Biologic Orthopedic Journal

Original Article

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

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Submitted: December 24, 2019. Accepted: May 31, 2020. Published: June 23, 2020.

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.[1] 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.[2]

Early knee OA is treated non-operatively with physical therapy and anti-inflammatory medications,[3] 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.[4] Additionally, patients younger than age 50 may have concerns related to the potential need of revision surgery in the future.[4] 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,[5] often much lower than more inflammatory arthritic diseases like Rheumatoid Arthritis (RA).[6] 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.[7]

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.[8] With the increased progression of OA, catabolic factors begin to dominate as pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and multiple chemokines drive production of matrix metalloproteinases and other enzymes which promote extracellular matrix (ECM) destruction and chondrocyte apoptosis.[7] 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.[9] Synovium hypertrophy and inflammation also contributes to joint pain and disease progression via cartilage destruction mediated by similar pro-inflammatory factors and proteases.[10]

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.[11–13] 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.[14,15] MSCs are derived from perivascular cells called pericytes, commonly found in the trabeculae of bone marrow cavities or near blood vessels within adipose tissue.[16] 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.[17] MSCs are capable of self-renewal and differentiation into muscle, bone, and cartilage, making them particularly useful for preventing the progression of OA.[18] 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

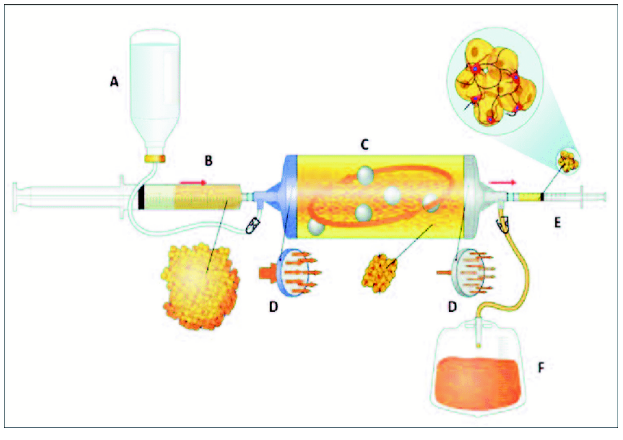
Micro-Fragmented Adipose Tissue

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."[19] 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.[19]

A commercially available device that complies with these regulations[19,20] 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.[21] During the 20-minute procedure, the processed fat is only subjected to 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.[22] This method yields a greater concentration of pericytes in a more time-efficient manner when compared to the enzymatic method of obtaining AD-MSCs.[19] 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.

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.[91]



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.[23] 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.[22–24] 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.[25] Alternatively, the AD-MSCs can be isolated from the SVF after removing the no-adhesion-plastic cells after 24 hours of culture and subsequent expansion in vitro.[26] 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,[27–31] this technique has its challenges. In addition to concerns related to high-costs, safety, and contamination,[32,33] this method of processing does not meet the standard of "minimal manipulation"[34] and has been classified as "more than minimal manipulation.[35]" Without a much more stringent Biological Licensing Agreement (~5-year process), these methods cannot be commercially manufactured for clinical use.[34]

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.[24,36,37] The enzymes digest the ECM surrounding the cells and damage the adipose cells themselves, thus affecting secretory functions and exosomes during processing.[21] 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 α-smooth muscle actin when compared with fat tissue aspirate in vitro.[19] 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-β1, with most of them positive for the pericyte marker NG2.[38] Carelli et al. also found that MFAT positively stained for S-100, vimentin, β-tubulin III, and fatty acid-binding protein 4.[37] 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.[39] 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.[40] This suggests a role CD10-expressing cells may have in directing the decreased inflammation and pain associated with OA.

