

Adipose Derived Stem Cells in Orthopaedics: History and Current Applications

Brian Johnson, Rajul Gupta, Bret Betz, Henry Kuechly*, Sarah Kurkowski, Brian Grawe

Department of Orthopaedic Surgery and Sports Medicine, University of Cincinnati, Cincinnati, Ohio, USA

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*Correspondence:

*Mr. Henry Kuechly, Department of Orthopaedic Surgery and Sports Medicine, University of Cincinnati, Cincinnati, Ohio, USA; Email: kuechlyh@ucmail.uc.edu

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Keywords

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Abstract

Context: Some studies have shown promising results with adipose-derived stem cell treatments for orthopaedic problems as a nonsurgical treatment option or an augmentation of surgical treatment.

Purpose: Review of the history and background, preparation methods, and current applications of adipose-derived stem cells in orthopedics. Provide critical appraisal of the available evidence for the use of Adipose Derived Stem Cells.

Results: Most of the studies utilizing adipose-derived stem cells are case series or meta-analyses with a small number of studies, therefore presenting a risk of selection bias. In cases of femoral head avascular necrosis and meniscal repair, no systematic review or meta-analysis has been published and available evidence is derived from smaller studies. Almost every review article concluded that large, multicenter, randomized control trials are needed to establish the value of adipose-derived stem cells in orthopaedics.

Conclusion: There is a need in orthopaedics for treatment modalities that increase biological healing potential for some pathologies and adipose derived stem cells represent a potential modality for such a purpose. However, there is a lack of high quality and robust evidence regarding the efficacy and safety of this treatment modality in orthopaedic applications. The use of adipose derived stem cells in orthopaedics requires additional studies of higher quality before they can be considered an appropriate treatment option.

Strength of Recommendation: Level C for use of Adipose-derived stem cells in orthopaedics.

Introduction

Many common orthopaedic complaints related to musculoskeletal pathology often improve with various operative and non-operative treatment options. While some treatments provide many patients with relief of their symptoms, the fundamental lack of healing potential in tendinous, cartilaginous, and other connective tissues presents a challenge to orthopaedic clinicians in the treatment of some patients. Additionally, surgical treatment options for these pathologies may not be appropriate or possible for all patients for a variety of reasons. This challenge represents a present need in orthopaedics for additional treatment options that introduce added biology to the site of pathology that promotes the healing potential of the tissues. Stem cell therapy is a modality of interest in the treatment of orthopaedic problems, with adipose tissue being a source of mesenchymal stem cell isolation. In the present article, we review the history and background of adipose derived stem cells, isolation procedures, current applications in orthopaedics, and a

current appraisal of the evidence for their use in the field of orthopaedics.

Methods

PubMed was used to search manually with varied search terms for peer-reviewed articles. The literature search ranged from January 1st, 1978 to July 31st, 2022. Articles related to the basic science, methods of preparation, and clinical application of adipose derived stem cells in orthopaedics were reviewed for inclusion in the narrative review and citations in those articles were reviewed for any relevant gaps in knowledge provided.

History

In 1978, adipocyte precursor cells were isolated and cultured for the first time from human and animal omental tissue. These precursors were obtained by culturing stromal vascular fraction (SVF) – a component of adipose tissue derived by collagenase digestion and centrifugation¹. While these cells were obtained from patients undergoing abdominal surgery, techniques to extract fatty tissues were developed to extract adipose tissue without extensive incisions, for the purpose of body contouring, in the plastic surgery arena and was termed lipoaspiration (LA)². In 2002, Zuk and colleagues demonstrated the processed lipoaspirate (PLA) contained a stem cell population which was immunophenotypically similar to bone marrow mesenchymal stem cells (BM-MSCs). These cells were demonstrated to differentiate into osteogenic, chondrogenic, myogenic and neurogenic lineages in presence of specific growth factors³.

