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STEM CELLS IN ORTHOPAEDIC SURGERY (J DRAGOO AND K JONES, SECTION EDITORS)

# Adipose-Derived Mesenchymal Stem Cell Treatments and Available Formulations

Kyle N. Kunze[1] · Robert A. Burnett[1] · Joshua Wright-Chisem[2] · Rachel M. Frank[3] · Jorge Chahla[1]

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## Abstract

**Purpose of Review** The use of human adipose-derived mesenchymal stem cells (ADSCs) has gained attention due to its potential to expedite healing and the ease of harvesting; however, clinical evidence is limited, and questions concerning optimal method of delivery and long-term outcomes remain unanswered.

**Recent Findings** Administration of ADSCs in animal models has been reported to aid in improved healing benefits with enhanced repair biomechanics, superior gross histological appearance of injury sites, and higher concentrations of growth factors associated with healing compared to controls. Recently, an increasing body of research has sought to examine the effects of ADSCs in humans.

**Summary** Several available processing techniques and formulations for ADSCs exist with evidence to suggest benefits with the use of ADSCs, but the superiority of any one method is not clear. Evidence from the most recent clinical studies available demonstrates promising outcomes following treatment of select musculoskeletal pathologies with ADSCs despite reporting variability among ADSCs harvesting and processing; these include (1) healing benefits and pain improvement for rotator cuff and Achilles tendinopathies, (2) improvements in pain and function in those with knee and hip osteoarthritis, and (3) improved cartilage regeneration for osteochondral focal defects of the knee and talus. The limitation to most of this literature is the use of other therapeutic biologics in combination with ADSCs. Additionally, many studies lack control groups, making establishment of causation inappropriate. It is imperative to perform higher-quality studies using consistent, predictable control populations and to standardize formulations of ADSCs in these trials.

**Keywords** Adipose · Stem cells · ADSCs · Biologics · Formulations · Regenerative medicine

## Introduction

The use of biologic adjuncts as both operative and non-operative treatments for orthopedic pathologies has become of recent interest due to potential healing benefits. [1–5] Administration of progenitor cells, defined as pluripotent cells that have the ability to differentiate into several lineages, is one such adjunct with increasing interest as they can be utilized for clinical benefit by expediting physiological processes responsible for healing. Several sources from adult progenitor cells have been reported in the literature including bone marrow mesenchymal stem cells and peripheral blood stem cells; [6, 7] more recently, adipose-derived mesenchymal stem cells (ADSCs) have been recognized as an alternative source of stromal cells thought to have equivalent differentiation capacity.

A primary reason for increased interest in the use of ADSCs for clinical applications manifests from the ease with which they are harvested relative to other stromal cells, as subcutaneous stores in the infrapatellar fat pad and buttocks/flank allow for a less invasive isolation process with a similar multi-lineage potential. [8] Indeed, these minimally invasive harvesting techniques are associated with lower donor site morbidity and pain and fewer complications than the harvesting of other stromal cells, and lipoaspirate has been demonstrated to result in higher

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✉ Jorge Chahla
jorge.chahla@rushortho.com

[1] Department of Orthopaedic Surgery, Division of Sports Medicine, Rush University Medical Center, Chicago, IL, USA

[2] Department of Orthopaedic Surgery, Hospital for Special Surgery, New York, NY, USA

[3] Department of Orthopaedic Surgery, Division of Sports Medicine, University of Colorado School of Medicine, Boulder, CO, USA

progenitor cell yields than bone marrow aspirates [9]. Other proposed benefits of ADSCs include greater availability, with reports of up to 10% nucleated cells from ADSCs versus 0.001–0.01% of bone marrow-derived mesenchymal stem cells, a low rate of complications, and high proliferative potential and rapid expansion. [10–13]

Despite increasing use, several limitations to ADSCs still exist. While controlled laboratory studies involving the use of ADSCs as an augment for the treatment of common orthopedic pathologies such as focal osteochondral defects and rotator cuff tendinopathy have reported promising results in animal models, [14–17] few studies involving human subjects exist. [18–21] Furthermore, most of these studies have inconsistent protocols and formulations of ADSCs, making it difficult to compare results between studies. Additionally, it is still unclear if ADSCs act directly by repairing the tissue or through signaling environmental molecules to aid the repair of the tissue. [22] The current article presents a comprehensive review of the mechanisms of action of ADSCs from the basic science literature and available formulations of ADSCS. Additionally, this review discusses evidence for the safety and efficacy of ADSCs for orthopedic pathologies and injuries in both animal models as well as clinical trials with an emphasis on clinical and functional outcomes as well as variability in harvesting and processing protocols. Finally, future directions for the use of ADSCs in the context of the current evidence in this review will be presented.

## Proposed Mechanisms of Action from the Basic Science Literature

The multipotent nature of ADSCs allows for the potential to differentiate into several different lineages including osteogenic, chondrogenic, adipogenic, and myogenic cell lines. Given the proposed beneficial effects of the use of ADSC in bone, tendon and muscle healing, a better understanding of the downstream effects induced by ADSC at the molecular level is imperative to clarify how these outcomes are mediated. Furthermore, such clarification will help better understand ADSCs as a biologic adjunct in general, the spectrum of their use in repair of common musculoskeletal injuries, and potentially aid in the optimization of ADSCs formulations.

### Proposed Mechanisms of Osteogenesis

While the specific mechanisms of osteogenesis from ADSCs are complex and not completely understood, there are several proposed mechanisms by which osteogenic differentiation is thought to occur. Recent research has focused specifically on signaling pathways and molecular biology. In particular, osteogenic differentiation is thought to proceed through the upregulation of various growth factors and acceleration of osteogenic-specific molecular pathways which utilize migration, molecular adhesion, and differential signaling to increase bone production. [22] Most theories accept the premise that these cascades begin with a commitment step from the stem cell precursor into the osteogenic lineage through molecular signals including transforming growth factor (TGF)-β, bone morphogenic protein (BMP) family, fibroblast growth factor (FGF), and Wnt/β-Catenin proteins. [22] The cellular and molecular mechanisms that underlie osteogenic differentiation via ADSCs are complex and out of scope of the current review; however, few major mechanisms and prevalent theories of osteogenic differentiation are presented below.

BMP has been implicated as one of the primary initiators of the signaling cascade for ADSC-mediated osteogenesis. Studies have shown that BMP initiates the cascade via ligand binding of serine/threonine kinase cell surface receptors. [23] The next step involves these activated receptor kinases phosphorylating transcription factor SMADs, which subsequently form a heterodimeric complex and promote gene expression and osteogenic differentiation. [24] BMP-2 induces the phosphorylation of Smad 1/5/8 and the transactivation of a BMP/Smad-responsive construct (12xSBE-Oc-pGL3). [25] Furthermore, Ducy et al. [26] showed that BMPs were able to increase the transcription of core-binding factor-1/Runt-related family 2(Cbfa1/Runx2), a molecule that is well known to be essential for the commitment process of osteoblastic lineage. The Notch pathway has also been found to be heavily implicated in the role of osteogenic differentiation through interactions with BMP-2, which results in increased expression of Delta1/Jagged1-activated Notch. [25, 27] Interestingly, overexpression of the Notch intracellular domain (NICD) impairs osteogenesis. Notch has been demonstrated to oppose the effects of BMP-2 and Wnt-3a on alkaline phosphatase activity, which is normally upregulated by these markers. Furthermore, Notch overexpression decreases the transactivating effect of Wnt-3a, cytoplasmic β-catenin levels, and Wnt-dependent gene expression. NICD overexpression prevents BMP-2 and Wnt biological effects by suppressing Wnt, but not BMP signaling. [25] This is a clinically relevant consideration in appropriately culturing and manipulating cellular conditions to optimize osteogenic differentiation from ADSCs.

