119,120]

Table 3. List of studies that demonstrated the myocardial regeneration after myocardial infarction (MI) with exosomes from adipose tissue-derived stem cells (ADSCs)

Study model	Exosome contents	Activating factors	Route administration	Effect	Reference
In vivo (male SD rats) and *in vitro* (H9c2 cells, cardiac fibroblasts, and HAP)	–	SK1/S1PR1	IV	Decreases myocardial fibrosis and apoptosis and promotes macrophage M2 polarization	Deng et al.,[103]
In vivo (male SD rats) and *in vitro* (H9c2 cells)	–	Bcl-2, Bax, Caspase 3, Wnt/β-catenin signaling	IV	Antiapoptotic and pro-survival effects on cardiomyocytes	Cui et al.,[104]
In vivo (C57BL/6JNifdc mice0 and *in vitro* (CP-M138 and CP-M073)	miR-671 mimic	TGFBR2/Smad2 axis	IMC	Decreases myocardial fibrosis and apoptosis.	Wang et al.,[105]
In vivo (male C57BL/6J mice) and *in vitro* (primary NRVMs)	miR-214 mimic	Bcl2l11 and Slc8a1	IMC	Antiapoptotic and pro-survival effects on cardiomyocytes	Eguchi et al.,[106]
In vivo (male SD rats) and *in vitro* (NRCMs)	–	VEGF, bFGF, and HGF	IMC	Suppresses the apoptosis of cardiomyocytes and promotes the angiogenesis	Xu et al.,[107]
In vivo (male SD rats) and *in vitro* (H9c2 cells)	miR-146a mimic	EGR1/TLR4/NF-κB axis	IMC	Suppresses the apoptosis, inflammatory response, and fibrosis	Pan et al.,[108]
In vivo (male SD rats) and *in vitro* (H9c2 cells)	miR-126 mimic	IL-1β, IL-6, and TNF-α	IV	Prevent cardiomyocyte ischemia-induced mitochondrial dysfunction and reactive oxygen species production, increases angiogenesis, and promotes macrophage M2 polarization. Reduces and reverses myofibroblast activation and decreases collagen expression	Luo et al.,[109]
In vivo (wild type and CXCR7$^{-/-}$ mice) and *in vitro* (PBMCs) and EPCs)	–	IRT1 and CXCR7	IMC	Positive effect on the recovery and reconstruction of cardiac function, anti-inflammation effect and promotes the angiogenesis	Huang et al.,[110]

(*Continued on next page*)

Table 3. *Continued*

Study model	Exosome contents	Activating factors	Route administration	Effect	Reference
In vivo (male SD rats)	–	NOX-1/2/4, mitochondrial-Bax/caspase 3/PARP/p53/ cytosolic-cytochrome-C, IL-1β/TNF-α/NF-κB/MMP-9, PI3K/Akt/GSK3β *p*-mTOR, SIRT1/3, IL-10, and IKB-α/p-AMKP/mitochondrial-cytochrome-C	IC	Prevent cardiomyocyte ischemia-induced mitochondrial dysfunction and reactive oxygen species production, increases angiogenesis, and promotes macrophage M2 polarization. Reduces and reverses myofibroblast activation and decreases collagen expression	Chai et al.,[111]
In vivo (C57BL/6 wild-type mice) and *in vitro* (HMEC-1 cells and NRCMs)	miR-205 inhibitor	HIF-1α and VEGF	IMC	Reduces myocardial fibrosis and inhibits myocardial apoptosis	Wang et al.,[112]
In vivo (C57BL/6 wild-type mice) and *in vitro* (H9c2 cells)	miR-221/222 mimic	PUMA, ETS-1 and AKT/NF-κB	IMC	Suppresses the apoptosis, inflammatory response, and fibrosis	Lai et al.,[113]
In vivo (male SD rats *in vitro* (HL-1 cells)	circ_0001747	miR-199b-3p/MCL1 signaling	–	cardio-protective activity against hypoxia/ reoxygenation-mediated damage	Zhou et al.,[114]

