



## CHEMICAL PROFILE AND CLINICAL EFFICACY OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELL THERAPY IN THE TREATMENT OF KNEE OSTEOARTHRITIS

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### ABSTRACT

Intra-articular injections of adipose-derived mesenchymal stromal cells (AD-MSCs) have emerged as a regenerative therapy to combat the progression of knee osteoarthritis (OA). These multipotent cells have been shown to alter the inflammatory processes inside the knee joint at the cellular level, thus creating a treatment option that both modifies the underlying causes of OA and benefits those who are not surgical candidates. This review article serves to present the following objectives: (1) to summarize the techniques used to harvest AD-MSCs, via micro-fragmentation and enzymatic processing, (2) to characterize the chemical profile and immunomodulatory role of these cells from the current literature, (3) to comprehensively review the clinical efficacy of these interventions, from animal to human studies that investigate the safety concerns, biomolecular changes, and key functional outcomes, and (4) to present areas of future research needed to optimize these interventional regenerative therapies in the treatment of knee OA.

**Keywords:** *Adipose-Derived Stromal Cells, Knee Osteoarthritis, Intra-Articular Injection, Regenerative Medicine, Micro-Fragmented Adipose Tissue (MFAT)*

As the US population continues to age, the prevalence of osteoarthritis (OA) is expected to increase in the coming decades. By the year 2040, an estimated 78.4 million adults, 25.9% of the projected total adult population in the US, will have doctor-diagnosed arthritis.<sup>1</sup> Nearly half of these individuals will report activity limitations related to their degenerative joint conditions. While the exact mechanisms of OA are

not fully understood, this condition is a whole joint disease defined by progressive degeneration of articular cartilage, synovial inflammation, subchondral bone remodeling, and soft tissue damage.<sup>2</sup>

Early knee OA is treated non-operatively with physical therapy and anti-inflammatory medications,<sup>3</sup> neither of which treat the underlying causes of the disease. Once a patient fails this conservative treatment

and progression to advanced knee arthritis (Kellgren-Lawrence grade 3 or 4) is evident on radiographic imaging, total knee arthroplasty is typically performed. However, some patients may not be amenable to surgery given the potential for complications, including deep vein thrombosis, pulmonary embolism, nerve injury, and infection.<sup>4</sup> Additionally, patients younger than age 50 may have concerns related to the potential need of revision surgery in the future.<sup>4</sup> Therefore, there is a clear demand for a treatment option that both modifies the underlying causes of OA and benefits those who are not surgical candidates.

Often considered to be a simple “wear and tear” joint process over time, OA has much more complicated pathogenesis defined by several biomechanical, pro-inflammatory, and cellular mechanisms. OA is not associated with markedly elevated numbers of leukocytes in the joint space, as leukocyte levels are typically less than 2000 cells per milliliter,<sup>5</sup> often much lower than more inflammatory arthritic diseases like Rheumatoid Arthritis (RA).<sup>6</sup> However, the critical inflammatory process in OA is better explained by pro-inflammatory mediators which lead to the production of proteolytic enzymes responsible for degrading extracellular matrix and subsequently eroding joint tissue.<sup>7</sup>

Articular cartilage is made of hyaline cartilage, which allows for a smooth surface between bones and the gliding motion of joints throughout the body. As OA pathogenesis begins, chondrocytes in the articular cartilage initially proliferate in response to the loss of matrix. These chondrocytes are the source of collagens, proteoglycans, and hyaluronan, which maintain this extracellular matrix.<sup>8</sup> With the increased progression of OA, catabolic factors begin to dominate as pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and multiple chemokines drive production of matrix metalloproteinases and other enzymes which promote extracellular matrix (ECM) destruction and chondrocyte apoptosis.<sup>7</sup> The limited vascular supply to cartilage reduces the regenerative capacity in these tissues. Loss of articular cartilage and narrowing of joint spaces lead to friction between bones and subsequent joint pain with limited mobility. Also, osteophytes form joint margins as subchondral bone attempts to remodel during the progression of

the disease.<sup>9</sup> Synovium hypertrophy and inflammation also contributes to joint pain and disease progression via cartilage destruction mediated by similar pro-inflammatory factors and proteases.<sup>10</sup>

To prevent the progression of OA, several regenerative medicine interventions have been developed over the past two decades to restore articular cartilage homeostasis. These interventions, including platelet-rich plasma, bone marrow aspirate concentrate (BMAC), and micro-fragmented adipose tissue (MFAT), have grown in popularity for patients who do not respond to conservative treatment and are not yet surgical candidates.<sup>11–13</sup> Mesenchymal Stromal Cells (MSCs) are multipotent adult stromal cells with multiple utilities for cell-based therapy, including differentiation, the release of regenerative growth factors, and immunomodulation.<sup>14,15</sup> MSCs are derived from perivascular cells called pericytes, commonly found in the trabeculae of bone marrow cavities or near blood vessels within adipose tissue.<sup>16</sup> Once isolated, these cells can be injected into a site of interest, such as an osteoarthritic knee. Compared to BMAC, AD-MSCs are present in higher numbers per unit volume of tissue, more rapidly proliferate in culture, and are less susceptible to senescence secondary to culture expansion, thus resulting in the growing interest in AD-MSCs for regenerative medicine techniques.<sup>17</sup> MSCs are capable of self-renewal and differentiation into muscle, bone, and cartilage, making them particularly useful for preventing the progression of OA.<sup>18</sup> This article serves to review the current literature for the processing of AD-MSCs, the chemical profile and anti-inflammatory properties of AD-MSCs, and the clinical efficacy in the treatment of knee OA.