Grottkau et al. [28] harvested ADSCs from normal rats and attempted to utilize BMP-2 enhanced ADSCs to restore critical size cranial defects. There were three populations studied: one group with deficits filled with alginate gel alone, another with alginate gel and normal ADSCs, and finally alginate gel with BMP-2 transfected ADSCs. The study concluded that alginate gel with BMP-2-enhanced ADSCs was necessary for critical size defect repair.

While molecular influences are essential for the differentiation of ADSCs, the mechanical environment can play a role in osteoblastic differentiation. [29] Rath et al. demonstrated that mechanical stimulation may function as an anabolic

stimulus for the osteogenic differentiation of mesenchymal stem cells. [29] Yang et al. [30] demonstrated that mechanical influences are also implicated in the differentiation of ADSCs. This group osteo-induced ADSCs for 48 hours by culturing them in a medium containing α-MEM supplemented with 10% FBS, 0.1 μM dexamethasone, 10 mM glycerol phosphate, and 50 mM L-ascorbic acid-2-phosphate. Following osteoinduction, the authors then placed them under uniaxial loads of various short or long duration patterns. This study showed that with increased duration of continuous mechanical stress, gene expression of BMP-2 and Runx2 were significantly increased. Furthermore, they concluded that ADSCs may sense mechanical loading in a *duration-dependent* manner, which affects osteogenic differentiation of ASDCs via the BMP-2 pathway. In addition to tensile loading, fluid flow and shear stress have been shown to be implicated in osteogenic proliferation of ADSCs. [31] In fact, pulse fluid flow (PFF) has been shown to directly stimulate osteogenic differentiation via fluid shear in ADSCs.

Another proposed mechanism of osteogenic differentiation via ADSC precursors is resultant expression and production of the bone marker proteins including alkaline phosphatase, type I collagen, osteopontin, and osteocalcin through the aforementioned molecular pathways. These markers are involved in mineralized matrix production. In a study comparing in vitro differentiation potential between various mesenchymal stem cell types, ADSCs induced the expression of alkaline phosphatase and upregulated Runx2 gene expression, both of which are highly implicated in regeneration and mineralization. [32] D'Alimonte et al. [33] sought to compare the effects of human ADSCs and dental-pulp stem cells (DPSCs) and found that ADSCs conferred greater osteogenic differentiation as measured by higher levels of expression of various early osteogenic markers including Runx2 and alkaline phosphatase at days 3–14, in addition to extracellular matrix mineralization at days 14–21. Furthermore, the authors found that ADSCs demonstrated faster doubling time and colony-forming ability when compared to DPSCs. The authors concluded that ADSCs could be effective in regenerative orthopedics given these properties.

Culture formula and conditions may also play a role in favoring osteogenic differentiation. Based on the fact that mesenchymal precursor cells must undergo proliferation and differentiation and can be manipulated to do so by varying formula conditions, Gu et al. [34] sought to investigate the use of osteogenic formula induction on ADSCs. After inducing ADSCs, the authors found increased c-Jun N-terminal kinase (JNK) activation at days 13–17, and this was associated with extracellular matrix synthesis and increased calcium deposition, the two hallmarks of bone formation. The authors proposed that the MAP kinase pathway was therefore a potential mechanism in ADSC proliferation and differentiation and that extracellular receptor kinase (ERK) signaling helps to govern this process. Indeed, it appears that function of the JNK pathway has a large role in committing to osteogenic differentiation of ADSCs in vitro. [34]

Interestingly, it has also been demonstrated that ADSCs have the potential to seed and integrate into porous metal prosthetics after osteogenic induction. Lewallen and colleagues [35] cultured human ADSCs on surgical-grade porous titanium disks as models for orthopedic implants and osteogenically induced them. They found that ADSCs adhered well to the porous surface and grew into the porous microenvironment compared to controls. Together, this suggests that ADSCs may have a role in clinical cases where enhanced fixation of surgical components is required. Namely, if there is significant bone loss or deformity, it is possible that ADSCs are pre-programmed to express an osteoblastic phenotype to help restore these deficits.

## Proposed Mechanisms of Chondrogenesis

There are at least four major mechanisms by which ADSCs may be manipulated towards chondrogenic differentiation and may result in a clinically relevant cell source for regenerative medicine: (1) response to specific growth factors, (2) utilization of specific culture media, (3) induction by low oxygen tension, and (4) culture with novel biomaterial scaffolds.

The first mechanism through which chondrogenic differentiation may be selected for is by subjecting ADSCs to a specific growth factor milieu. Similar to osteogenesis, the propensity for ADSCs to differentiate into chondrocytes is highly dependent on culture conditions. Most standard protocols utilize growth factors from the TGF-β superfamily to promote chondrogenesis; [36] however, numerous protocols utilizing a wide variety of culture conditions exist, most of which require 3 to 4 weeks for complete differentiation. It is thought that the TGF-β superfamily of growth factors facilitates the differentiation of ADSCs through several signaling pathways including extracellular signal-related kinase-1 and 2, SMAD, and c-Jun-N-terminal kinase. [37–39] Indeed, culture with TGF-β and BMP-6 has been shown to induce cartilage-specific proteins including type II collagen. [39, 40] A recent study induced ADSCs with transforming growth factor beta-3 (TGF-B3) and bone morphogenetic protein-6 (BMP-6) and found that over 2–4 weeks, chondrocytes were formed and were surrounded by type II collagen and aggrecan. [41] Interestingly, a total of 10 major chondrogenic genes were upregulated in this study. Ultimately, histology, immunohistochemistry, and gene expression profiles confirmed that the ADSCs were transformed into hyaline-like cartilage in culture. The authors of this study proposed that ADSCs may have a significant role in joint restoration given the current challenges incurred with degenerative joint disease. Other growth factors which have demonstrated a strong chondrogenic

potential include BMP-4, sex determining region Y box 9 (SOX 9), and basic fibroblast growth factor.

The second mechanism through which chondrogenic differentiation may be selected for is by ADSCs is through the utilization of media which favors chondrogenesis. Three commonly used protocols are implicated in promoting the chondrogenic differentiation of ADSCs by manipulating the media to select for this lineage. Each protocol utilizes a combination of a medium with various supplements: (1) Dulbecco's modified Eagle's medium (DMEM) with TGF-β3, albumin (1.25 μg/mL), dexamethasone (10-7 M), ascorbic acid (6.25 μg/mL), transferrin, and insulin (6.25 μg/mL); [42] (2) DMEM + 1% fetal calf serum (FCS) with TGF-β1 (10 nh/mL), ascorbate-2-phosphate (50 nM), and insulin (6.25 μg/mL); [43] and (3) OriCell (Cyagen, GUXMX-90041, Santa Clara, CA, USA) with TGF-β3, dexamethasone, ascorbic acid, ITS cell culture supplement, sodium pyruvate, and proline.