Despite these findings, there was an underappreciation of adipose tissue as a source of stem cells⁴. Adipose tissue has been demonstrated to produce a much higher stem cell yield compared to bone marrow from the same donor⁵. BM-MSC comprise a relatively small number of cells obtained in bone marrow aspirate (BMA)⁶. Additionally, BM-MSC become senescent much earlier than Adipose Derived Stem Cells (ADSC)^{7,8}. The process of bone marrow aspiration is more painful for the patient compared to lipoaspiration, often requiring intravenous or spinal anesthesia, compared to tumescent anesthesia needed to extract appropriate quantities of lipoaspirate⁹. These factors, when combined, arguably make adipose tissue an optimal tissue source for stem cell harvesting.

Growth factor secretome and the biological properties of adipose stromal cells

In order to separate mesenchymal stem cells (MSCs) from the stromal vascular fraction, immunophenotyping using positive and negative selection has been used by various research groups. A recent systematic review found D90, CD44, CD29, CD105, CD13, CD34, CD73, CD166, CD10, CD49e and CD59 to be the most frequently reported positive

markers and CD31, CD45, CD14, CD11b, CD34, CD19, CD56 and CD146 to be the most commonly reported negative markers¹⁰. Though this profile has significant overlap with BM-MSCs there are well defined surface markers to differentiate between the two, including CD106 and CD49d⁵. Flow cytometry is the most common laboratory method to verify and characterize MSCs. Experiments have elaborately described the growth factor secretome (the proteins secreted by biologic cells) of ADSCs¹¹. When cultured in hypoxic conditions or along with endothelial cells¹², the quantity of growth factors (VEGF, FGF, HGF) secreted by ADSCs increased 5-fold. This also correlated with increased perfusion in ischemic hindlimbs in animal studies^{12,13}. Other growth factors such as Nerve Growth Factor (NGF), IL-1, and IL-6, among others are secreted at higher levels by ADSCs as compared to BM-MSCs. Further, adipose derived stem cells are minimally immunogenic as demonstrated by a lack of T cell proliferation to allogeneic ADSCs as well as ASC-mediated suppression of lymphocyte reaction^{14,15}. Finally, there is substantial evidence of anti-inflammatory properties of stromal vascular fraction evidenced by suppression of pro-inflammatory IL-6 and TNF- α expression and higher levels of anti-inflammatory cytokines such as IL-10 in fat transplant models^{16,17}.

Nomenclature

It is important at this point to clearly identify the different contents of adipose tissue with their relevance in regenerative medicine.

- *Manipulated Lipoaspirate* is a product of manipulation of lipoaspirate. This can be done by enzymatic methods, such as enzymatic digestion, or mechanical methods, such as ultrasonic cavitation¹⁸. Recently issued FDA guidelines make “more than minimally manipulated” lipoaspirate, defined as processing adipose tissue for use other than its role as a structural support tissue, subject to a new set of regulations¹⁹.
- *Stromal vascular fraction (SVF)* is a heterogenous cell mixture²⁰. While commonly derived from adipose tissue by manipulation of lipoaspirate, it can also be isolated from bone marrow²¹. Manipulation of lipoaspirate yields adipose derived-SVF (AD-SVF). This SVF is profoundly depleted in adipocytes but abundant in adipose stromal, hematopoietic stem cells (HSCs), endothelial cells, fibroblasts, lymphocytes and macrophages, as well as a variety of growth factors.
- *Mesenchymal stem cells (MSCs)* are spindle shaped cells which can be identified by plastic adherence and by presence of antigen Mab STRO-1 from many sources including bone marrow (BM), adipose tissue (AT), dental pulp, peripheral and cord blood and placental tissue^{10,22}. Stromal vascular fraction contains a wide variety of cells, connective tissue and blood vessels in addition to MSCs²³.

- ADSCs are defined as mesenchymal stem cells derived from adipose tissue. They are isolated by seeding SVF into culture, resulting in a population of elongated cells that adhere to the wall of the container, and further purified by standardized methods to deplete hematopoietic cells. This population is comprised of different types of cells but is much less heterogenous than SVF^{23,24}. This population is comprised of different types of cells but is much less heterogenous than SVF^{23,24}.