## PROCESSING ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS

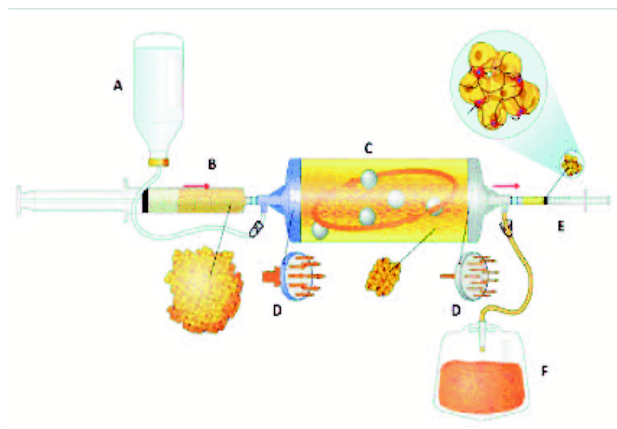
### *Micro-Fragmented Adipose Tissue*

The United States Food and Drug Administration (FDA) heavily regulates the use of human cells, tissues, or cellular or tissue-based products (HCT/P) for clinical use (see Code of Federal Regulations Title 21, Part 1271). Biologic products used clinically (i.e., not for research purposes) must be autologous and no more than “minimally manipulated.”<sup>19</sup> According to the FDA, minimal manipulation refers to the process

of altering the biological characteristics of the original tissue. If processed correctly, the preparation of micro-fragmentation of adipose tissue does not alter these characteristics, and thus is FDA-compliant if the tissue is extracted, processed, and implanted within the same individual (i.e. autologous use) during the same surgical procedure.<sup>19</sup>

A commercially available device that complies with these regulations<sup>19,20</sup> is used to cleanse and gently resize adipose tissue while maintaining the tissue's original microarchitecture, including the stromal vascular niche after processing. Following a minimally-invasive incision to the patient's abdomen, fat tissue is harvested into a completely closed, low-pressure liquid environment, where it is mechanically micro-fragmented and separated from pro-inflammatory oils and blood residues (Figure 1). The final product contains a variety of cell types, including adipocytes, blood vessel endothelial cells, fibroblasts, macrophages, and pericytes within the intact stromal vascular niche, ready to dynamically interact with the patient's local environment after transplant, where the pericytes are subsequently activated as MSCs.<sup>21</sup> During the 20-minute procedure, the processed fat is only subjected to

**FIG. 1** Micro-fragmenting of lipoaspirate after washing of oil, blood, and cellular debris. (A) Sac with a physiologic solution; (B) Syringe with lipoaspirate clusters; (C) Washing chamber containing marbles for the emulsion of fluid and elimination of oil and blood against gravity; (D) Mechanical filters; (E) Syringe with clusters of reduced size; (F) Sac with waste oil and blood. Reproduced from Tremolada et al.<sup>91</sup>



light mechanical forces, with no detrimental effects on the integrity of the stromal vascular niche and/or the structural microarchitecture of the tissue itself, a critical component towards increasing the effectiveness of the tissue in the recipient environment.<sup>22</sup> This method yields a greater concentration of pericytes in a more time-efficient manner when compared to the enzymatic method of obtaining AD-MSCs.<sup>19</sup> With this technique, the collected sample of MFAT can provide damaged tissues with a regenerative environment via one-step autologous grafting in a relatively quick outpatient visit.

### ***Enzymatically-Processed Adipose Tissue***

Alternatively, the stromal vascular fraction (SVF) can be isolated from subcutaneous adipose tissue samples through various enzymatic techniques. Enzymes such as collagenase or trypsin are commonly used to create the single-cell suspensions.<sup>23</sup> Enzymatic SVF isolation techniques vary but typically follow a common standard procedure, including centrifugation to eliminate adipocytes, an erythrocyte lysis step to prevent contamination of cells of hematopoietic origin, several washings, and cryopreservation.<sup>22-24</sup> At this point, there are two options for SVF preparation before joint injection. The SVF, containing a variety of cells including preadipocytes, fibroblasts, AD-MSCs, and white blood cells, can be injected as is.<sup>25</sup> Alternatively, the AD-MSCs can be isolated from the SVF after removing the non-adhesion-plastic cells after 24 hours of culture and subsequent expansion *in vitro*.<sup>26</sup> While some studies have demonstrated the clinical value of both types of autologous adipose SVF, alone or in combination with platelet-rich plasma (PRP) or hyaluronic acid, for joint conditions such as osteoarthritis,<sup>27-31</sup> this technique has its challenges. In addition to concerns related to high-costs, safety, and contamination,<sup>32,33</sup> this method of processing does not meet the standard of “minimal manipulation”<sup>34</sup> and has been classified as “more than minimal manipulation.”<sup>35</sup> Without a much more stringent Biological Licensing Agreement (~5-year process), these methods cannot be commercially manufactured for clinical use.<sup>34</sup>

### **BIOCHEMICAL PROFILE**

After injection of AD-MSCs, the tissue and reparative cells produce a variety of bioactive molecules

that are secreted via exosomes – lipid vesicles used to transport cellular proteins and genetic information to signal nearby cells in the affected joint space. The exosome content is more concentrated in micro-fragmented adipose tissue compared to the enzymatic method.<sup>24,36,37</sup> The enzymes digest the ECM surrounding the cells and damage the adipose cells themselves, thus affecting secretory functions and exosomes during processing.<sup>21</sup> Minimally manipulated adipose tissue preserves the structural niche around the pericytes, which helps to preserve their paracrine effectiveness in the affected joint space.

