

Review Article

Therapeutic applications of adipose-derived stem cells in cardiovascular disease

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Abstract: Cardiovascular disease (CVD) is the number one cause of death globally, and new therapeutic techniques outside of traditional pharmaceutical and surgical interventions are currently being developed. At the forefront is stem cell-centered therapy, with adipose derived stem cells (ADSCs), an adult stem population, providing significant clinical promise. When introduced into damaged heart tissue, ADSCs promote cardiac regeneration by a variety of mechanisms including differentiation into new cardiomyocytes and secretion of paracrine factors acting on endogenous cardiac cells. We discuss the application of ADSCs, their biochemical capabilities, availability, ease of extraction, clinical trial results, and areas of concern. The multipotent capacity of ADSCs along with their ability to secrete factors promoting cell survival and regeneration, along with their immunosuppressive capacity, make them an extremely promising approach in the field of CVD therapy.

Keywords: Adipose derived stem cells, cardiovascular disease, myocardial infarction, regenerative medicine

Introduction

Cardiovascular disease (CVD) encompasses issues pertaining to the heart and associated blood vasculature including stroke, arrhythmia, valvular disease, and at the extreme, myocardial infarction [1]. One common cause of major CVD is atherosclerosis, a narrowing of the vasculature due to buildup of fatty plaque [2]. The narrowing of the vessel limits the ability of oxygen-rich blood to travel through the body, affecting flow to the heart and associated tissues. Myocardial infarction (heart attack) results from blocked arteries in the heart causing muscle tissue death and can result in heart failure [2]. Severe atherosclerosis can lead to coronary artery disease, kidney disease, or embolism, complications that can be exacerbated by risk factors such as hypertension, diabetes, obesity, smoking, or poor diet and exercise habits [2]. Smoking, hypertension, and hyperlipidemia are the main risk factors for CVD, and almost half of all Americans currently possess one of the three [3]. Currently CVD is the leading cause of death in the U.S. for both sexes, over 610,000 individuals die each year due to

heart disease and stroke in the U.S. alone, with 33% of those deaths being preventable with simple lifestyle changes [3].

Due to the importance of proper blood flow, heart attacks are traditionally treated with pharmacological or surgical interventions [2]. These methods work to either dissolve atherosclerotic clots or reroute vessels for increased flow to hypoxic regions, respectively [2]. Although heart attacks are not always fatal, an initial attack greatly increases the chances of a second [3]. Over 28% of the 735,000 Americans who have a heart attack each year have already had one, indicating that traditional treatments are not always effective [3]. However, more recent approaches involve the use of stem cells, which may have additional advantages. Using these cells, the goal is to not only save viable cardiac tissue, but to regenerate damaged or dead myocardium, preventing or reducing inflammation that leads to scarring and long-term impairment [4]. Given the increased incidence of heart disease worldwide and our increased understanding of stem cell therapies for CVD, we discuss recent findings

from the literature along with clinical data, and also recommendations for next steps.

Stem cell research

A stem cell is a non-specialized cell that has two capabilities. First, it has the potential to undergo multiple cell divisions in order to expand clonally within the body. Additionally, it can differentiate from an unspecialized cell to one that performs a specific functional role [5]. Stem cell types currently under investigation include embryonic, cardiac progenitor, bone marrow, induced pluripotent, and adipose-derived stem cells (**Table 1**).

While embryonic stem cells (ESC) have strong regenerative and pluripotent capacities, meaning they can differentiate into any cell type from any germ layer, they are the most ethically controversial [6]. This moral dilemma lies in the invasive method required to harvest ESCs, which involves destroying human embryos without consent from the developing embryo itself. Strict governmental regulations greatly limit the amount of research that can be performed with these cells, which is limited to specific pre-established lines [6]. Furthermore, because these stem cells originate from a different person, transplanting them to a recipient comes with serious risk of immune rejection [6]. These patients are required to be put on a myriad of immunosuppressive medications for the rest of their lives to allow the embryonic stem cells to survive.