The third mechanism through which chondrogenic differentiation may be selected for is through subjecting ADSCs to low oxygen tension. Creating a hypoxic environment in conjunction with specific culture conditions, studies have demonstrated that restricting oxygen during this process may enhance the yield and selectivity of chondrogenesis. [39] Specifically, restricting ADSC culture conditions to 5% oxygen has been demonstrated to be selective for chondrogenesis. However, there is currently no evidence to suggest that this effect remains in vivo. [44]

The fourth mechanism by which ADSCs can be manipulated to favor the pathway of chondrogenesis is by promoting growth by utilizing scaffolds and mechanical forces. [39] It is proposed that mechanical forces take advantage of influencing ADSC cell morphology through responses to extracellular stress. [45] This may be accomplished through applying pressure to cell cultures with dynamic stamps or centrifugal forces. Likewise, commercial systems exist which are designed to take advantage of this mechanical force concept. The FlexCell system (FlexCell Tension System FX-5000T, Dunn Labortechnik GmbH, Asbach, Germany) depends on seeding ADSCs onto silicone membranes which are stretched in a static or cyclic manner by changing the pressure environment. Three-dimensional scaffolds and gel materials may also be used to overcome growth inhibition imparted by cell-cell contact in vitro by mimicking the physiologic milieu or utilizing chemotactic agents to direct migration. [46, 47]

As the process of chondrogenesis proceeds, numerous laboratory techniques are capable of monitoring the differentiation process, [48] and in some cases identify which stage the ADSCs are currently in with respect to completion of the differentiation process. These methods utilize detection of genes or markers expressed by ADSCs that are indicative of the stages of chondrogenic differentiation. Most commonly, the following are used: (1) immunostaining for collagen types I, II, X, and keratin and chondroitin sulfate; (2) polymerase chain reaction; (3) Western blot analysis and ELISA; and (4) RNA microarrays. Genomic analysis is capable of identifying the stages of chondrogenic differentiation as differential gene expression is indicative of specific stages within the chondrogenic lineage. [46] Early in stage 1, SOX 4, BMP-2, and collagen I and VI are expressed; in stage 2, collagen XI, SOX 9, and COMP are expressed; in stage 3, Homeobox 7, Wnt, chondroadherin, and Indian hedgehog are expressed; and as chondrogenesis approaches completion in stage 4, osteocalcin, fibromodulin, alkaline phosphatase, aggrecan, PTHrP, and collagens type II, IX, and X are expressed.

Taken together, chondrogenic differentiation of ADSCs is an intricate process highly dependent on growth factor milieu and culture conditions; although common methods exist, there is no consensus as to which combination of conditions optimizes the yield of ADSCs. Chondrogenic differentiation may also be tracked by identifying differential gene expression at various stages over the 3–4-week culture length.

## Available Formulations

Bone marrow-derived MSCs (BM-MSCs) are traditionally harvested from cancellous bone within the iliac crest, proximal tibia, proximal humerus, and calcaneal tuberosity. However, utilization of BM-MSC has recently come under scrutiny. The harvesting of these cells is invasive and initial studies suggest that cell yield is lower than ADSCs. [49–54] Moreover, there is concern regarding the pluripotency of BM-MSC, with evidence suggesting an inverse relationship between differentiation potential and donor age. [55] For the above-mentioned reasons, alternative sources of multipotent stem cells, such as ADSCs, are of great interest to orthopedic surgeons and patients alike (Table 1).

ADSCs are a particularly promising therapy given the lower morbidity profile associated with the harvesting procedure, often being completed as an in-office procedure. Additionally, initial in vitro studies boast greater yields in ADSC harvests compared to bone marrow harvests. [53] BM-MSCs constitute only 0.002% of total stromal cell populations. [56] ADSCs, however, are considered to be the highest yield of all tissue types, with nearly 2% of MSC in the stromal vascular fraction. [57] ADSCs have been isolated from the abdominal

**Table 1** Benefits of ADSC relative to traditional BM-MSC use

- Higher cell yield
- Less invasive harvesting
- Lower risk of donor site infection
- More predictable cellular differentiation
- Less donor site pain

area, buttocks, flank, thigh, as well as infrapatellar fat pad (IFP). [49] The buttocks are a common location to harvest ADSCs due to high yield with similar differentiation potential. [19] Dragoo et al. demonstrated that IFP is a convenient harvest location for patients already undergoing knee arthroscopy with a mean yield of $4.86 \pm 2.64 \times 10^5$ cells/g of SVF tissue, sufficient for an autologous source of stem cells. [10] ADSCs isolated from the buttock/flank and IFP offer a stable population with low levels of senescence that can differentiate in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. [9, 58]

The harvesting methods for ADSCs are well described and continue to see advancements as new technologies become available. [59] In 1994, Coleman et al. described a technique for harvesting subcutaneous adipose tissue which is still utilized today. [60] A small incision is made over the desired region of adipose tissue, typically in the buttocks or flank. A 3-mm, blunt-edged, 2-hole cannula connected to a 10-mL syringe is used to suction fat manually with a plunger, advancing the cannula through the harvest site while applying negative pressure to isolate the adipose tissue in the syringe. [60] The cannula is replaced with a Luer-Lock aperture and the aperture is removed prior to the device being placed in the centrifuge. [60] This method can be performed via a "wet" or "dry" method. [61] The "wet" method utilizes an injection in the donor site that contains 0.9% NaCl, epinephrine, and a local anesthetic, whereas the "dry" method does not employ use of tumescent fluid. [61] Illouz and de Villers demonstrated that the "wet" technique can facilitate fat aspiration via hydrodissection. [62] IFP is harvested arthroscopically with use of a shaver and curette. The adipose tissue is removed from the intra-articular space through arthroscopic portal and placed into a reservoir for fat isolation. The benefit of this procedure is that it can often be associated with arthroscopic knee surgery, thus avoiding a separate liposuction procedure.

Significant variety exists among protocols for ADSC isolation; however, the technique described below offers a fairly standard method of enzymatic preparation in the United States. [59] Typically, the lipoaspirate is mixed with a collagenase mixture consisting of 0.1% (*w*/*v*) collagenase (Worthington Biochemical Products, Lakewood, NJ), 1% (*v*/*v*) bovine serum albumin (fraction V) (Atlas Biologicals, Fort Collins, CO), and 2 mM calcium chloride in phosphate-buffered saline (PBS, Gibco, Fisher Scientific). [59] Alternative enzymes used for fat separation include trypsin and dispase. [63] After a period of incubation, the blood layer is aspirated off leaving a layer of fat, which is then washed several times with D-PBS (1:1 volume) for further isolation. Occasionally, an erythrocyte lysis step is included to extract erythrocyte contamination and to decrease the amount of hematopoietic progenitor cells. The fat layer is then mixed 1:1 (*v*/*v*) with warm D-PBS/collagenase solution and subjected to several cycles of centrifugation with serial aspiration of the supernatant and resuspension of concentrated pellets. The liposuction aspirate can then be expanded under sterile laboratory conditions with sequential media refeedings over the next 7 days. [59] The cells are harvested via trypsinization and pelleted via centrifugation. From here, the isolated ADSC sample can now be considered a dynamic source of multipotent stem cells that can be utilized in many different ways: (1) further passage (up to 4 passages before concern for senescence), (2) freezing for further use, or (3) osteogenic or chondrogenic differentiation in pellet culture or alginate beads (Pronova LVG USP). [59, 64, 65]

The sources of variability in different enzymatic isolation techniques primarily involve the following steps during processing: (1) number of washing steps, (2) enzyme concentrations, (3) centrifugation parameters, (4) erythrocyte lysis methods, and (5) culture conditions. [63, 66–68] Concerns over the high cost, as well as senescence and decline in multipotency with enzymatic processing techniques, have encouraged the development of other technologies that rely on mechanical means of isolation. [63, 69] As discussed in the future directions section, reducing variability in these protocols will be imperative to allow for homogenous comparisons between studies when considering the efficacy and safety as well as outcomes for the use of ADSCs in clinical trials.