Several studies have characterized MFAT by immunohistochemical analysis and found a higher number of cells positive for CD34, CD146, S-100 protein, and  $\alpha$ -smooth muscle actin when compared with fat tissue aspirate in vitro.<sup>19</sup> Ceserani et al. found that MFAT was composed of an abundant number of microvascular endothelial cells, positive for CD31, CD34, and CD146, surrounded by several stromal cells expressing mesenchymal markers including CD44, CD105, and TGF- $\beta$ 1, with most of them positive for the pericyte marker NG2.<sup>38</sup> Carelli et al. also found that MFAT positively stained for S-100, vimentin,  $\beta$ -tubulin III, and fatty acid-binding protein 4.<sup>37</sup> Furthermore, AD-MSCs express markers such as CD13, CD29, CD44, CD63, CD73, CD90, and CD105. These cells are also negative for markers associated with hematopoietic antigens, such as CD14, CD31, CD45, and CD144.<sup>39</sup> These markers serve to identify AD-MSCs that are present in MFAT and suggest the relationship between pericytes and MSCs post-injection after the procedure. Furthermore, Kouroupis et al. recently demonstrated in a rat model of acute synovitis that MSCs from the infrapatellar fat pad, when primed with pro-inflammatory and pro-fibrotic factors, display a sharp increase in CD10 expression and a concomitant decrease in Substance P, which is a crucial modulator in propagating the inflammatory and nociceptive pathways in OA.<sup>40</sup> This suggests a role CD10-expressing cells may have in directing the decreased inflammation and pain associated with OA.

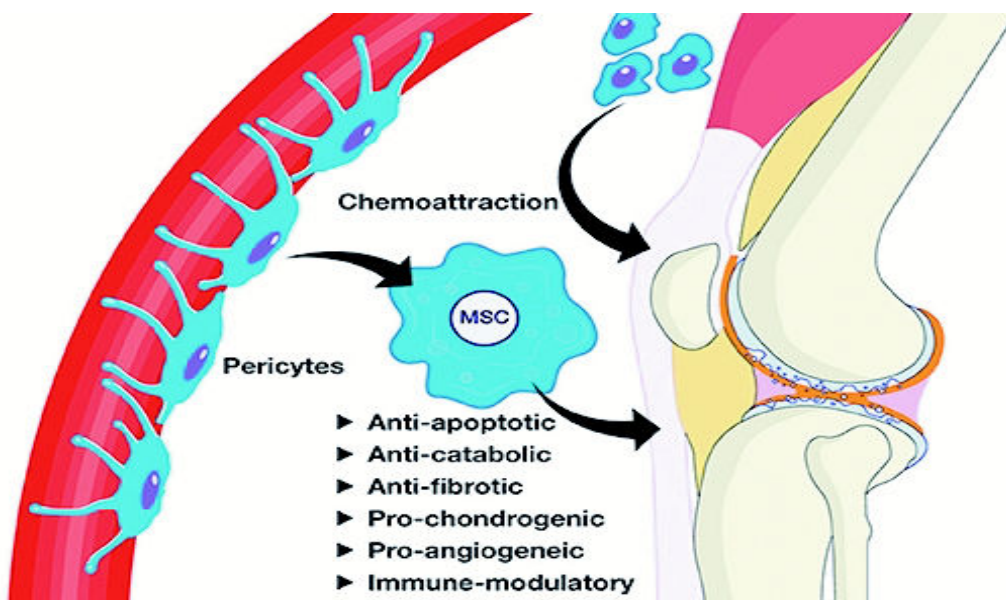
In addition to their cell markers, the bioactive molecules secreted by AD-MSCs have also been recently described (Table 1). These paracrine factors serve to trigger and sustain angiogenic, neurogenic,

osteogenic, antifibrotic, antiapoptotic, antimicrobial, and immunomodulatory responses in the affected tissues (Figure 2).<sup>19,22,36,38,41</sup> Specifically, AD-MSCs have been shown to secrete vascular endothelial growth factor and hepatocyte growth factor, both of which promote neovascularization for host tissue repair.<sup>42</sup> These cells have also shown the ability to attenuate lymphocyte proliferation by secreting anti-inflammatory and immunosuppressive factors such as prostaglandin E2, iNOS, IDO, TSG6, HO1, TGF- $\beta$ , IL-10, and galectins.<sup>43–46</sup> By preventing excessive inflammation, AD-MSCs can promote tissue repair and regeneration by limiting further immune-mediated tissue damage.<sup>47,48</sup> Recently, Nava et al. found that when comparing MFAT and fat lipoaspirate, MFAT cells have longer-lasting anti-inflammatory activity and cytokine production, presumably due to the long-term survival of its MSC content supported by the stromal vascular niche.<sup>46</sup>

Another key role of AD-MSCs specific to OA involves synovial macrophages (SMs). In joints affected by OA, SMs have been shown to produce catabolic mediators, namely matrix metalloproteinases (MMPs) and aggrecanases, and pro-inflammatory mediators, such as IL-6, IL-1 $\beta$ , TNF- $\alpha$ , CCL2/MCP-1, and CCL3/MIP-1 $\alpha$ .<sup>49</sup> Recent studies have found that the secreted factors from MSCs can influence macrophage function and polarization to an anti-inflammatory phenotype via PGE2, resulting in increased secretion of IL-10 and decreased secretion of pro-inflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-12).<sup>50,51</sup> Paoletta et al. found that MFAT in coculture with synoviocytes from osteoarthritic patients produced low levels of catabolic and pro-inflammatory mediators such as CCL5/RANTES, CCL2/MCP-1, CCL3/MIP-1 $\alpha$ , and MMP-9. MFAT also increased levels of TIMP-1, an MMP-9 inhibitor, suggesting its ability to turn destructive macrophages off via TLR4 and NF $\kappa$ B signaling.<sup>52</sup> These results parallel those of Ceserani et al., who found that MFAT can down-modulate several macrophage functions involved with inflammation, including migration, cellular adhesion, and secretion of CCL2/MCP-1 and CCL5/RANTES.<sup>38</sup> Also, Nava et al. found that MFAT-conditioned medium was able to inhibit U937 monocyte/macrophage migration and reduce CCL2/MCP-1 and CCL5/RANTES secretion