In vitro and in vivo differentiation capabilities of ADSCs

Since the seminal work in 2002, multiple research groups have advanced the findings and clinical applications of ADSCs with respect to their differentiation capabilities. Osteogenic differentiation *in vitro* can be induced by dexamethasone, 1,25-dihydroxyvitamin D₃, carbon nanotubes and graphite surfaces²⁵. Inducible osteogenic differentiation was demonstrated in human critical size defects in bone using ADSC with beta-tricalcium phosphate²⁶. Other *in vivo* osteogenic growth factors include PGA scaffolds²⁷ and rhesus Bone Morphogenic Protein-2²⁸. Similarly, *in vitro* chondrogenic differentiation was induced by insulin, ascorbate, and TGF beta-1³. Since then, multiple naturally occurring (Silk scaffold)²⁹ and synthetic (magnetic iron nanoparticles)³⁰ materials have been shown to induce chondrogenic differentiation. In concordance with multiple *in vitro*³¹ and animal studies³², clinical studies demonstrated clinical improvement in knee osteoarthritis using PRP as a stimulator of cartilage healing by SVF^{33,34}. Finally, hydrocortisone has been used to induce myogenic differentiation of adipose stem cells *in vitro*, while multiple other mediums and growth factors have been used to induce myogenic differentiation *in vivo*³⁵. Multiple other tissue lineages have been derived from ADSC, further demonstrating the differentiation capabilities, but this discussion is focused on applications in orthopaedics and musculoskeletal medicine.

Stem cell yield from Bone Marrow versus Adipose tissue

It is important to differentiate the terms “cell yield” and “stem cell yield”. Not all plastic adherent cells from the stromal vascular fraction will express MSC surface markers. Hence, the term “cell yield” as compared to “stem cell yield” is preferable when comparing different counting methods immediately after SVF isolation, since around 5 – 10 percent of isolated cells demonstrate MSC markers on flow cytometry or form colonies after 14 days in culture³⁶. Stem cell yield from adipose tissue is 20 to 2000-fold higher than that from bone marrow. It is noteworthy that excision of adipose tissue yields twice the number of stem cells from adipose tissue compared to lipoaspiration but carries with it inherently more risks to the patient⁸. Commercially

available cell counters and light microscopy can be used to assess cell counts in SVF³⁶.

Methods of preparation

Lipo-aspiration

Lipo-aspiration can be done under intravenous or tumescent anesthesia⁹. Since SVF and ADSCs preparation require removal of a relatively small amount of subcutaneous fat, tumescent anesthesia is preferred unless the patient is also undergoing another reconstructive or reparative procedure requiring regional or general anesthesia⁹. Briefly, skin of the harvest site (typically, but not limited to, abdomen, groin, or thigh) is infiltrated with a mixture of 1:100 000 epinephrine and 0.9 percent sodium chloride solution by an infiltration canula of around 2.5 mm diameter and allowed to stand for 20 minutes. The volume of infiltrated solution is equal to the volume harvested. Use of a 5 mm blunt lipo-aspiration canula results in better SVF yield³⁷. Lower suction pressure around 30 KPa yields improved SVF cellularity³⁸. When the aspirate is allowed to stand for 10-30 minutes, supernatant separates from sedimented fat³⁹. This fat graft is used to isolate SVF by enzymatic or mechanical methods.

Enzymatic Methods

The methods used for enzymatic processing may follow a slight variation of the standard technique³⁹. Fat graft is washed with isotonic and iso-osmotic solution (Hank's balanced salt, Ringer's lactate, Phosphate-Buffered Saline) to remove erythrocytes and fat debris. The fat tissues are mixed with 0.075% to 0.1 % GMP grade collagenase for up to 2 hours for digestion^{39,40}. This is then centrifuged (at varying speed and duration), yielding 3 distinct layers. Fatty and aqueous layers are discarded to retain the pellet containing SVF. While enzymatic digestion is reported to yield a higher concentration of progenitor cells, it is more expensive, time consuming, and subject to variability of enzyme quality despite GMP certification⁴¹.