Potential non-enzymatic solutions for separation of ADSCs from lipoaspirate include use of shear force, gravity separation, centrifugal force, vibration energy, and pressure. A number of automated devices on the market claim to offer a reproducible, viable SVF sample for injection into the diseased or injured tissue of interest (Table 2).

Centrifugation is a mechanical technique that capitalizes on the varying molecular weights of the lipoaspirate contents to separate the SVF from residual lipids and inflammatory markers. The AdiPrep® Adipose Transfer System (Adiprep, Harvest Technologies Corporation, Plymouth, Mass.) is a commercially available system which isolates adipose tissues from lipoaspirate using a proprietary lipid barrier disc technology that aims to achieve more effective layer isolation than standard gravity decantation methodologies. [10, 77] Dragoo et al. [10] described a promising novel application of this technique using modern instrumentation for isolating ADSCs from arthroscopically harvested IFP and surrounding synovium. The IFP-derived sample is collected into a lipoaspirate filtration system (AquaVage, M.D. Resource, Livermore, Calif.) followed by fat fractionization with a syringe emulsification technique and finally concentrated with the AdiPrep® Adipose Transfer System. They report successful SVF isolation with mean yield of $4.86 \pm 2.64 \times 10^5$ cells/g of tissue and a mean viability of $69.03\% \pm 10.75\%$, achieving the standard cutoffs for therapeutic benefits of treatment. This method provides physicians with a simple means of obtaining autologous sources or stem cells for regenerative procedures.

Puregraft ® 250 System (Puregraft LLC, Solana Beach, CA), and Lipogems (Lipogems, Norcross, GA) are specific

**Table 2** Enzymatic and mechanical methods of ADSC isolation

| | Product | Company | Design | Publication/ patent |
|---|---|---|---|---|
| Mechanical | Harvest® AdiPrep® | | | |
| | Cha-Station™ | Somnotec http://www.somnotec.net | Syringe Emulsification and Centrifugation | [10] |
| | Sieve Shaker | PNC International Co., Ltd. http://inphronics.com.sg/equipment/cha-station | Semiclosed, semiautomated | [70] |
| | Octagon D200 | Endecotts Ltd. *https://www.endecotts.com* | Electromagnetic vibration machine | [71] |
| | Lipokit with Maxstem | Medi-Khan *http://www.medikanint.com/* | Semiclosed, manual, centrifugation | [70] |
| | Puregraft 250 | Puregraft LLC *http://www.puregraft.com* | Semiclosed, manual, filtration | [65] |
| | Lipogems | Lipogems *https://understandlipogems.com* | Closed, filtration | [72] |
| Enzymatic | Rigenera cons | HBW srl *https://rigenerahbw.com/* | Single-use, closed, centrifugation | [60] |
| | Sepax™ C-Pro | GE Healthcare *https://www.gelifesciences.com* | Semiclosed, semiautomated | [73] |
| | Celution® 800/CRS | Cytori Therapeutics, Inc. *http://www.cytori.com* | Semiclosed, automated, decantation, +collagenase | [70] |
| | GID SVF-1 | GID Group, Inc. *http://www.thegidgroup.com/* | Closed, manual, filtration, +collagenase | [74] |
| | Tissue Genesis® Icellator | Tissue Genesis, Inc. *http://www.tissuegenesis.com/icellator* | Semiclosed, semiautomate, +adpiase | [75] |
| | Cellthera Kit I and II | Cellthera, Ltd. *https://www.cellthera.org* | Closed, automated, +collagenase | [76] |

technologies designed to isolate ADSCs through non-invasive, mechanical means. Studies have shown washing with filtration in a closed system, such as Puregraft, produces a fat graft with higher tissue viability with few tissue contaminants. [65] In basic science studies, Lipogem technology touts preserved exosome activity given an intact stromal vascular niche, which increases bioactivity in a paracrine fashion following MSC activation. The proposed benefit of this method is cartilage restoration that mimics adjacent tissue. [53, 72, 78, 79]

Newer technologies are currently under development to procure ADSCs in a mechanical fashion. One such tool, Rigenera cons (HBW srl, Turin, Italy), is a disposable tool that decreases the dimension of adipose tissue scraps in a non-enzymatic fashion and produces a cellular suspension injectable with a needle, known as an adipose micro-graft. [80] The use of vibrational energy to isolate ADSCs has been described and theoretically may provide surgeons with another non-enzymatic method to produce isolated ADSCs. The initial study on this technology demonstrated that ADSCs treated with a vibration machine, Sieve Shaker Ocatgon D200 (Endecotts Ltd., London, United Kingdom), did not affect the viability or proliferation of the cultured ADSCs. [71] However, the initial study failed to isolate ADSCs from IFP utilizing vibrational energy alone, suggesting that ADSCs from IFP may require alterations in the vibration frequencies utilized in the initial study.

As various enzymatic and non-enzymatic technologies continue to be developed, the most successful technique will likely be the one that allows for the greatest amount of multipotent differentiation while ensuring ease and reliability in obtaining tissue. Bertozzi described a hybrid method for harvesting adipose tissues and isolating MSCs using a combination of mechanical and enzymatic methods that produces ready-to-use ADSCs pellet in 80 min. [81]

Another area that holds promise is augmentation of the AVF isolate with biologics. Sceldis® (ED Co. Ltd. & Purebiotech Co., Ltd., South Korea/Medica Group, United Arab Emirates) is one such technology that has been used in orthopedics in which autologous SVF is injected along with PRP, hyaluronic acid, and $CaCl^2$ to treat knee pain following meniscus tears. [82, 83]

There is currently limited literature comparing the various technologies available. Moreover, there is a paucity of prospective studies evaluating the benefit of these preparations in isolated injections. Future research is warranted to better clarify the components of these formulations and steps in preparation as to standardize this process. Standardization and promulgation of these methods will allow for safer use of ADSCs and for more homogenous comparisons of outcomes between studies.

## Evidence from Animal Models

### Therapeutic Mechanisms in Achilles Tendon Injury Models

The use of ADSCs has been studied in various models of induced injuries in animals. Lee and colleagues [84] obtained

ADSCs from the lipoaspirate of human subcutaneous fat tissue of healthy donors. They washed lipoaspirates with PBS and digested them in PBS containing 1% bovine serum albumin and 0.025% collagenase type I. Next, the isolated SVF was cultured to obtain a sufficient number of cells for injection. Finally, ADSC were harvested by trypsinization, suspended, and tested for purity. Using these ADSCs, the authors found that implantations of ADSCs in Sprague-Dawley rats with full-thickness rectangular defects in the Achilles tendon led to better gross morphological and biomechanical recovery than those in both the fibrin and sham groups. In addition, they found that ADSCs significantly increased the expression of human-specific type I collagen and tenascin-1. The authors concluded that one of the benefits of ADSCs was the propensity to secrete proteins and provide potential for therapeutic healing.