**TABLE 1** Summary of the Biochemical Profile of Adipose-Derived Mesenchymal Stromal Cells

MFAT Component	Cell Markers	Source
Microvascular Endothelial Cells	CD31, CD34, CD146	36
Stromal Cells (+)	CD13, CD29, CD44, CD63, CD73, CD90, CD105, TGF- $\beta$ 1	35-36
<i>Pericytes</i>	NG2	36
Stromal Cells (-)	CD14, CD31, CD45, CD144	35
Process	Molecule or Signaling Pathway	Source
Decreasing Pain	Substance P	38
Neovascularization	VEGF, HGF	40
Anti-Inflammatory/ Immunosuppressive	E2, iNOS, IDO, TSG6, HO1, TGF- $\beta$ , IL-10, galectins	41-44
Interaction with Macrophages	PGE2, TLR4 and NF $\kappa$ B signaling	48-50
<i>Downregulated Pro-Inflammatory Factors</i>	IL-6, TNF- $\alpha$ , IL-12, CCL5/RANTES, CCL2/ MCP-1, CCL3/MIP-1 $\alpha$ , MMP-9	36, 44, 48- 50

**FIG. 2** Proposed mechanisms of action for tissue repair by endogenous MSCs; reproduced from Mancuso et al.<sup>89</sup>

after 28 days of culture, whereas the anti-inflammatory activity of fat lipoaspirate conditioned medium was reduced after just 7 days of culture, further suggesting the long-lasting anti-inflammatory capabilities of MFAT.<sup>46</sup> Furthermore, Carelli et al. recently found that the mechanical shaking of adipose tissue is a key driver of both increased production of anti-inflammatory proteins and decreased inflammatory activity of THP-1 macrophages.<sup>53</sup> In summary, AD-MSCs have a well-characterized clinical profile and play a clear role in modulating the inflammatory processes of OA at a cellular level.

### CLINICAL EFFICACY IN TREATING KNEE OSTEOARTHRITIS

A growing body of research in both human and animal models has been conducted to support the role of MFAT in the treatment of osteoarthritic knees. Using a rat OA model, Li et al. were one of the first to associate the proliferation of AD-MSCs in knee joints with the duration of cell therapy efficacy.<sup>54</sup> They detected fluorescent-labeled AD-MSCs in soft tissue structures 10-weeks after injection, in addition to demonstrating increased cartilage thickness and improved tissue preservation. Toghraie et al. demonstrated that rabbits receiving AD-MSCs had lower degrees of cartilage degeneration, osteophyte formation, and subchondral sclerosis than the non-AD-MSC control group at 16 and 20 weeks,<sup>55</sup> as well as significantly decreased severity of cartilage OA lesions by Mankin scoring.<sup>56</sup> Studies on mice have also indicated that AD-MSCs are joint protective in the setting of increased synovial inflammation.<sup>57,58</sup> Furthermore, AD-MSCs have been shown to influence cartilage repair in addition to reducing OA progression.<sup>59,60</sup> In a study by Zeira et al. of 130 dogs with spontaneous knee OA treated with intra-articular MFAT injections, 88% of the dogs showed significant improvement in orthopedic examination scores six months post-treatment and 92% showed significant improvement in Helsinki chronic pain index scores as reported by their owners.<sup>61</sup>

As for human studies, Jo et al. led a proof-of-concept clinical trial that showed intra-articular injections of high-dose, culture-expanded AD-MSCs ( $1.0 \times 10^8$  cells in 3 mL of saline) into osteoarthritic knees improved

function and pain without causing adverse effects.<sup>62</sup> Using MFAT, Russo et al. conducted a retrospective observational study with 30 patients affected by diffuse degenerative chondral lesions.<sup>63</sup> They evaluated the 1-year safety and outcome of a single intra-articular injection of MFAT, finding no major complications at either the knee or harvest site. They also published 3-year follow-up data, further suggesting that MFAT injections are a safe treatment option in the mid-term.<sup>64</sup> More recently, Panchal et al. demonstrated the safety and significant improvements in pain, quality of life, and function at 12 months after ultrasound-guided injection of MFAT in elderly individuals (age 54–78) with severe refractory knee OA.<sup>65</sup> In 38 subjects treated with MFAT associated with an arthroscopic chondral shaving procedure, Cattaneo et al. found a statistically significant improvement in all measured clinical scores,<sup>66</sup> as well as 100% patient satisfaction and no adverse complications. These findings suggest that MFAT is a safe and potentially efficacious treatment for knee OA, both as a stand-alone treatment and adjuvant for surgical procedures.

Intra-articular adipose injections have been shown to attenuate knee OA progression by inducing structural changes, such as limiting joint damage and cartilage degeneration,<sup>62,67,68</sup> as well as increasing cartilage tissue repair.<sup>67–69</sup> For example, Koh et al. demonstrated an improvement in MRI markers for knee cartilage health and overall knee pain scores after intra-articular injections of AD-MSCs, indicating both radiological and clinical benefits.<sup>67</sup> One year later, a subsequent study found that 87.5% of elderly adults (14/16) had improved or unchanged cartilage status after AD-MSC treatment, as assessed via arthroscopy.<sup>68</sup> Hudetz et al. observed decreases in pain scores and increases in the glycosaminoglycan (GAG) content of hyaline cartilage located in specific areas of the MFAT-treated knee joints in 17 patients with OA.<sup>70</sup> In a subsequent prospective study consisting of 20 MFAT-treated patients with late-stage knee OA, Hudetz et al. reported significant improvement in functional outcome scores in 85% of their patients.<sup>71</sup> Flow cytometry analysis of their micro-fragmented lipoaspirate specimens revealed a substantial number of endothelial progenitor cells, suggesting their synergistic involvement with AD-MSCs in regenerating cartilage tissue.