Mechanical Methods

Minor variations in technique aside, mechanical methods typically involve vibrating and centrifuging washed lipoaspirate⁴². While this method is cheaper and requires less processing time, it yields more blood cells and less ADSCs. Further, many collagen bonds remain intact^{40,43}. A novel mechanical method with stem cell yields equal to enzymatic method has been described by Amirkhani et al⁴¹. After Phosphate Buffered Saline wash, tissues are dissected in a blender-like device for a short time. This is followed by ultrasonic cavitation and centrifugation. Further processing includes isolation of the SVF pellet, suspension with ammonium chloride (to lyse RBCs) for 5 minutes and additional centrifugation. While the cell yield is optimal,

osteogenic potential is potentially less compared to enzymatic digestion⁴².

Preparations

Although both enzymatic and mechanical methods of LA processing yield SVF, collagenase preparation falls outside what the USFDA defines as minimal manipulation. In contrast, the processes used to manufacture SVF that are usually intended for autologous use in the same-day surgical setting may fall within the FDA guidelines of minimal manipulation of human tissue^{44,45}. If one wishes to isolate and expand ADSC from SVF, as mentioned above, it requires some sort of cell expansion in culture media. Once expanded, frozen cells can be preserved for up to 2 weeks⁴⁵. A depiction of the two methods to isolate SVF and ADSC is provided in Figure 1. Administration at the target tissue is done in conjunction with carrier media such as autologous conditioned serum⁴⁵. Other scaffolds have been proposed for osteogenic⁴⁶, neurogenic^{47,48} and tenogenic differentiation⁴⁹. These preparations of ADSC are also considered more than “minimally manipulated” by FDA guidelines due to the alteration of the original characteristics of adipose tissue relating to utility in tissue support and cushioning.

Current Applications of ADSCs in Orthopaedics

Rotator Cuff Disease

Though current treatments for rotator cuff disease have relatively good results, a persistently unmet need for introduction of favorable biology for healing remains for both partial and full thickness tears. Some studies have aimed to evaluate the safety and efficacy of introducing ADSCs at the site of rotator cuff disease both in operative

and nonoperative management. In 2017, Kim et al. demonstrated a lower retear rate in patients undergoing rotator cuff repair with no difference in functional outcomes at two-year follow-up⁵⁰. Though a small study, a randomized controlled trial showed improved shoulder function and pain relief after ADSC injection for management of partial thickness tears without adverse events⁵¹. Another small RCT in 2020 demonstrated a safety profile similar to that of corticosteroid injections with improved functional outcome scores after treatment of partial thickness tears with ADSC injection⁵². A recent RCT evaluating augmentation of arthroscopic rotator cuff repair with ADSCs showed a reassuring safety profile, however any additional benefit in functional outcome scores were only seen in short term follow-up⁵³.

Meniscal Disease

Literature related to treatment of meniscus pathology and augmentation of meniscus repair with ADSCs is sparse. One small case series showed two patients with repaired degenerative meniscus tears after intraarticular knee injection of ADSCs for primary osteoarthritis when viewed directly with arthroscopy⁵⁴. A single patient report in 2014 showed apparently healed meniscus tear on MRI after injection of ADSCs, however this formulation included PRP and other ingredients and does not represent injection with pure ADSCs⁵⁵.

Knee Osteoarthritis

Cartilage degeneration in osteoarthritis of the knee represents a significant need for improvements in biologically active therapies that directly promote and provide the necessary materials for regeneration of articular