SD rats, Sprague-Dawley rats; NRVMs, Ventricular cardiomyocytes from neonatal rats; PBMCs, Peripheral blood mononuclear cells; EPCs, Endothelial progenitor cell; IV, Intravenous; IM, Intramyocardial; IC, Intracoronary; S1P, Sphingosine-1-phosphate receptor 1; SK1,Sphingosine kinase 1; S1PR1, Sphingosine-1-phosphate receptor 1; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-like protein 4; TGFBR2, Transforming growth factor (TGF) beta receptor 2; Bcl2l11, Bcl2-like 11; Slc8a1, Solute carrier family 8 member A1; VEGF, Vascular endothelial growth factor; bFGF, basic fibroblast growth factor ; HGF, Hepatocyte-growth factor; EGR1, Early growth response protein 1; TLR4,Toll like receptor 4; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor alpha; IRT1, Iron-regulated transporter 1; CXCR7, C-X-C motif chemokine receptor 7; NOX-1/2/4/, NADPH1/2/4 oxidase; PARP, Poly(ADP-Ribose) polymerase 1; IL-1β, Interleukin-1β; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; MMP9, Matrix metalloproteinase 9; PI3K, Phosphoinositide 3-kinases; Akt, Protein kinase B; GSK3β, Glycogen synthase kinase-3 beta; *p*-mTOR,Phosphorylated mammalian target of rapamycin; SIRT1/SIRT3, Sirtuin 1/3; IL-10, Interleukin-10; IKB-α, I-kappa-B-alpha; *p*-AMKP, Phosphorylated AMP-activated protein kinase; HIF-1α, Hypoxia-inducible factor-1 alpha; VEGF, Vascular endothelial growth factor; PUMA, p53-upregulated modulator of apoptosis; MCL1, Myeloid cell leukemia 1.