In a controlled laboratory study, Oshita et al. [14] investigated the effects of ADSCs on Achilles tendon healing in 16 F344/NSIc rats that underwent collagenase injections in the Achilles tendon to simulate tendinopathy. One week after this injection, eight rats received ADSCs while eight received saline. ADSCs were harvested from the inguinal fat pads of two F344/NSlc rats. Processing of ADSC consisted of finely mincing and enzymatically digesting the fat pads using 0.15% type I collagenase at 37 °C and adding an equal volume of DMEM containing 10% FBS for neutralization. At this point, the cells were centrifuged at 1500 rpm for 5 min and seeded at a density of 105 cells/100-$mm^2$ tissue culture plate and maintained in control medium of DMEM with 10% FBS and 1% antibiotic-antimycotic solution at 37 °C and 5% $CO_2$. Finally, cells were passaged using 0.25% trypsin when they reached 90% confluency. At both 4 and 12 weeks after treatment, the authors found that the ADSC group showed a significantly lower degree of tendon degeneration than the saline group. Furthermore, the type III to type I collagen ratio was significantly lower in the ADSCs group and this continued to decrease relative to the saline group. The authors concluded that ADSCs result in significant improvements in pathological findings associated with tendinopathy (inflammation and micro-injury in tendons during the acute stage and a decrease in type I collagen in tendons).

## Therapeutic Mechanisms in Flexor Tendon Healing

ADSCs have also been studied in controlled environments to investigate their ability to aid tendon regeneration. The following study did not report their method of ADSC harvesting and processing. Tendon regeneration in one study was stimulated using connective tissue growth factor (CTGF) with or without ADSC. The ADSCs were applied to the repair surface of a canine flexor tendon using cell sheets, and the CTGF was delivered via porous structures. At 14 days following repair, both treatments reduced inflammatory markers and matrix degradation gene expression, while increasing collagen synthesis in comparison to controls. However, they showed that the addition of ADSCs was more effective than CTGF alone in reducing inflammatory markers, increasing collagen, and increasing tendon stem/progenitor cells at the tendon surface and along suture tracks. The authors proposed that this combined approach using ADSCs may promote expedited flexor tendon healing and warrants further investigation. [17]

## Therapeutic Mechanisms in Rotator Cuff Injury Models

Chen et al. [85] investigated the effects of ADSCs in a rat model of collagenase-induced rotator cuff injury and compared their results to a control group that received a saline placebo injection. In this study, ADSCs were provided by GWOXI Applied Technology Co., Ltd. (Hsinchu, Taiwan) from a 3-g tissue sample from the infraumbilical region of a 46-year-old Asian male. Processing was performed in a third-party laboratory where isolated ADSCs were cultured in Keratinocyte-SFM (Gibco, Grand Island, NY, USA), 10% FBS (Hyclone, UT, USA), 50 μg/mL bovine pituitary extract (Gibco), 5 ng/mL human recombinant epidermal growth factor (Gibco), 2 mM N-acetyl-l-cysteine (Sigma), and 0.2 mM l-ascorbic acid 2-phosphate (Sigma) under an atmosphere of 5% $CO_2$ at a temperature of 37 °C. The authors found that at 14 days post-ADSC injection, inflammatory cells were significantly decreased in this group compared to controls and that the fiber arrangement and tendon organization were also superior histologically. They also found that the load to failure of the ADSC group was significantly greater than in the control group ($15.87 \pm 2.20$ N vs. $11.20 \pm 1.35$ N; $p < 0.001$), suggesting superior supraspinatus tensile strength conferred by the addition of ADSCs. Furthermore, on day 28, the injured tendon had a tensile strength equivalent to an uninjured tendon, suggesting full recovery.

The rotator cuff has also been the subject of several other studies. Notably, in one study concerning 50 Sprague-Dawley rats that underwent detachment and repair of the supraspinatus tendon, animals were randomized to receive a collagen carrier alone or collagen carrier with ADSCs. ADSCs were harvested from subcutaneous adipose tissue of two rats, after which the lipoaspirate was washed with PBS and digested at 37 °C for 30 min with collagenase. Ten percent FBS was used to neutralize enzymatic activity, and the mixture was then centrifuged at 300*g* for 10 min. The resultant pellet was treated with 160 mM ammonium chloride for 10 min and then washed and suspended in DMEM plus 10% FBS. This was centrifuged again and the resulting cell suspension was filtered through a 70-μm nylon mesh and the material obtained was resuspended in DMEM with glucose and pyruvate, 10% FBS, 2 mM glutamine, 1% streptomycin 10 μm/mL, and penicillin 1 UI/mL. The cells were then plated in 100-mm tissue-culture dishes at

10 to 15 × 10 3 cells/mL and cultured at 37 °C in a humid atmosphere with 5% carbon dioxide in DMEM containing 10% FBS and 1% penicillin/streptomycin. The medium was changed to remove non-adherent cells 24 h after seeding, and every 4 days thereafter. After their experiment, the authors found no differences in biomechanical variables, but histologic examination demonstrated significantly less inflammation and edema in the ADSCs group. The authors proposed that this may lead to a more elastic repair and less scarred healing. [16]

Similarly, Kim et al. [86] created bilateral supraspinatus tears in 11 rabbits and repaired them after 3 weeks. They randomized which supraspinatus received ADSCs at the time of repair and injected the ADSCs at the muscle belly near the musculotendinous junction and saline in the control side. ADSCs were harvested from subcutaneous adipose tissue of New Zealand male rabbits and digested with a type I collagenase solution (Invitrogen, Carlsbad, CA, USA) with gentle agitation for 1 h at 37 °C. They separated the upper adipocyte fractions from stromal fractions by centrifugation at 1200*g* for 10 min. The remaining stromal fractions were treated with 3-mL red blood cell lysis buffer (Sigma-Aldrich) for 10 min at room temperature, filtered through a 100-μm nylon mesh, and centrifuged at 1200*g* for 10 min. The combined stromal fractions of the samples were re-suspended and cultured in DMEM with 5% FBS and cells were allowed to adhere to the flask for 24 h at which point fresh media were added. The cells were incubated at 37 °C and 5% $CO_2$, and culture media were changed every 2–3 days. To perform the transplantation, cells were suspended to a concentration of $1 \times 10^7$ labeled cells/500 μL of Hank's balance salt solution (HBSS) and then were injected. The authors found significantly increased expression of both IFG-1 and myosin heavy chain (MyHC) in the ADSCs-injected rotator cuff, suggesting potential anabolic effects of ADSCs use in rotator cuff pathology.

## Cartilage Regeneration in Animal Models

ADSC therapy for treatment of osteoarthritis (OA) and chondral defects in animal models has been well documented. [87] Toghraie et al. [88] used ADSCs derived from infrapatellar fat pad (IFP) in rabbits subsequently administered to rabbits with OA-induced knees. They processed these ADSCs as follows: the fat pad was minced and washed three to four times with PBS after which it was suspended in an equal volume of PBS with 1% FBS and 0.1% collagenase type I prewarmed to 37 °C. The tissue was subjected to continuous agitation for 3 h in a shaking bath and centrifuged for 5 min at 300*g* at room temperature. The supernatant was removed, and the pelleted stromal connective tissue fraction was resuspended in culture medium (DMEM, 10% FBS, penicillin–streptomycin, actinomycine) and plated for culture. Following 48 h of incubation at 37 °C/5%$CO_2$, the cultures were washed with PBS and maintained in stromal medium. Non-adherent cells were aspirated with the medium after 24 h of cultivation and fresh medium was added and changed every 3 days. The cells were sub-cultured three times by using standard methods of trypsinization and cells achieved confluence after 10 to 20 days when they were separated from the culture flask by treating with 0.25% trypsin. The authors found that administration of these ADSCs delayed the progression of osteoarthritic changes, including cartilage thinning, osteophyte formation, and subchondral sclerosis in the joint.