Another stem cell type that has been explored is the cardiac progenitor cell (CPC), which has been shown to have the inherent ability to differentiate into cardiac tissues including cardiac muscle, vascular, and endothelial cells [7]. The issue surrounding this type of stem cell is the availability of the cells themselves. CPCs are not found in large quantities in healthy hearts. In fact, there are very few CPCs unless the heart has suffered some kind of injury, which even then induces only moderate proliferation [7]. There are also many factors that can affect the functionality of CPCs, such as the age of the heart or any pre-existing diseases in the donor or recipient. The implications of these predisposing factors in regards to the therapeutic performance of CPCs have not been studied further [7].

Bone marrow-derived mesenchymal stem cells (BM-MSC) are a well-characterized adult stem cell type. This population encompasses several cell precursors, including hematopoietic and skeletal progenitor cells. Current research indicates that these cells can induce repair of scarred cardiac tissue, likely through paracrine effects, but the exact mechanism of this repair is still being investigated [8-10]. Because of the predominate effects of paracrine signaling, researchers have questioned the ability of BM-MSCs to fully differentiate into cardiomyocytes *in vivo* [9]. However, some research suggests these cells form a donor BM-MSC/recipient cardiomyocyte hybrid, which accounts for some of the tissue repair [9]. Differentiated and hybrid cells likely have different therapeutic potential, so more research needs to be done to better predict the outcome of this treatment [9]. Additionally, there are serious risks to the *in vitro* expansion of BM-MSCs, such as contamination and potential immunogenicity associated with exposure of stem cells to animal-based supplements [11]. This cell type is also difficult to harvest, producing limited cell numbers, viability, and low extraction rates [11].

Induced pluripotent stem cells (iPSC) are another category of stem cells, created from the somatic cells of donors induced to re-assume stem cell fates. These iPSCs have the same benefits as CSCs and BM-MSCs in that they originate from the patient themselves, avoiding the problem of immune rejection. iPSCs have been specifically shown in CVD to differentiate into cardiomyocytes and improve ventricular remodeling and function [12]. However, there are many challenges facing iPSCs, including current methods of extraction and processing being tainted by unwanted cell heterogeneity, cardiomyocytes with poor purity, and an inability to differentiate between highly proliferative and potential teratoma forming iPSCs [12].

Adipose derived stem cells (ADSCs) offer broad therapeutic capacity with similar multipotent capacity to other mesenchymal stem cells [4, 13]. With data from over 130 ongoing clinical trials, ADSC's successful ability has been tested in skeletal repair, multiple sclerosis, myocardial infarction, and beyond [4]. Therefore, ADSCs appear to be the most promising option for CVD therapy. While concerns regarding their limited retention and low survival rates in a car-

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Table 1. Definitions and major advantages and disadvantages of the five main sources of stems cells found in the human body

Name of Cell Type	Definition	Advantages	Disadvantages
<i>Embryonic stem cell</i>	Pluripotent stem cell derived from inner cell mass of blastocyst in embryo	Great pluripotent and regenerative properties	Ethical constraints due to embryo destruction; potential immune rejection during transplant to recipient
<i>Induced pluripotent stem cells</i>	A somatic (adult) cell that is induced to show embryonic cell-like properties	No donor-to-recipient immune activation issues; ability to differentiate into cardiomyocytes	Poor purity; difficulties working with unwanted heterogeneity and teratoma-forming cells
<i>Cardiac progenitor stem cells</i>	Cells in the heart that can differentiate, after injury, into new cardiac-specific cells	Inherent ability to differentiate into cardiac muscle, vascular, or endothelial cells	Extracted in low numbers; unknown potential for interactions with donor and recipient heart conditions
<i>Bone marrow stem cells</i>	A stem cell found in the bone marrow that can form erythrocytes, leukocytes, and platelets	Well-known with multiple cell precursors	Extracted in low numbers; take on fused donor/recipient characteristic; potential contamination issues during <i>in vitro</i> expansion
<i>Adipose-derived stem cells</i>	Cells derived from adipose lipoaspirates	High multipotent potential and no ethical issues; easily harvested with minimal effort	Potentially tumorigenic; limited understanding of mechanisms involved in cardiac repair

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diac environment exist, they provide the most viable therapeutic option, as will be explored further in this review [14]. The major advantages and disadvantages of each stem cell type have been summarized in the accompanying table (Table 1).