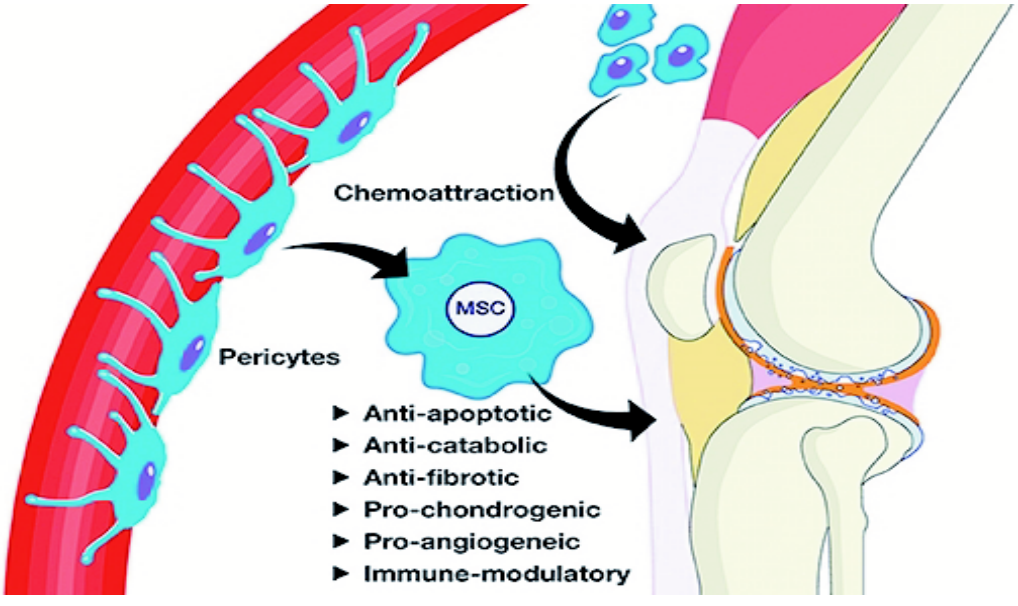
In addition to their cell markers, the bioactive molecules secreted by AD-MSCs have also been recently described (Table 1). These paracrine factors serve to trigger and sustain angiogenic, neurogenic, osteogenic, antifibrotic, antiapoptotic, antimicrobial, and immunomodulatory responses in the affected tissues (Figure 2).[19,22,36,38,41] 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.[42] 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- β, IL-10, and galectins.[43–46] By preventing excessive inflammation, AD-MSCs can promote tissue repair and regeneration by limiting further immune-mediated tissue damage.[47,48] 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.[46]

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-1β, TNF-α, CCL2/ MCP-1, and CCL3/MIP-1α.[49] 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-α, and IL-12).[50,51] Paolella 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-1α, 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 NFκB signaling.[52] 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.[38] 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-β1	35-36
Pericytes	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- β, IL-10, galectins	41-44
Interaction with Macrophages	PGE2, TLR4 and NFκB signaling	48-50
Downregulated Pro-Inflammatory Factors	IL-6, TNF-α, IL-12, CCL5/RANTES, CCL2/ MCP-1, CCL3/MIP-1α, MMP-9	36, 44, 48-50

FIG. 2 Proposed mechanisms of action for tissue repair by endogenous MSCs; reproduced from Mancuso et al.[89]



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.[46] 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.[53] 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.[54] 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,[55] as well as significantly decreased severity of cartilage OA lesions by Mankin scoring.[56] Studies on mice have also indicated that AD-MSCs are joint protective in the setting of increased synovial inflammation.[57,58] Furthermore, AD-MSCs have been shown to influence cartilage repair in addition to reducing OA progression.[59,60] 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.[61]

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 (1.0×10^8 cells in 3 mL of saline) into osteoarthritic knees improved function and pain without causing adverse effects.[62] Using MFAT, Russo et al. conducted a retrospective observational study with 30 patients affected by diffuse degenerative chondral lesions.[63] 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.[64] 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.[65] 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,[66] 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,[62,67,68] as well as increasing cartilage tissue repair.[67–69] 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.[67] 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.[68] 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.[70] 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.[71] 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.[30,63,65–68,72–77] 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.[78] 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.[79] 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.[27,30,31,80–83] 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.[27] 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.[27,84] 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.[83] 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.[85] 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.[86] 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,[87] and the efficacy of adjunctive therapy with PRP in vivo.[88] 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.[89] 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.[90] Further research is necessary to investigate these affects 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.[70] 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.

FUNDING

N/A

ACKNOWLEDGEMENTS

N/A

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