Ude et al. [89] administered intra-articular injections of labeled autologous ADSCs into osteoarthritic sheep knees to study their effect on cartilage regeneration. They harvested adipose tissue from the right infra patella fat pad of the sheep during the surgical resections at week 1. Processing of the adipose consisted of mincing with a surgical blade before digestion with an equal volume of 0.6% collagenase II (Gibco) in an orbital incubator at 37 °C with 21-g force for 2 h. Subsequently, the digest was filtered with a cell strainer of 100 μm pore size and centrifuged at a 4724-g force for 5 min at 37 °C. The resultant pellet was washed with PBS twice and basal medium before culture. The authors of this study found that ADSCs preferentially populated to areas with osteoarthritic change and decreased the progression of OA throughout the knee. Furthermore, histological staining revealed loosely packed matrices of de novo cartilage, while immunostaining demonstrated the presence of the cartilage specific proteins collagen II and SOX9.

Dasandro et al. [90] harvested adipose from the inguinal zone of adult male New Zealand rabbits. This group processed the adipose by treating it with 0.4 U/mL NB4 collagenase standard grade, after which they re-suspended the SVF in α-MEM (Gibco) supplemented with 1 U/mL heparin (Sigma, St Louis, MO, USA), 2% platelet growth factor enriched plasma (PGFEP), and 0.05 g/mL penicillin G (Gibco). After culturing, cells were harvested and seeded at a density of 2000 cells/$cm^2$ for expansion. The selection of ADSCs was determined by ability to adhere to the plastic, to form colonies, and to differentiate into chondrogenic and osteogenic lineages. After intra-articular administration into osteoarthritis-induced knees of adult male New Zealand rabbits, the authors found that labeled ADSCs were detected in the synovial membrane and medial meniscus but not in the cartilage tissue at 3 to 20 days after injection. At these same time periods, white cartilage with no degenerative noticeable macroscopic evidence was observed in the ADSCs-treated groups. Furthermore, a significant reduction of the fibrillin index and significant increase in cartilage thickness was found in the ADSC group compared to the control group. The decrease in OA progression with ADSC suggests that there may be some trophic mechanism of action by release of growth factors and cytokines that stimulate healing and chrondrogenesis.

These animal studies suggest that ADSC therapy may offer patients who suffer from arthritis a non-invasive, safe, and easy option for treatment of arthritis.

Together, the proposed evidence from the basic science literature confirms the propensity for ADSCs to differentiate into several biologic lineages which may serve therapeutic functions in the realm of clinical sports medicine and preservation efforts. Multiple studies within the past 5 years that have investigated the effects of ADSCs in animal models have demonstrated the efficacy of this biologic and its potential for clinical use. [15, 85, 91, 92] Indeed, in many aspects of sports medicine where solutions for preservation are scarce, such as cartilage regeneration and bone remodeling, ADSCs represent an area of potential to aid the controlled restoration of normal biological growth to promote healing. As discussed above, few studies currently exist which have investigated the use of ADSCs as a source of mesenchymal stem cells in particular; however, the ease of harvesting human ADSCs in conjunction with these early preliminary results suggest that future investigations are worthwhile.

## Evidence from Clinical Studies

The subjects of clinical studies which have investigated the effects of ADSCs have primarily been restricted to the Achilles tendon, knee osteoarthritis, and osteochondral defects of the knee (Table 3). These studies represent heterogeneous populations in which many variations of ADSC formulations are employed. Despite these limitations, the current evidence suggests potential benefits for the use of ADSCs.

### Treatment of Achilles Tendinopathies

Usuelli and colleagues [93] sought to compare the efficacy of PRP with the stromal vascular fraction of ADSCs (SVF) in 44 patients with Achilles tendinopathy in a randomized, controlled trial (RCT). Adipose tissue was harvested from each subject via lipoaspiration of abdominal subcutaneous tissue. It was also noted that two very thin patients were required to have their adipose tissue harvested from the medial aspect of their thighs. All adipose tissue was processed with the FastKit system (Corios, San Giuliano Milanese, Italy) and mechanically digested by rubbing tissue down through a 120-μm internal filter. The SVF was collected and centrifuged at 400*g* for 10 min after which the resulting bottom phase was transferred to a new syringe and used for injections. Postoperatively, the authors found a significant benefit for the SVF group compared to the PRP group when analyzing the VISA-A, VAS pain, and AOFAS score at 15 and 30 days; however, this effect did not persist past this time point. Given that all patients experienced improvement from baseline, the authors concluded that both PRP and SVF were safe and effective treatments for Achilles tendinopathy and that patients with SVF may obtain faster results.

### Treatment of Rotator Cuff Tendinopathy

Clinical studies investigating the therapeutic effects of ADSCs on rotator cuff tendinopathy are sparse. Striano et al. [94] injected micro-fragmented adipose tissue harvested from the abdomen and processed with Lipogems® (Lipogems USA, Atlanta Ga.) into 18 shoulders with varying cuff pathology. At 1-year follow-up, significant improvement in National Pain Scale (NPS) and American Shoulder and Elbow Surgeons Score (ASES) were reported, from 7.5 to 3.6 and 33.7 to 69.2, respectively. Prospectively designed studies with large numbers of participants are required to further confirm the benefits of ADSCs in rotator cuff tendinopathy.

### Treatment of Osteoarthritis

Clinical studies involving ADSCs therapy suggest that these cells may offer a non-surgical treatment option for degenerative joint disease. A number of clinical studies have been conducted to understand the potential benefit of these interventions in treating various degrees of osteoarthritis.

Pers et al. [95] conducted a bicentric, uncontrolled phase I clinical trial in which 18 patients with severe osteoarthritis (OA) received a single intra-articular injection of autologous ADSC following. The study design consisted of a dose escalation stratification. Three consecutive cohorts (six patients each) were created in which the first six received a low dose ($2 \times 10^6$ cells) injection of ADSCs, the next six received a medium dose ($10 \times 10^6$), and the third six received a high dose ($50 \times 10^6$). All patients underwent outpatient liposuction for adipose harvesting, and ADSCs were processed at a single facility (Etablissement Français du Sang Midi-Pyrénnées, France) using a previously established and meticulous protocol. [107] At 6-month follow-up, the procedure was found to be safe and associated with improved pain and function levels compared to baseline in all patients. [95] Panchal et al. [96] reported improvement in pain, quality of life, and functional outcome scores at 12 months following ultrasound-guided injection of micro-fractured adipose tissue into 25 arthritic knees (Kellgren-Lawrence, grade of 3 or 4). The average Knee Society Score (KSS) improved from 74 to 82, while the lower extremity activity scale (LEAS) saw improvements from 36 to 47 at the 1-year post-injection timepoint. Additional studies involving intra-articular injections of ADSCs into OA knees have reported associations with reduced pain and improved knee function. [19, 20]

Cattaneo et al. [97] sought to investigate the effects of autologous micro-fragmented adipose tissue injections in conjunction with arthroscopic procedures (meniscectomy or chondral shaving) for 38 patients with symptomatic knee