Adipose-derived stem cells

In 2002, Zuk et al. published the finding that human adipose tissue is a promising source of mesodermal multipotent stem cells. Obtained from lipoaspirate (liposuction waste) by collagenase treatment and centrifugation to obtain the stromal vascular fraction, these cells, also referred to as processed lipoaspirate (PLA), retain multipotent differentiation capacity [13]. These adipose derived stem cells can be induced to differentiate *in vitro* into different lineages depending on specific factors added to the cell culture media [15]. For example, exposure to 5-azacytidine (a demethylating agent), angiotensin II (Ang-II: a vasoconstrictive peptide hormone), and transforming growth factor beta-1 (TGF β -1: a cytokine involved in cell cycle regulation) produces cardiomyocytes; exposure to insulin-like growth factor-1 (IGF-1: a growth hormone), vascular endothelial growth factor (VEGF: an angiogenic signaling protein), and basic fibroblast growth factor (bFGF: a mitogenic signaling protein) yields endothelial cells; transfection of the *TBX18* gene (of the T-box family) creates pacemaker cells; and treatment with thromboxane A2 (TXA2: a vasoconstrictive signaling molecule) forms vascular smooth muscle cells [14]. Therefore, like cardiac progenitor cells, ADSCs have the inherent ability to differentiate into numerous cells of the cardiovascular system, including cardiomyocytes, vascular smooth muscle cells, pacemaker cells, and endothelial cells [14]. Other hormones can induce differentiation into adipose, bone, cartilage, or even neuron-like lineages [13].

Mesenchymal stem cells derived from adipose tissue, bone marrow, and cord blood all exhibit the same surface antigen markers [16]. Yet a unique gene expression profile has been identified for ADSCs, including several proteins such as N-cadherin, VE-cadherin, cadherin 11, and fibronectin [16]. Proteins characteristic of cellular division are preferentially expressed in ADSCs compared to stem cells derived from bone marrow and cord blood, suggesting a

higher proliferative potential in ADSCs [16]. ADSC functionality is not only limited to successful differentiation but to the paracrine effects, with the cells secreting chemokines such as vascular endothelial growth factor (VEGF) and others [14], which will be discussed later in the review.

Availability, extraction, and isolation of ADSCs

Another important consideration in stem cell research is the clinical applicability of the population of stem cells under investigation. Obtaining a sufficient quantity of stem cells is a concern for both research and clinical cell therapies, and adipose tissue is fortunately characterized by a large pool of stem cells. In fact, the concentration of stem cells extracted from adipose is significantly greater than that obtained from bone marrow: 5% vs 0.01% [14]. West et al. isolated a concentrated population of cells with an average yield of 9.3 million perivascular stem cells (PSC) per 100 mL of lipoaspirate. These PSCs are indistinguishable from conventional mesenchymal stem cells in their differentiation potential [11]. Thus, the high quantities of ADSCs obtainable from processed lipoaspirate makes adipose tissue a promising candidate for extensive stem cell research and therapeutic use in the future. With the increased rate of obesity in the United States and countries around the world, adipose tissue is not in short supply [15]. However, the relationship between body mass index (BMI) and number of ADSCs extracted is debated in the literature. West et al. extracted ADSCs from donors with a broad spectrum of BMIs, and reported no significant differences in the number of viable PSCs obtained with varying BMI, age (between 22-64 years old), or gender [11]. However, other studies have concluded that lipoaspirates from obese patients tend to yield fewer stem cells, and those that are extracted do not differentiate as well [17].