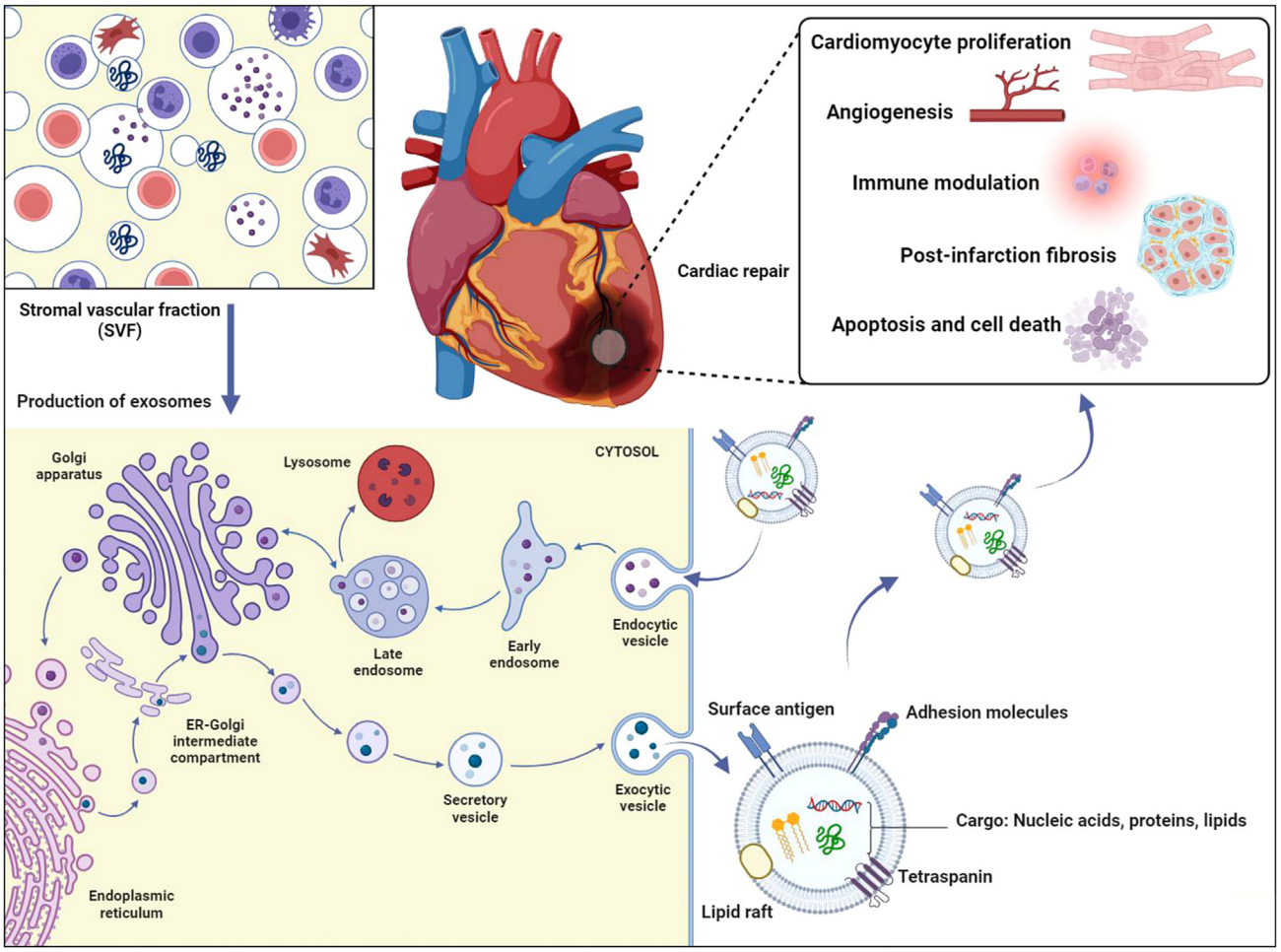

Figure 6. Therapeutic potential of stromal vascular fraction (SVF) cell population-derived exosomes as an effective therapeutic tool for myocardium regeneration after ischemia

SVF-derived exosomal cargo act on the repair process of ischemic heart injury.

In addition to regional anatomical differences in fat composition, for example, the molecular nature of abdominal adipose tissue is different from adipose tissue taken from the medial thigh, and there are also differences between brown adipose tissue and white adipose tissue.[121] Some studies have demonstrated that SVF isolated from white adipose tissue contained more hematopoietic cells, macrophages, hematopoietic progenitor cells, and immature cells, which together contributed to a higher degree of plasticity than the cell population of SVF isolated from brown adipose tissue. White adipose tissue is not strictly limited to subcutaneous areas. White adipose tissue concentrated around internal organs, also called visceral adipose tissue, is often found in excessive amounts in obesity and metabolic disorders. Increasing your visceral fat stores can also increase your risk of developing cardiovascular disease, endocrine disease, and digestive disorders, to name a few. Given that excessive visceral fat storage is detrimental, it is reasonable to ask whether SVF isolated from visceral fat, compared with SVF from subcutaneous adipose tissue, is equally harmful or dysfunctional.[122] Some studies have demonstrated that SVF from visceral adipose tissue promotes inflammation, potentially due to a higher proportion of macrophages, natural killer cells, and T cells compared with SVF from subcutaneous fat. Also, SVF isolated from the omentum or even from the serous fluid of the peritoneum was rich in T cells and $CD45^{+}$ leukocytes, respectively.[93,123]