Several recent studies have indicated that intra-articular MFAT injections improve several pain and functional performance measures in knee OA.<sup>30,63,65–68,72–77</sup> Schiavone Panni et al. recently studied a retrospective cohort of 52 patients with early knee OA (Kellgren-Lawrence grade 0-2) who were treated with arthroscopic debridement followed by percutaneous injection of MFAT.<sup>78</sup> All pain and function scores demonstrated improvement over an average 15.3-month follow-up. Moreover, patients with pre-operative VAS scores greater than 8 were found to show greater clinical and functional benefits compared to those with VAS scores less than 8, suggesting that patients with later stages of knee OA may experience more dramatic relief in pain symptoms after MFAT treatment. These findings further support the need for larger clinical trials, wherein subgroup differences in efficacy can be explored. A 2019 study by Mautner et al. compared pain and functional outcome scores in 76 OA patients treated with either MFAT or BMAC injections over a mean follow-up time of 1.09 years and 1.80 years, respectively.<sup>79</sup> While both patient groups demonstrated significant improvement in knee pain and knee-related quality of life, there was no significant difference in the same post-procedure scores when comparing the MFAT and BMAC treatment groups.

Concerning the enzymatic technique for SVF isolation, several studies have demonstrated its clinical role in the treatment of knee osteoarthritis.<sup>27,30,31,80–83</sup> However, there is limited research comparing this method to the minimal manipulation techniques associated with MFAT. Desando et al. recently explored the migration patterns of culture-expanded AD-MSCs, SVF, and MFAT in a rabbit knee OA model.<sup>27</sup> They found that at 7 days, the culture-expanded AD-MSCs have a higher tropism for the synovial membrane, whereas MFAT had a higher tropism for cartilage. Possible suggestions for this contrasting migration pattern include the physical differences in cell preparations, giving heterogeneous MFAT the ability to survive in hypoxic cartilage tissue, and ensure a gradual release of cytokines.<sup>27,84</sup> Yokota et al. compared the clinical outcomes of knee OA treatment with intra-articular injections of cultured AD-MSCs or SVF. Both treatment options improved pain and clinical outcome scores, but the cultured AD-MSC-treated group reported an

earlier reduction of symptoms and pain with less comorbidity.<sup>83</sup> Of note, a recent prospective double-blind placebo-controlled randomized clinical trial by Garza et al. looked at thirty-nine knee OA patients treated with high-dose SVF, low-dose SVF, or placebo.<sup>85</sup> At both 6- and 12-months follow-up, both SVF groups had dose-dependent statistically significant improvements in total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores for subjective pain and functionality. While promising, there is a clear need for future research that directly compares these adipose processing techniques and patient outcomes.

Further research is needed to investigate intra-articular MFAT injection therapy as a whole via randomized control studies with larger sample sizes to truly establish efficacy.<sup>86</sup> Future areas of interest include the standardization of MFAT-based therapy with intervention timing and study endpoints, quantifying the cellular and acellular components at the time of MFAT injection, optimization of MFAT therapy with cellular preconditioning,<sup>87</sup> and the efficacy of adjunctive therapy with PRP *in vivo*.<sup>88</sup> Recent research has looked at the adult stem cell secretome, the bioactive molecules inside the exosomes of MSCs, as an acellular intra-articular injection alternative to cell-based therapy in regenerative medicine. Results have been promising in animal OA models, where increased chondrocyte proliferation and delayed cartilage damage have been documented.<sup>89</sup> However, the secretome alone may not be as effective in immunomodulation when compared to cell-based therapy, as cell-cell contact may still be required to affect lymphocyte proliferation and function.<sup>90</sup> Further research is necessary to investigate these effects in human OA models and to directly compare injections with MFAT and the acellular MSC secretome for their chemical profiles and clinical outcomes in knee OA. These future studies are a crucial next step towards optimizing regenerative therapy in knee OA to enhance functional outcomes for these patients.

## CONCLUSION

Management of early knee osteoarthritis is controversial as conservative management can be limited while replacement surgery can be premature.<sup>70</sup> Tissue- and cellular-based regenerative therapies have recently emerged as a treatment option for these patients,

although cellular-based therapies are not currently approved in the United States. There is a plethora of research to suggest these AD-MSc therapies have a well-documented immunomodulatory function and may be clinically effective in delaying or preventing the onset of late-stage knee osteoarthritis. However, large, randomized, controlled clinical trials must be conducted with these devices and techniques to scientifically validate these claims and establish efficacy. There are multiple ways to retrieve AD-MSCs, yet further research is needed to compare these methods based on clinical outcomes, optimal dosages, and the degree to which these methods can be tailored to different groups of patients.

#### AUTHOR'S CONTRIBUTION

G.M. devised the project design. J.H. led the literature review and wrote the manuscript. J.H., N.H., and G.M. shared responsibility for the revision and submission of the manuscript.

#### CONFLICTS

Dr. Malanga receives honoraria from Lipogems for education and speaking. Dr. Hogaboom and Mr. Haberl report no conflicts.