**Table 3** Evidence from human clinical studies for the use of ADSC

| | Study design | Subjects | Location | Comparison | Results | Conclusion |
|---|---|---|---|---|---|---|
| Tendinopathy | | | | | | |
| Usuelli et al. [93] | RCT | 44 | Achilles | ADSC + PRP vs. PRP alone | Early VAS pain, AOFAS, and VISA-A score benefits | PRP and SVF safe and effective; SVF may provide faster results |
| Striano et al. [94] | Case Series | 18 | Rotator Cuff | None | Improvements in National Pain Scale and American Shoulder and Elbow Surgeons Score (ASES) | Use of ADSCs resulted in significant improvements in pain, function and quality of life at 12 months |
| Osteoarthritis | | | | | | |
| Pers et al. [95] | Case Series | 18 | Knee | None | Improvements in KOOS, SAS, WOMAC VAS Pain | ADSCs are safe and well tolerated in patients with knee osteoarthritis |
| Panchal et al. [96] | Case Series | 17 | Knee | None | Improvements in numerical pain rating scale, knee society score, and lower extremity activity scale | The injection of autologous, micro-fractured, minimally manipulated adipose tissue appears to be a safe and effective treatment option for patients with refractory, severe (grade 3 or 4) knee OA. |
| Cattaneo et al. [97] | Case Series | 38 | Knee | None | Improvements in KOOS and WOMAC scores | Micro-fragmented adipose tissue injection associated with arthroscopic procedures is a safe and benefical adjunct for symptomatic knee OA |
| Dall'Oca et al. [98] | Case Series | 6 | Hip | None | Improvements in HHS and WOMAC scores | MSC Lipogems is a fairly easy technique and no adverse effects were observed. Preliminary results showed a positive outcome. |
| Hudetz et al. [99] | Case Series | 17 | Knee | None | Increases in contents of cartilage glycosaminoglycans; improved VAS pain scores; no differences in N-glycan or IgG synovial concentrations | The use of autologous and microfragmented adipose tissue in patients with knee OA (measured by dGEMRIC MRI) increased glycosaminoglycan (GAG) content in hyaline cartilage, which is in line with observed clinical results |
| Russo et al. [100] | Case Series | 22 | Knee | None | Improvements in KOOS Lysholm, VAS Pain, and IKDC scores | Autologous and micro-fragmented adipose tissue injection is an innovative and safe approach for the management of diffuse knee OA in the mid-term. |
| Panni et al. [101] | Case Series | 52 | Knee | None | Improvements in IKS and VAS pain scores | Injection of ADSCs during arthroscopic debridement increased clinical and functional scores in patients with early knee OA at a mid-term follow-up, especially those with higher pre-operative VAS scores. |
| Jo et al. 2014 [20] | Case Series | 18 | Knee | None | Improvement in WOMAC score; size of cartilage defect decrease; volume of cartilage increase; thick, hyaline-like cartilage regeneration. | ADSC improved function and pain of the knee joint without causing adverse events; reduced cartilage defects |
| Koh et al. 2013 [19] | Case Series | 18 | Knee | None | Improvement in WOMAC and Lysholm scores; decrease in VAS pain; improved cartilage whole-organ MRI score | ADSC effective and safe for improving function, reducing pain |
| Kim et al. 2015 [102] | Cohort | 20 | Knee | ADSC+PRP injection vs. ADSC implantation alone | Improved IKDC, Tegner scores in implantation group | |

**Table 3** (continued)

| | Study design | Subjects | Location | Comparison | Results | Conclusion |
|---|---|---|---|---|---|---|
| | | | | | | ADSC implantation for knee OA resulted in better clinical and second-look arthroscopic outcomes than an ADSC injection. |
| OCD | | | | | | |
| Kim et al. [18] | Case Series | 55 | Knee | None | Improved IKDC, Tegner scores | Encouraging clinical outcomes |
| Kim et al. [103] | Cohort | 49 | Talus | Marrow stimulation alone | Improved VAS pain, AOFAS, and Tegner, cartilage restoration | Encouraging results, even in setting of poor prognostic factors |
| Kim et al. [104] | Case Series | 24 | Knee | None | Improved IKDC, Tegner scores; improved cartilage lesion grade on MRI | ADSC useful for repairing cartilage lesions |
| Koh et al. [21] | RCT | 80 | Knee | Microfracture alone | Improved KOOS, cartilage signal intensity; 65% in ADSC group with complete cartilage coverage vs. 45% in control group | ADSC provided radiologic. KOOS pain and symptom subscore improvements; no differences in activity, sports, or quality-of-life |
| Freitag et al. [105] | Case Report | 1 | Knee | None | Improvement in NPRS pain score, Global WOMAC score, and all components of the KOOS; MRI showed complete filling of the chondral defect; modified ICRS score improved from Grade 3 to Grade 0. | The use of injection of autologous ADSCs following a traumatic chondral defect of the patella resulted in significant pain and functional improvements and complete regeneration of hyaline-like cartilage within the defect. |
| D'Ambrosi et al. [106] | Case Series | 4 | Talus | None | Improvements in the American Orthopedic Foot and Ankle Society score and VAS pain score; no complications. | Autologous microfractured, purified adipose tissue is safe and effective for osteochondral lesions of the talus. |

OA. Adipose tissue was obtained using a minimal manipulation technique from the lower or lateral abdomen. The authors processed all harvested adipose tissue using the Lipogems® processing kit. At a minimum 12-month follow-up, patients on average demonstrated statistically significant improvements from baseline in all components of the KOOS questionnaire and the WOMAC score. They also reported that all 38 patients claimed to be satisfied with the treatment and that none were symptomatic, with the exception of one patient when performing squatting. No complications or adverse events were documented.

Dall'Oca et al. [98] retrospectively studied the outcomes of six consecutive patients with hip OA who were treated with intra-articular injections of autologous ADSCs. Adipose tissue was harvested from the abdominal wall and was processed using the Lipogems® processing kit. At 6-month follow-up, patients demonstrated mean improvements in the Harris hip score (67.2 vs. 84.6, $p < 0.001$), the WOMAC score (36.3 vs. 19.8, $p < 0.001$), and the VAS pain score (4.6 vs. 1.5, $p < 0.001$). One patient developed a hematoma on the abdomen at the site of adipose tissue harvest.

Hudetz et al. [99] investigated the effects of intra-articular injections of microfragmented adipose tissue on proteoglycan synthesis in patients with knee OA. The authors enrolled 17 patients in a prospective, single-center, open-label clinical trial and assessed through clinical and imaging outcomes as well as synovial fluid analysis at 12 months post-operatively. Adipose harvest was procured from abdominal subcutaneous adipose tissue and was processed using the Lipogems® processing kit. At follow-up, the authors reported (1) decreases in VAS pain scores (3.9 vs. 0.6, $p < 0.001$), (2) no significant differences in N-glycan or IgG synovial concentrations, and (3) increases in cartilage glycosaminoglycans via dGEMRIC index values.

A recent study by Russo et al. [100] suggested that the therapeutics effects of ADSCs for knee OA may persist as long as 3 years. Adipose tissue was harvested from the lateral or lower abdomen and was processed using the Lipogems® processing kit. They followed the outcomes of 30 patients, seven of which were excluded because they underwent additional procedures during the postoperative period (hyaluronic acid or platelet rich plasma injections), while another additional patient was lost to follow-up. In the remaining 22 patients, the authors reported median improvements in KOOS scores and Lysholm Knee scores of 20 points and 7 points, respectively. Furthermore, no adverse events were observed either at the harvest sites or related to the knee joint.