Recent years have seen increasing recognition of the influence of age and gender in the field of cell therapy research. Indeed, the National Institutes of Health has now mandated the inclusion of these biological variables in all funded research under its recently approved "rigor and transparency" guidelines. Recent studies show that the regenerative properties of SVF decline with age. Liu et al. demonstrated a decrease in colony-forming unit (CFU) and SVF cell yield in elderly patients compared with middle-aged patients.[124] Age comparisons of C57Bl/6 mice in the study by Frazier et al. show that SVF obtained from inguinal and epididymal white adipose tissue, as well as dorsal interscapular adipose tissue from younger males, contained a significantly higher percentage of preadipocytes, hematopoietic stem cells and $CD25^{-}$, FoxP3+ Tregs compared with SVF from mice middle age.[125]

The SVF, with its rich diversity of cell types offers a multifaceted approach to tissue regeneration and healing in MI. The successes reported by Hamdi et al. and others in using SVF cells for repairing cardiac tissue in animal models highlight the cells' capacity not only for improving survival rates post-MI but also for encouraging the formation of new blood vessels, a process critical for the recovery of ischemic tissues.[56] The challenge, however, lies in harnessing the full therapeutic potential of these cells. The variability in cell composition, which can vary widely from one preparation to another and across different passages in culture, poses a significant obstacle in standardizing treatments and understanding the mechanisms at play. This variability can influence the efficacy of SVF cell-based therapies and complicates the replication of successful outcomes across different studies. To overcome these challenges, it is essential to explore innovative strategies that can optimize the regenerative capacity of SVF cells. The three proposed methods aim to address some of the key issues in the field: (1) using freshly isolated SVF cells ensures that the cells are as close to their natural state as possible, potentially enhancing their regenerative abilities; (2) freshly isolated cells may better mimic the cellular interactions and signaling pathways that occur *in vivo*, leading to more effective tissue repair and regeneration; и providing an appropriate growth environment for the cells is crucial for maintaining the viability and functional properties of the diverse cell subpopulations within the SVF. This involves not only the physical scaffolding that supports three-dimensional tissue formation but also the biochemical signals that guide.

Despite the superiority of SVF compared to the ADSCs described previously, there are few studies comparing these two different types of adipose tissue-derived cells to effectively treat MI. More research is needed not only to study the effectiveness of SVF in the treatment of MI, but also to conduct systematic studies in comparison with ADSCs therapy. Nevertheless, based on the previous discussion, the interrelations between each subpopulation of SVF contribute to the process of neovascularization and more significant immune modulation, and SVF may be better than ADSCs at improving LVEF after AMI.

The growing number of clinical studies using SVF represents a shift from studying cultured and homogeneous cell populations to a heterogeneous mixture of SVF cells. Clinical trials registered on ClinicalTrials.gov are investigating the use of a heterogeneous population of SVF cells in the treatment of various human diseases: musculoskeletal diseases, trauma, autoimmune diseases, etc. Ongoing clinical trials, most of which are in Phase 1 or Phase 2, are seeking demonstrate the safety and effectiveness of SVF therapy in humans.[8] Considering the promising results of these clinical studies on the use of SVF in therapy for various pathologies, they prove to us that SVF has great potential for use as cell therapy in patients after MI.

Conclusion

Thus, many details aimed at cardiac recovery after MI in both acute and chronic periods during SVF transplantation have not been established. At the same time, it can be assumed with a certain degree of confidence that the implanted SVF will perform a supporting function, limiting post-infarction cardiac remodeling, and the intrinsic contractility of the implanted cells can improve systolic function. Also, the secretion of growth factors and cytokines with a paracrine effect from the SVF stimulates angiogenesis and inhibits postinfarction fibrosis, cell apoptosis, and the inflammatory process, which certainly improves the recovery of cardiac function after MI. In general, despite rapid progress, many fundamental issues of SVF-based therapy for MI remain unresolved and to date there is no convincing clinical data proving its effectiveness, which requires additional research and observations.

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AUTHOR CONTRIBUTIONS

I.G.: conceptualization, writing – original draft, writing – review and editing, and project administration. O.B.: investigation, resources, and data curation. T.I. and A.A.: validation and visualization. V.C. and H.S.: project administration and supervision. H.S.: funding acquisition. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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