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#### REFERENCES

1. Hootman JM, Helmick CG, Barbour KE, et al. Updated projected prevalence of self-reported doctor-diagnosed arthritis and arthritis-attributable activity limitation among US adults, 2015-2040. *Arthritis Rheumatol* 2016;68:1582–87.
2. Loeser RF, Goldring SR, Scanzello CR, et al. Osteoarthritis: A disease of the joint as an organ. *Arthritis Rheumatis* 2012;64:1697–707.
3. McAlindon TE, Bannuru RR, Sullivan MC, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthr Cartil* 2014;22:363–88.
4. Bourne RB, Chesworth BM, Davis AM, et al. Patient satisfaction after total knee arthroplasty: who is satisfied and who is not? *Clin Orthop Relat Res* 2010;468:57–63.
5. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;29:1039–49.
6. de Jonge R, Brouwer R, Smit M, et al. Automated counting of white blood cells in synovial fluid. *Rheumatology (Oxford)* 2004;43:170–73.
7. Liu-Bryan R, Terkeltaub R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat Rev Rheumatol* 2015;11:35–44.
8. Bhosale AM, Richardson JB. Articular cartilage: structure, injuries and review of management. *Br Med Bull* 2008;87:77–95.
9. Sharma A, Jagga S, Lee S-S, et al. Interplay between cartilage and subchondral bone contributing to pathogenesis of osteoarthritis. *Int J Mol Sci* 2013;14:19805–30.
10. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nat Rev Rheumatol* 2010;6:625–35.
11. Malanga GA, Bemanian S. Microfragmented adipose injections in the treatment of knee osteoarthritis. *J Clin Orthopaed Trauma* 2019;10:46–48.
12. Vincent H, Sampson S. The Orthobiologic Institute 9th Annual PRP & Regenerative Medicine Symposium (TOBI 2018): Compendium of Abstracts. *Bio Ortho Journal* 2019;1(1): e2-e17.
13. Kumar A, Kadamb AG, Ghosh Kadamb K. Hope, Hype, Hurdles & Future Perspective for PRP, PRP versus Hyaluronic Acid Injection in Osteoarthritis of Knee: A Review Article. *Biologic Ortho J* 2020;2(1): e1-e12.
14. Malanga G, Abdelshahed D, Jayaram P. Orthobiologic interventions using ultrasound guidance. *Phys Med Rehabil Clin N Am* 2016;27:717–31.
15. Caplan AI. Mesenchymal stem cells: Time to change the name! *Stem Cells Transl Med* 2017;6:1445–51.
16. Caplan AI. The new MSC: MSCs as pericytes are sentinels and gatekeepers. *J Orthop Res* 2017;35:1151–59.
17. Im G-I. Bone marrow-derived stem/stromal cells and adipose tissue-derived stem/stromal cells: Their comparative efficacies and synergistic effects. *J Biomed Mat Res Part A* 2017;105:2640–48.
18. Malanga G, Ibrahim V. *Regenerative Treatments in Sports and Orthopedic Medicine*. New York, NY: Springer Publishing; 2018.



19. Bianchi F, Maioli M, Leonardi E, et al. A new non-enzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplantation* 2013;22:2063–77.
20. Maioli M, Rinaldi S, Santaniello S, et al. Radioelectric asymmetric conveyed fields and human adipose-derived stem cells obtained with a nonenzymatic method and device: A novel approach to multipotency. *Cell Transplantation* 2014;23:1489–1500.
21. Tremolada C, Colombo V, Ventura C. Adipose Tissue and mesenchymal stem cells: State of the art and Lipogems® technology development. *Curr Stem Cell Rep* 2016;2:304–12.
22. Vezzani B, Shaw I, Lesme H, et al. Higher pericyte content and secretory activity of microfragmented human adipose tissue compared to enzymatically derived stromal vascular fraction. *Stem Cells Transl Med* 2018;7:876–86.
23. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013;15:641–48.
24. Oberbauer E, Steffenhagen C, Wurzer C, et al. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. *Cell Regeneration* 2015;4:4:7.
25. Miana VV, González EAP. Adipose tissue stem cells in regenerative medicine. *Ecancermedicalscience* 2018;12:822.
26. Chu D-T, Nguyen Thi Phuong T, Tien NLB, et al. Adipose tissue stem cells for therapy: An update on the progress of isolation, culture, storage, and clinical application. *J Clin Med* 2019;8(7):917.
27. Desando G, Bartolotti I, Martini L, et al. Regenerative features of adipose tissue for osteoarthritis treatment in a rabbit model: enzymatic digestion versus mechanical disruption. *Int J Mol Sci* 2019;20(11):e2636.
28. Pak J, Lee JH, Park KS, et al. Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications. *J Biomed Sci* 2017;24:9.
29. Pak J, Lee JH, Pak N, et al. Cartilage regeneration in humans with adipose tissue-derived stem cells and adipose stromal vascular fraction cells: updated status. *Int J Mol Sci* 2018;19(7):e2146.
30. Bansal H, Comella K, Leon J, et al. Intra-articular injection in the knee of adipose derived stromal cells (stromal vascular fraction) and platelet rich plasma for osteoarthritis. *J Translational Med* 2017;15(1):141.
31. Fodor PB, Paulseth SG. Adipose Derived Stromal Cell (ADSC) injections for pain management of osteoarthritis in the human knee joint. *Aesthet Surg J* 2016;36:229–36.
32. Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *Springerplus* 2015;4:713.
33. Shah FS, Wu X, Dietrich M, et al. A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. *Cytotherapy* 2013;15:979–85.
34. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research Center for Devices and Radiological Health Office of Combination Products Research. Regulatory Considerations for Human Cells, Tissues, and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use Guidance for Industry and Food and Drug Administration Staff, <http://www.fda.gov/regulatory-information/search-fda-guidance-documents/regulatory-considerations-human-cells-tissues-and-cellular-and-tissue-based-products-minimal> (2017, accessed 8 August 2019).
35. Lysaght T, Campbell AV. Regulating autologous adult stem cells: the FDA steps up. *Cell Stem Cell* 2011;9:393–96.
36. Garcia-Contreras M, Messaggio F, Mendez AJ, et al. Metabolomic changes in human adipose tissue derived products following non-enzymatic microfacturing. *Eur Rev Med Pharmacol Sci* 2018;22:3249–60.
37. Carelli S, Messaggio F, Canazza A, et al. Characteristics and properties of mesenchymal stem cells derived from microfragmented adipose tissue. *Cell Transplant* 2015;24:1233–52.
38. Ceserani V, Ferri A, Berenzi A, et al. Angiogenic and anti-inflammatory properties of micro-fragmented fat tissue and its derived mesenchymal stromal cells. *Vascular Cell* 2016;8:3.
39. Baer PC. Adipose-derived mesenchymal stromal/stem cells: An update on their phenotype in vivo and in vitro. *World J Stem Cells* 2014;6:256–65.
40. Kouroupis D, Bowles AC, Willman MA, et al. Infrapatellar fat pad-derived MSC response to inflammation and fibrosis induces an immunomodulatory phenotype involving CD10-mediated Substance P degradation. *Sci Rep* 2019;9:10864.