In the U.S. alone, there are roughly 400,000 liposuction surgeries every year [15]. Each liposuction surgery removes on average 100 milliliters to 3 liters of lipoaspirate and ADSCs are found in the stromal vascular fraction, or densest portion, of the lipoaspirate [15]. Lipoaspirate is routinely disposed of after surgery, making adipose a bountiful source of stem cells in otherwise underutilized tissue [15]. Additionally, it

has been determined that the anatomical source region of the adipose does not affect the number of viable cells that can be retrieved. Studies have extracted samples from the knee, abdominal region, and hip, among others [18].

Although a standardized method of obtaining ADSCs during liposuction has not been established, various methods have been used [11]. In one, the region of interest was infused with a cold saline solution alongside 15 cc of adrenaline and 20-30 cc of 0.5% lidocaine per 500 ccs. The tissue was removed using a cannula and syringe [18]. Given the heterogeneity of the population of cells collected, which include inflammatory cells, cellular debris, and fibroblasts, a method of separation was necessary to isolate the regenerative cells. The standard method of isolating ADSCs from adipocytes is through a collagenase digestion followed by centrifugation, which helps separate tissue from water and oil layers. Expansion of ADSCs occurs *in vitro* in basal medium supplemented with 10% fetal bovine serum medium or human platelet lysate [4].

Of the processing methods currently under investigation, it has become clear that liposuction-derived ADSCs have numerous benefits compared to the process currently used for obtaining other stem cells types. For example, the method by which BM-MSCs are collected is extremely painful and invasive and is accompanied by a low yield of viable stem cells [11, 14]. However, the processing of BM-MSCs is well-established and in accordance with the Good Manufacturing Practice (GMP) compliant conditions, despite being expensive, labor intensive, and time-consuming [11]. There is a need to streamline an efficient protocol for the collection, processing, and delivery of ADSCs for therapeutic uses. One recent approach used a computerized optimization technique to implant differentiated ADSCs intraoperatively into radiolesions, which are ischemic tissue in various regions of the body resulting from radiation damage (typically from external oncologic radiation therapy) [18]. In this study an imaging algorithm simulated the potential tissue growth pathways, enabling workers to identify key points for targeting by injection [18]. ADSCs were able to increase the regeneration and perfusion of these damaged tissues, leading to an alleviation of clinical symptoms in some cases [18].

Therapeutic uses of ADSCs

Myocardial infarction leads to the death of myocardial tissue, which can result in reduced left ventricular function and eventual heart failure [14]. There have been numerous studies in animal models demonstrating the ability of ADSCs to improve cardiac function and induce repair [19-23]. The sources of these improvements fall into three categories: differentiation of stem cells into new cardiac tissue [24]; paracrine effects (secretion of beneficial cytokines) leading to vascularization of infarcted tissue [25]; and paracrine effects for increasing tissue survival or preventing cell death [4, 26].

Zhu et al. showed that the paracrine effects of these cells may be more therapeutically important than actual regeneration of tissues [23], consistent with previous research with other types of adult mesenchymal stem cells [21, 27]. The paracrine effects of ADSCs include inducing angiogenesis, recruiting local stem cells, reducing fibrosis, and limiting apoptosis [25]. The paracrine growth factors secreted by ADSCs include endothelial growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF-1), and microRNAs [28]. More recently, an endothelial-derived factor, Neuregulin-1 (NRG1) has been shown to synergize with the ADSCs in cardiac repair. NRG1 belongs to a family of cell-to-cell signaling molecules which act through receptor ErbB tyrosine kinases [29-31]. On its own, activation of the NRG1/ERBB signaling is effective for cardiovascular therapy and repair, shown in both animal models [32-37] and human clinical trials [38, 39]. In two animal studies the combination of ADSCs with NRG1 along with microparticle technology (ADSC-NRG-MPs) have demonstrated a substantial improvement in heart regeneration capacity [40, 41]. One study has already shown that induced expression of NRG1 from mesenchymal stem cells themselves, rather than on microparticles, was also correlated with improved cardiac regeneration efficacy [42]. These studies suggest that the ADSC-NRG1 combination could be used for future clinical studies, perhaps with NRG1 expressed from the ADSCs directly, or from co-administered microparticles.