Several studies have looked at injection of ADSCs following arthroscopic debridement. Panni et al. [101] retrospectively analyzed 52 patients with knee OA who received percutaneous injection of ADSC following arthroscopic debridement. Adipose tissue was harvested from the abdomen, and adipose was processed using the Lipogems® processing kit. At 15-month follow up, the mean IKS knee score improved from 37.4 to 62.6 and the IKS function score improved from 57.2 to 83 at latest follow up. Kim et al. [102] in 2015 performed a match-paired cohort study of 40 patients in which one group received arthroscopic debridement and injection of ADSCs with PRP ($n = 20$), while another group underwent debridement and implantation of ADSCs alone ($n = 20$). Adipose was harvested 1 day prior to surgery via tumescent liposuction of each patient's buttocks. Mature adipocytes were processed and separated from the SVF via a centrifugation protocol reported by Zuk et al. [43] and subsequently cultured. Using epitope profiles, ADSCs were isolated. It was found that IKDC and Tegner scores had improved for both groups at second look arthroscopy 1 year following the intervention. At 28.5 months, the mean IKDC and Tegner scores had improved to a significantly greater extent for the implantation group when compared to the group who received an injection. [102]

## Treatment of Osteochondral Defects

Several studies have focused solely on osteochondral defects (OCDs) and attempted to elucidate the benefit of ADSCs through patient reported outcomes, MRI findings, arthroscopic findings, and histologic analysis. Kim et al. isolated and processed ADSCs as above and showed that mean pre- and postoperative IKDC and Tegner activity scores significantly improved following arthroscopic debridement and ADSC injections in knees with isolated full-thickness cartilage lesions. Forty-one of 55 patients (74.5%) reported excellent/good results following surgery, with only 3 (5.5%) patients stating they had poor results. [18] A study by the same author reported on functional outcomes at 2 years following arthroscopic marrow stimulation with injection of SVF containing ADSCs versus marrow stimulation alone in osteochondral lesions of the talus. At mean follow-up of 21.9 months, all outcomes including the VAS pain, AOFAS, and Tegner scores improved in the ADSC-intervention cohort compared to the marrow stimulation-only group. [103] The authors then divided patients by median values for variables such as age, BMI, duration of symptoms, and lesion size to determine predictors of outcomes. MRI follow-up scans showed more extensive cartilage restoration in the ADSC group, even in the setting of poor prognostic factors such as older age (> 46.1 years), large lesion size (> 1.5 cm), and presence of subchondral cysts.

MRI follow-up studies have reported cartilage restoration with ADSCs. Kim et al. isolated and processed ADSCs from patients as reported in the previous studies by this group [18, 102, 103] and reported on isolated OCDs in 24 knees in which ADSC were implanted in a fibrin glue scaffold. Clinical outcomes measured with IKDC and Tegner activity scale scores improved and significantly correlated with similar improvements in cartilage lesion grades on MRI at 2-year follow-up. [104]

Koh et al. [21] reported the results of a prospective RCT in which patients with a symptomatic cartilage defect (> 3 $cm^2$) were randomized to receive ADSCs with fibrin glue with microfracture treatment ($n = 40$) or microfracture alone ($n = 40$) for the treatment of osteochondral lesions of the talus. This group harvested subcutaneous adipose tissue from the buttocks of each patient 1 day prior to surgery and isolated the SVF and ADSC per the protocol outlined by Zuk et al. [43] where following isolation ADSCs were characterized by expression of the surface markers CD90 and CD105 and absence of CD34 and CD14. At 2-year follow-up, 26 patients (65%) in the ADSC group had complete cartilage coverage of the lesion compared to 18 patients (45%) in the microfracture group alone. Better signal intensity was observed in the ADSC group as well. Additionally, KOOS pain and symptom sub-scores were improved. There was no reported difference in activity, sports, and quality of life sub-scores. [21] Furthermore, the authors histologically examined microfracture-repaired OCDs with and without ADSC and found that the mean International Cartilage Repair Society II score was 1054 for ADSC group with microfracture compared to 967 for microfracture alone ($p = 0.036$). [21]

Freitag et al. [105] reported a case on the use of autologous ADSCs for a post-traumatic chondral defect of the patella in a 26-year-old female athlete. The patient initially underwent arthroscopic chondroplasty with chondral loose body removal but failed to symptomatically improve 1 year post-operatively. Repeat MRI at that point demonstrated a persistent patella chondral defect measuring 12 mm. At that point, the patient received intra-articular administration of ADSCs in conjunction with arthroscopic microfracture to the area of full-thickness chondral loss. The source of adipose tissue was the patient's abdomen and ADSCs were isolated by separating the SVF through enzymatic digestion and centrifugation. The SVF was subsequently cultured under hypoxic conditions in 10% FBS, after which non-adherent cells were removed by washing with PBS and expanded up to two passages. After passage 2, cells were harvested, washed three times, and cryopreserved in clinical-grade cryoprotectant media using a validated control rate freezing method of 1 °C/min. The patient experienced progressive improvement in pain (NPRS pain score improved from 8 to 2 within 3 months), quality of life (Global WOMAC score improved from 64 to 92 at 12 months), and all components of the KOOS. Furthermore, structural follow-up using MRI showed complete filling of the chondral defect and the modified ICRS score improved from grade 3 to grade 0.

D'Ambrosi et al. [106] used autologous microfractured and purified adipose tissue to treat four patients with osteochondral lesions of the talus. Adipose tissue was harvested from the abdomen of each patient and processed using the Lipogems® processing kit. At 6 months postoperatively, the authors reported that all patients demonstrated statistically significant improvements in the American Orthopedic Foot and Ankle Society score (46.8 vs. 83.8, $p < 0.05$) and VAS pain score (8 vs. 2.3, $p < 0.005$), and no patients experienced complications.

Together, the evidence from the few clinical studies available shows promising outcomes in the treatment of select musculoskeletal pathologies. The limitation to most of this published literature is the inclusion of other therapeutic biologics, such as PRP combined with ADSC, in the injection. Additionally, these studies lack a control group in most cases, making establishment of causation statistically inappropriate.

## Future Research

The small body of literature that exists on ADSCs in human studies suggests that there is an associated benefit with injection of these cells into diseased joints or tissues. Many studies have reported increased cartilage thickness and viability after treatment with ADSCs. With regards to OA, the majority of studies report clear and consistent benefits in terms of improvements in function and pain when administering ADSCs into the hip and knee joint. Similar benefits are demonstrated with studies concerning Achilles and rotator cuff tendinopathies. However, the drawback on the design of these studies is that most do not have a control group, and those that do often still have some intervention in the control group, whether that be PRP injection, microfracture, or some other type of preservation procedure. Furthermore, these studies often use different protocols to extract and prepare their ADSCs, and a variety of formulations have been found in the literature. The majority of these studies also use a case series design and cannot establish causation between ADSCs and these outcomes. As interest in the use of ADSCs continues to increase, it is imperative to both validate their safety and efficacy with higher quality, prospective studies using consistent, predictable control populations, and to better standardize formulations of ADSCs such that legitimate comparisons between studies can be made. The use of a standardized tool to improve transparency and consistency of the processing and harvesting of ADSCs, such as DOSES, [108] may be a first step towards better understanding the characteristics and uses of ADSCs in clinical settings.

## Conclusion

The proposed mechanisms of actions of ADSCs are in their inherent capacity to differentiate into cartilage and bone and to augment joint, muscle, and tendon preserving treatments. Currently, there exist many different formulations and protocols to prepare ADSCs, with little empirical evidence supporting or rejecting their use. The efficacy of ADSCs has

been consistently demonstrated in animal models for the treatment of many orthopedic pathologies, with few early human studies showing promising results.

## Compliance with Ethical Standards

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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