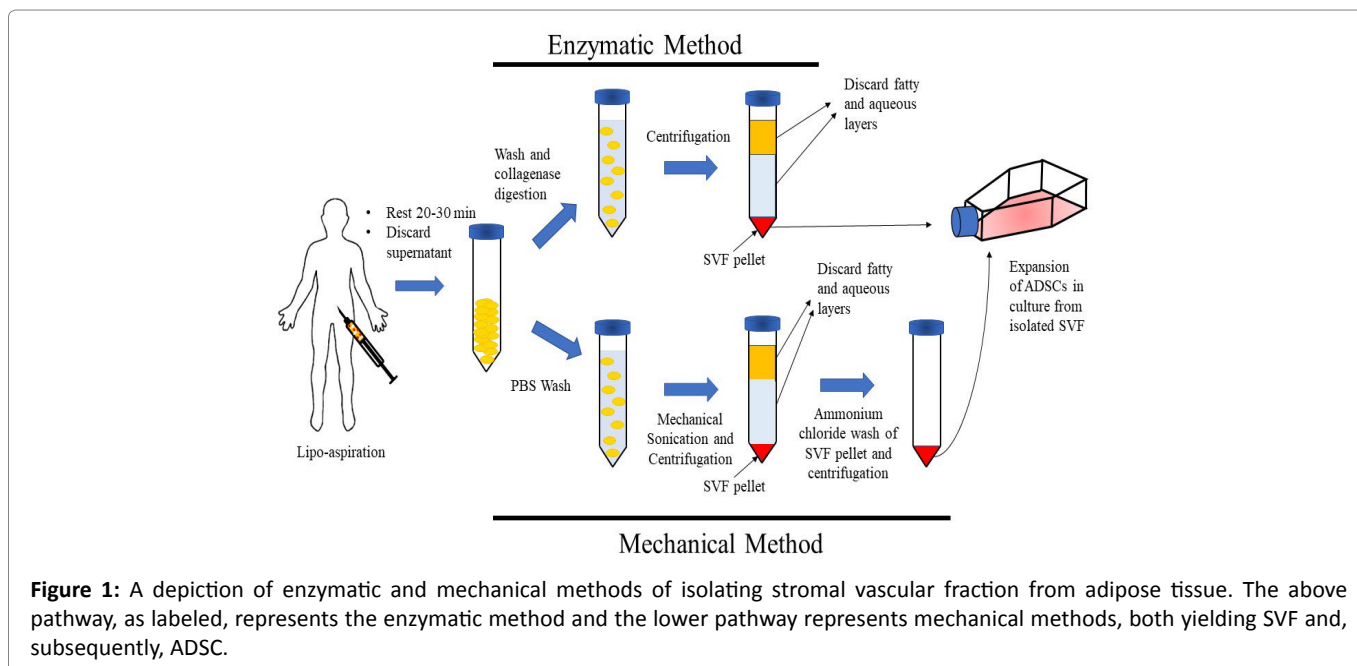


Figure 1: A depiction of enzymatic and mechanical methods of isolating stromal vascular fraction from adipose tissue. The above pathway, as labeled, represents the enzymatic method and the lower pathway represents mechanical methods, both yielding SVF and, subsequently, ADSC.

cartilage. Nonoperative treatments that are commonly used or are being used more frequently include injections with corticosteroids, platelet rich plasma, hyaluronic acid, bone marrow aspirate and ADSCs. The latter four treatment options involve a theoretical benefit of improved healing and regeneration of cartilage. In 2015, a systematic review of preclinical animal models for ADSC injection for knee OA demonstrated positive results with respect to delaying progression of OA with some evidence of improvement of cartilage features and regeneration⁵⁶. In 2017, Coughlin et al. published a stepwise technical approach to ADSC harvesting and intraarticular knee injections as a single procedure⁵⁷. Koh et al. published a randomized control trial in 2012 demonstrating short term benefits to functional outcomes scores compared to controls and a case-control trial in 2013 demonstrating improved pain and functional scoring, as well as improved MRI findings as 2 years^{33,58}, however these results are confounded by the inclusion of PRP in the ADSC injections. Lopa et al. reported in a 2019 review that though studies reported improvement of knee pain and function from ADSC treatment, most were small studies that did not compare to controls⁵⁹. A 2019 RCT from Lu et al. showed improved cartilage volume as measured on MRI when compared to patients receiving hyaluronic acid injection⁶⁰. Meta analyses from Anil et al. and Bolia et al. in 2021 and 2022, respectively, showed treatment with SVF from adipose tissue improved pain and functional outcomes at least in the short term but both concluded that presently available trials have significant variation in preparation techniques and study design^{61,62}.