Clinical trials

The APOLLO trial was the first instance where ADSCs were used in humans to treat myocar-

dial infarction, specifically patients with ST elevation, a conduction defect resulting from damaged myocardium. This is a Phase I clinical trial based in Spain with 9 analyzable patients [24]. The trial demonstrated that six months after intracoronary infusion of ADSCs, cardiac function improved (ejection fraction analyzed by CT scan), perfusion of the tissue increased, and infarct size reduced 50% on average, compared to placebo. Additionally, no serious side effects of this therapy were detected. While there is a very small sample size, this trial is a promising sign that the preclinical data can be transferable to humans. The follow up ADVANCE study is ongoing with no results posted at the time of writing [24].

The Precise Trial is a phase II clinical trial that was performed in Denmark and Spain. This trial looked at 27 patients suffering from chronic ischemic heart disease due to myocardial infarction. The trial was conducted at multiple facilities and used transendocardial injection of ADSCs to treat these patients. Using the New York Heart Association (NYHA) and Canadian Cardiovascular Society (CCS) classifications of heart disease, patients who received treatment improved modestly. This study saw small improvements in contractility (ejection fraction), scar size, and perfusion in groups treated with ADSCs. Importantly, the treated group was able to preserve cardiac function over a 2-year span while the placebo group deteriorated. No serious side effects were detected. Perin et al. emphasized that, “we have shown for the first time that the harvest and transendocardial injection of ADRCs [adipose derived regenerative cells, a synonym for ADSCs] are safe and feasible in no-option patients with ischemic cardiomyopathy”. This is a significant advancement for ADSC therapy in a larger, more prominent clinical trial [43].

The Athena program is a phase II clinical trial involving two parallel double-blind studies that took place in the United States to test ADSCs in myocardial ischemia. In these studies adipose cells were extracted from patients, processed for stem cells, and administered by intramyocardial injection. The trial was a double-blind placebo-controlled trial with 31 participants. In this trial ADSC treated patients showed improved perfusion, a drop in 12-month hospitalization rates, and improvements based on NYHA and CCS classifications of heart dis-

ease compared to the placebo group. In this trial, no difference was seen in left ventricular function between the ADSC treated and placebo groups. While it is important to note the small sample size, this trial is another demonstration that injected autologous ADSCs are a safe and viable treatment for ischemic heart disease [44].

In a phase I safety trial, ADSCs were administered to patients without matching patient serotypes [45], which relied on the immunosuppressive properties of ADSCs [14]. The precise immunosuppressive mechanisms involved remain controversial and more work is needed, but ADSCs exhibit low expression of class II Major Histocompatibility Complex (MHC-II) and apparently also directly inhibit T- and B-cell responses, among other potential pathways [24, 46, 47]. Kastrup et al., 2017 [45] showed some of the patients developed donor specific HLA antibodies after treatment, but this caused no clinical problems. After a 6-month period, mild improvements were seen in cardiac function, including contractility, filling, NYHA class, and exercise ability [45]. It is important to mention that there was no placebo group in this trial and the sample size was very small, so follow-up studies are necessary [45].