41. Dubey N, Mishra V, Dubey R, et al. Revisiting the advances in isolation, characterization and secretome of adipose-derived stromal/stem cells. *Internat J Molec Sci* 2018;19:2200.
42. Pak J, Lee JH, Lee SH. Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells. *BioMed Res Internat* 2014;2014:1–10.
43. Cui L, Yin S, Liu W, et al. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. *Tissue Eng* 2007;13:1185–95.
44. Yañez R, Oviedo A, Aldea M, et al. Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. *Exp Cell Res* 2010;316:3109–23.
45. DelaRosa O, Lombardo E, Beraza A, et al. Requirement of IFN-gamma-mediated indoleamine 2,3-dioxygenase expression in the modulation of lymphocyte proliferation by human adipose-derived stem cells. *Tissue Eng Part A* 2009;15:2795–806.
46. Nava S, Sordi V, Pascucci L, et al. Long-lasting anti-inflammatory activity of human microfragmented adipose tissue. *Stem Cells Internat* 2019;2019:1–13.
47. Puissant B, Barreau C, Bourin P, et al. Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells. *Br J Haematol* 2005;129:118–29.
48. Niemeyer P, Kornacker M, Mehlhorn A, et al. Comparison of immunological properties of bone marrow stromal cells and adipose tissue-derived stem cells before and after osteogenic differentiation in vitro. *Tissue Eng* 2007;13:111–21.
49. Manferdini C, Paoletta F, Gabusi E, et al. From osteoarthritic synovium to synovial-derived cells characterization: synovial macrophages are key effector cells. *Arthritis Res Ther* 2016;18:83.
50. Eggenhofer E, Hoogduijn MJ. Mesenchymal stem cell-educated macrophages. *Transplant Res* 2012;1:12.
51. Manferdini C, Paoletta F, Gabusi E, et al. Adipose stromal cells mediated switching of the pro-inflammatory profile of M1-like macrophages is facilitated by PGE2: in vitro evaluation. *Osteoarth Cartil* 2017;25:1161–71.
52. Paoletta F, Manferdini C, Gabusi E, et al. Effect of microfragmented adipose tissue on osteoarthritic synovial macrophage factors. *J Cellular Physiol* 2019;234:5044–55.
53. Carelli S, Colli M, Vinci V, et al. Mechanical activation of adipose tissue and derived mesenchymal stem cells: novel anti-inflammatory properties. *Internat J Molec Sci* 2018;19:267.
54. Li M, Luo X, Lv X, et al. In vivo human adipose-derived mesenchymal stem cell tracking after intra-articular delivery in a rat osteoarthritis model. *Stem Cell Res Ther* 2016;7:160.
55. Toghraie F, Razmkhah M, Gholipour MA, et al. Scaffold-free adipose-derived stem cells (ASCs) improve experimentally induced osteoarthritis in rabbits. *Arch Iran Med* 2012;15:495–99.
56. Toghraie FS, Chenari N, Gholipour MA, et al. Treatment of osteoarthritis with infrapatellar fat pad derived mesenchymal stem cells in rabbit. *Knee* 2011;18:71–75.
57. Schelbergen RF, Van Dalen S, Ter Huurne M, et al. Treatment efficacy of adipose-derived stem cells in experimental osteoarthritis is driven by high synovial activation and reflected by S100A8/A9 serum levels. *Osteoarth Cartil* 2014;22:1158–66.
58. Ter Huurne M, Schelbergen R, Blattes R, et al. Anti-inflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. *Arthritis Rheumatis* 2012;64:3604–13.
59. Hermeto LC, Derossi R, Oliveira RJ, et al. Effects of intra-articular injection of mesenchymal stem cells associated with platelet-rich plasma in a rabbit model of osteoarthritis. *Genet Mol Res* 2016;15(3):1–14.
60. Latief N, Raza FA, Bhatti F-U-R, et al. Adipose stem cells differentiated chondrocytes regenerate damaged cartilage in rat model of Osteoarthritis. *Cell Biol Int* 2016;40:579–88.
61. Zeira O, Scaccia S, Pettinari L, et al. Intra-articular administration of autologous micro-fragmented adipose tissue in dogs with spontaneous osteoarthritis: safety, feasibility, and clinical outcomes. *Stem Cells Transl Med* 2018;7:819–28.
62. Jo CH, Lee YG, Shin WH, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. *STEM CELLS* 2014;32:1254–66.
63. Russo A, Condello V, Madonna V, et al. Autologous and micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis. *J Exp Orthop* 2017;4:33.
64. Russo A, Screpis D, Di Donato SL, et al. Autologous micro-fragmented adipose tissue for the treatment of diffuse degenerative knee osteoarthritis: an update at 3-year follow-up. *J Exp Orthop* 2018;5:52.
65. Panchal J, Malanga G, Sheinkop M. Safety and efficacy of percutaneous injection of lipogems micro-fractured