Achilles Tendinopathy

Two randomized controlled trials have demonstrated injection of ADSC for Achilles tendinopathy improved functional outcomes sooner than treatment with PRP injections with similar outcomes at each study's endpoint^{63,64}. However, it is important to highlight that even the efficacy of treatment with PRP for Achilles tendon pathology is controversial^{65,66}.

Critical Appraisal and Current State of Acceptance

In the current regulatory environment, it is not difficult for the preparations of autologous cell therapies to fall outside FDA regulation. In general, "stem cell therapy" has received positive and optimistic coverage in the media. Concerns have been raised about the impact of stem cell research on the public by scientific societies, the research community, and regulatory authorities⁶⁷. The scientific enthusiasm surrounding these treatment modalities has given rise to some unproven therapies which are often expensive^{44,68,69}. The number of stem cell clinics has grown five-fold in the last 5 years. More than 80 percent of these treat pain-related conditions and nearly half of them have their focus on orthopaedic conditions. While cells derived

from bone marrow remain the most popular, the proportion of clinics using ADSC has increased⁴⁴. These clinics use patented processes that are significantly different from each other. Apart from orthopaedic conditions, stem cell treatment is being offered for many neurological conditions such as stroke, Alzheimer's disease, and dementia. Recently, due to the wide variety of conditions in which stem cell treatments are used, many clinicians have expressed skepticism about therapeutic effects.

Patients often desire to avoid surgery for degenerative conditions and some researchers have contacted stem cell clinics simulating patients seeking injection. In such a scenario, direct to patient advertising of "non-surgical treatment" often has a strong impact on the target population and patients then seek treatment in "stem cell clinics"⁶⁹. Such patients are often first seen by practitioners who are rarely qualified orthopaedic physicians. To circumvent strict FDA guidelines in the US, clinics sometimes transport lipoaspirate across the border into Mexico, where ADSC are isolated and cultured. Patients are then administered injections across the border in Mexico. There has been a report of multi-articular septic arthritis resulting from contamination during the obviously complex transportation procedure⁷⁰. The culture media used for ADSC culture may harbor pathogenic micro-organisms, which sometimes contaminate the final product. Fungal pathogens such as candida, aspergillus, and penicillium often contaminate stem cell cultures⁷¹. Even when minimally manipulated preparations are injected, some clinics mix a variety of adjuvants, potentially increasing the possibility of contamination and subsequent deep infections, as well as complicating any anecdotal conclusions made about treatment efficacy⁷². Fortunately, serious complications with nonexpanded and mechanically expanded SVF preparations have been rare. These complications mainly originate from the injection procedure rather than injectate. There are case reports of skin organisms causing septic arthritis and spondylodiscitis after "biologic" injections^{70,73}.

Most of the studies utilizing ADSCs or SVF are case series. Meta-analyses have been done to evaluate the effectiveness of adipose tissue preparations in knee arthritis^{61,62} and rotator cuff repair⁷⁴. Since these meta-analyses included a relatively small number of studies, there is a risk of selection bias. In cases of femoral head AVN and meniscal repair, no systematic review or meta-analysis has been published and available evidence is derived from smaller studies. Almost every review article concluded that large, multicenter, randomized control trials are needed to establish the value of ADSC in orthopaedics. Frequent use of adjuvants like PRP further adds to the heterogeneity of these studies.

The relative lack of such evidence in the literature for many applications in orthopaedics demonstrates the need

for conducting larger studies of higher quality to more definitively and effectively evaluate the efficacy and safety of this treatment modality in orthopaedic applications.

Conclusion

Regenerative medicine techniques have increased in popularity over the years. There is a need in orthopaedics for treatment modalities that increase biological healing potential for some pathologies. Adipose derived stem cells represent a potential modality for such a purpose with their ease of collection and favorable secretome profile. However, the evidence for the use of adipose derived stem cells in orthopaedics requires conducting of additional studies of higher quality before they can be considered an appropriate treatment option.

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Conflict of Interest

The authors have no conflict of interest to report.

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