Potential adverse effects

While no *in vivo* studies have shown a direct link between ADSC implantation and serious adverse effects, many have investigated the potential side effects that could arise if this treatment was used in a larger population. As with most stem cell treatments, the proliferative potential of cells is potentially linked to tumorigenesis. Most of the research supporting this has been done with BM-MSCs, and there are some concerning links to tumor induction and promotion of metastasis [48-51]. Much of the instability of stem cells is a result of the need to expand the cells in order to achieve therapeutic results, which leaves them susceptible to cancer-causing mutations. In animal models it has been shown that without careful monitoring of the chromosomal makeup of mesenchymal stem cells during expansion, the cells can form tumors once implanted [51, 52]. Additionally, lack of a controlled environment *in vivo* can allow cells to differentiate into a non-desired cell type, causing issues such as calcification/ossification in cardiac tissue [48].

In testing the paracrine effects of mesenchymal stem cells, one study showed that bone marrow-derived MSCs promoted the proliferation and metastasis of breast cancer cells when cultured together and injected into mice [50]. In a similar study using ADSCs and pancreatic cancer cells, the stem cells again increased the proliferation and invasiveness of the cancer cells, although this study only used *in vitro* assays [53].

It is important to note that the all *in vivo* studies describe mesenchymal stem cells, not ADSCs specifically. For these mesenchymal studies, there seems to be a difference between animal models, where tumorigenic side effects were observed, and the multiple published studies using mesenchymal stem cells in human patients with no reported tumor formation [24, 43, 49], suggesting that this is an area for further investigation. ADSCs may be safer than other mesenchymal stem cells because they are available in higher quantities, requiring less expansion and therefore less susceptible to mutation *in vitro* [11]. However, the potential relationship with cancer should be monitored as the use of ADSCs in humans continues in clinical trials.

Discussion

The goal of this review is to explain the advantages of ADSCs over other stem cell therapies in treating ischemic heart disease due to myocardial infarction. Stem cell treatment for cardiac disease is not new, but ADSCs are some of the most promising current therapies for acute myocardial infarction. ADSC-associated positive outcomes include better contractility and filling as well as improved cardiac function relative to placebo [43]. In total, four clinical trials have been completed with promising results. In order to fully assess the extent to which these treatments may be able to combat cardiovascular disease, additional research must be done.

The potential for negative side effects with this type of treatment also needs to be addressed. The primary issue of stem cell therapy lies in the possible induction of tumorigenic malignancies in those predisposed to proliferative cell growth [50, 53]. It remains to be seen, however, if the advantages of improving 5-year survival rates after myocardial infarction is worth

the potential for cancer later on. Ideally, a system to screen patients who have a history of cancer, skin disease, or connective tissue illness would help minimize risk of adverse events [18].

While other effective treatments exist for improving health post-myocardial infarction, they may not be able to fully repair the damaged tissue. Stem cells have emerged as a treatment that can restore the damaged tissue, whether through physical regeneration or paracrine effects on endogenous stem cells [26, 27, 54, 55]. For stem cell treatment to have the potential for widespread use, the availability should be high and cost needs to be kept low, a reason ADSCs have the potential to be a viable treatment for the general population [14].

Current practice in treatment of CVD includes drugs such as antiplatelet agents, beta blockers, and statins, which are limited to milder cardiac injury, not recovery of damaged heart tissue. Alternatively, heart transplantation is incredibly invasive and routinely blocked by shortages of viable organs. These individuals must be placed on immunosuppressive drugs for the rest of their lives [14]. In contrast, ADSCs possess some intrinsic immunosuppressive qualities that allow them to be well-tolerated by the recipient even without donor matching. Although one study noted that patients developed donor-specific HLA antibodies post-treatment, they also determined that these molecules posed no serious adverse events to the patient, and there was no need for immunosuppressive drugs for the long term [45]. As such, ADSCs may be able to overcome several important limitations of existing treatments for cardiac disease.

Adipose-derived stem cells are still in the preliminary phases of research, but due to their wide availability, limited ethical concerns compared to other stem cell treatments, and promising early results in clinical trials, they present a most promising avenue in treating cardiac disease.

Disclosure of conflict of interest

None.

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