- adipose tissue for osteoarthritic knees. *Am J Orthop* 2018;47(11).
66. Cattaneo G, De Caro A, Napoli F, et al. Micro-fragmented adipose tissue injection associated with arthroscopic procedures in patients with symptomatic knee osteoarthritis. *BMC Musculoskeletal Disorders* 2018;19:176.
  67. Koh Y-G, Jo S-B, Kwon O-R, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy* 2013;29:748–55.
  68. Koh Y-G, Choi Y-J, Kwon S-K, et al. Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc* 2013;23:1308–16.
  69. Pagani S, Borsari V, Veronesi F, et al. Increased chondrogenic potential of mesenchymal cells from adipose tissue versus bone marrow-derived cells in osteoarthritic in vitro models. *J Cell Physiol* 2016;232:1478–88.
  70. Hudetz D, Borić I, Rod E, et al. The effect of intra-articular injection of autologous microfragmented fat tissue on proteoglycan synthesis in patients with knee osteoarthritis. *Genes (Basel)* 2017;8(10):270.
  71. Hudetz D, Borić I, Rod E, et al. Early results of intra-articular micro-fragmented lipoaspirate treatment in patients with late stages knee osteoarthritis: a prospective study. *Croat Med J* 2019;60:227–36.
  72. Pers Y-M, Rackwitz L, Ferreira R, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. *Stem Cells Transl Med* 2016;5:847–56.
  73. Nguyen PD, Tran TD-X, Nguyen HT-N, et al. Comparative clinical observation of arthroscopic microfracture in the presence and absence of a stromal vascular fraction injection for osteoarthritis. *Stem Cells Transl Med* 2017;6:187–95.
  74. Striano RD, Battista V, Bilboo N. Non-responding knee pain with osteoarthritis, meniscus and ligament tears treated with ultrasound guided autologous, micro-fragmented and minimally manipulated adipose tissue. *Open J Regenerat Med* 2017;06:17–26.
  75. Adriani E, Moio M, Di Paola B, et al. Percutaneous fat transfer to treat knee osteoarthritis symptoms: preliminary results. *Joints* 2017;5:89–92.
  76. Barfod KW, Blønd L. Treatment of osteoarthritis with autologous and microfragmented adipose tissue. *Dan Med J* 2019;66(10):A5565.
  77. Michalek J, Vrablikova A, Darinskas A, et al. Stromal vascular fraction cell therapy for osteoarthritis in elderly: Multicenter case-control study. *J Clin Orthop Trauma* 2019;10:76–80.
  78. Schiavone Panni A, Vasso M, Braile A, et al. Preliminary results of autologous adipose-derived stem cells in early knee osteoarthritis: identification of a subpopulation with greater response. *Internat Orthopaed* 2019;43:7–13.
  79. Mautner K, Bowers R, Easley K, et al. Functional outcomes following microfragmented adipose tissue versus bone marrow aspirate concentrate injections for symptomatic knee osteoarthritis. *Stem Cells Transl Med* 2019;8(11):1149–56.
  80. Tran TDX, Wu C-M, Dubey NK, et al. Time- and Kellgren Lawrence grade-dependent changes in intra-articularly transplanted stromal vascular fraction in osteoarthritic patients. *Cells* 2019;8(4):308–24.
  81. Hong Z, Chen J, Zhang S, et al. Intra-articular injection of autologous adipose-derived stromal vascular fractions for knee osteoarthritis: a double-blind randomized self-controlled trial. *Int Orthop* 2019;43:1123–34.
  82. Yokota N, Yamakawa M, Shirata T, et al. Clinical results following intra-articular injection of adipose-derived stromal vascular fraction cells in patients with osteoarthritis of the knee. *Regen Ther* 2017;6:108–12.
  83. Yokota N, Hattori M, Ohtsuru T, et al. Comparative clinical outcomes after intra-articular injection with adipose-derived cultured stem cells or noncultured stromal vascular fraction for the treatment of knee osteoarthritis. *Am J Sports Med* 2019;47:2577–83.
  84. Swärd P, Wang Y, Hansson M, et al. Coculture of bovine cartilage with synovium and fibrous joint capsule increases aggrecanase and matrix metalloproteinase activity. *Arthritis Res Ther* 2017;19:157.
  85. Garza JR, Campbell RE, Tjoumakaris FP, et al. Clinical efficacy of intra-articular mesenchymal stromal cells for the treatment of knee osteoarthritis: a double-blinded prospective randomized controlled clinical trial. *Am J Sports Med* 2020;48(3):588–98
  86. Jones IA, Wilson M, Togashi R, et al. A randomized, controlled study to evaluate the efficacy of intra-articular, autologous adipose tissue injections for the treatment of mild-to-moderate knee osteoarthritis compared to hyaluronic acid: a study protocol. *BMC Musculoskeletal Disord* 2018;19(1):383.
  87. Ferreira JR, Teixeira GQ, Santos SG, et al. mesenchymal stromal cell secretome: influencing therapeutic potential by cellular pre-conditioning. *Front Immunol* 2018;9:2837.

88. Jayaram P, Ikpeama U, Rothenberg JB, et al. Bone marrow-derived and adipose-derived mesenchymal stem cell therapy in primary knee osteoarthritis: a narrative review. *PM&R* 2019;11:177–91.
89. Mancuso P, Raman S, Glynn A, et al. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. *Front Bioeng Biotechnol* 2019;7:9.
90. Conforti A, Scarsella M, Starc N, et al. Microvesicles derived from mesenchymal stromal cells are not as effective as their cellular counterpart in the ability to modulate immune responses in vitro. *Stem Cells Dev* 2014; 23: 2591–99.
91. Tremolada C, Allegri M, and Slevin M. Mesenchymal stromal cells and micro fragmented adipose tissue: new horizons of effectiveness of lipogems. *J Stem Cell Res Dev Ther* 2019;5:017.