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Adipose-derived regenerative therapies for the treatment of knee osteoarthritis

Epanomeritakis IE *et al.* Adipose-derived regenerative therapies for knee osteoarthritis

Abstract

Knee osteoarthritis is a degenerative condition with a significant disease burden and no disease-modifying therapy. Definitive treatment ultimately requires joint replacement. Therapies capable of regenerating cartilage could significantly reduce financial and clinical costs. The regenerative potential of mesenchymal stromal cells (MSCs) has been extensively studied in the context of knee osteoarthritis. This has yielded promising results in human studies, and is likely a product of immunomodulatory and chondroprotective biomolecules produced by MSCs in response to inflammation. Adipose-derived MSCs (ASCs) are becoming increasingly popular owing to their relative ease of isolation and high proliferative capacity. Stromal vascular fraction (SVF) and micro-fragmented adipose tissue (MFAT) are produced by the enzymatic and mechanical disruption of adipose tissue, respectively. This avoids expansion of isolated ASCs *ex vivo* and their composition of heterogeneous cell populations, including immune cells, may potentiate the reparative function of ASCs. In this editorial, we comment on a multicenter randomized trial regarding the efficacy of MFAT in treating knee osteoarthritis. We discuss the study's findings in the context of emerging evidence regarding adipose-derived regenerative therapies. An underlying mechanism of action of ASCs is proposed while drawing important distinctions between the properties of isolated ASCs, SVF, and MFAT.

Key Words: Knee osteoarthritis; Mesenchymal stromal cells; Adipose tissue; Stromal vascular fraction; Micro-fragmented adipose tissue; Regeneration

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Core Tip: Adipose tissue products are becoming increasingly popular regenerative therapies for treating knee osteoarthritis. Encouraging results have been demonstrated in numerous observational studies and randomized trials. However, it is important to

distinguish among isolated adipose-derived mesenchymal stromal cells (ASCs), stromal vascular fraction (SVF), and micro-fragmented adipose tissue (MFAT) to avoid study heterogeneity and improve the quality of evidence regarding these therapies. Different modes of preparation, cell composition, and physical properties are likely to influence the regenerative function of ASCs. To elucidate which adipose-derived therapy is superior for cartilage regeneration, randomized trials are needed to compare ASCs, SVF, and MFAT as distinct therapies.

INTRODUCTION

Knee osteoarthritis is a degenerative joint disease that causes significant morbidity globally^[1]. Currently, no disease-modifying therapies are available for this condition. Nonoperative measures, including exercise, analgesics, and intraarticular corticosteroids, provide temporary symptomatic relief^[2,3]. Arthroscopic procedures such as debridement of torn menisci to alleviate mechanical symptoms provide little or no clinically important benefit^[4]. The poor intrinsic healing ability of hyaline cartilage means that definitive treatment of the condition ultimately requires joint replacement^[5].

Therapies capable of regenerating knee cartilage and avoiding knee replacement would lead to a significant reduction in financial and clinical costs^[6]. Therapies aimed at cartilage regeneration include intraarticular platelet-rich plasma, mosaicplasty, and microfractures. These therapies may provide symptomatic relief and lead to radiographic improvements^[3,7-9]. However, evidence for their use is inconsistent, and the lifespan of the repair is limited.

The search for regenerative therapies has led to the increasing use of tissue engineering techniques, including autologous chondrocyte implantation (ACI) and mesenchymal stromal cells (MSCs)^[5]. Embedding cells in scaffolds allows targeted cell delivery to injury sites and improves cell retention and survival within the graft^[10,11]. ACI and MSCs have proven promising in clinical trials for the treatment of knee osteoarthritis, as evidenced by patient-reported outcomes and radiographic evidence^[12,13]. However, obstacles to the widespread adoption of cell therapies include

the potential for donor site morbidity and the need to perform two separate procedures^[14], which can reduce the acceptability of the therapy to patients. In addition, the isolation and expansion of cells in culture is expensive and can be met with regulatory restrictions on cell differentiation *ex vivo*^[15].

MSCs have greater proliferative capacity than chondrocytes, are more widely available, and more often promote the formation of hyaline rather than fibrocartilage^[10,15,16]. Their regenerative functions are likely due to the secretion of a combination of chondroprotective, pro-reparative, and immunomodulatory cytokines^[15]. MSCs can be isolated from numerous sites, including the bone marrow, adipose tissue, umbilical cord, and synovium, but it remains unclear which MSC source is optimal for cartilage regeneration^[17]. While bone marrow has traditionally been used as an abundant source of MSCs, adipose-derived MSCs (ASCs) have gained increasing popularity. Adipose tissue is easier to access and has less potential for donor site morbidity, and ASCs have a greater proliferation capacity than bone marrow-derived MSCs (BMSCs) *in vitro*^[17]. Their ability to improve symptoms in patients with knee osteoarthritis has been proven superior to that of BMSCs^[13].

Therapies that avoid the need for cell expansion *ex vivo* can facilitate a 'one-step' treatment and undergo less stringent regulatory processes^[15]. In addition to isolated culture-expanded ASCs, preparations of adipose tissue that retain heterogeneous cell populations and do not involve cell culture have been tested for cartilage regeneration^[18,19]. Stromal vascular fraction (SVF) is produced by the enzymatic digestion of fat tissue and contains a heterogeneous mix of ASCs, pericytes, immune cells, fibroblasts, erythrocytes, and endothelial cells^[20]. Micro-fragmented adipose tissue (MFAT) is another relatively novel technique that has proven safe and effective in treating knee osteoarthritis^[21]. MFAT involves mechanical, rather than enzymatic, disruption of adipose tissue and contains a similarly heterogeneous cell population^[22,23]. Unlike SVF, the lack of enzymatic digestion maintains an intact extracellular matrix (ECM) that is more akin to that found *in vivo*.

In a multicenter, randomized trial, Wu *et al*^[24] investigated the efficacy of MFAT performed in conjunction with arthroscopic surgery in treating patients with Kellgren-Lawrence grade 2-3 osteoarthritis of the knee^[25]. Compared with an intraarticular hyaluronic acid (HA) control, they demonstrated the efficacy of MFAT at 24 months of follow-up, as evidenced by patient-reported outcome measures (PROMs). This editorial reviews the trial by Wu *et al*^[24] in the context of current evidence regarding adipose-derived regenerative therapies for knee osteoarthritis. Comparisons were made between ASC, SVF, and MFAT treatments to elucidate important distinctions and how the underlying mechanisms of action of ASCs may be affected by their differing compositions.

PATHOPHYSIOLOGY OF OSTEOARTHRITIS

Understanding the mechanisms governing osteoarthritis allows an understanding of the mechanisms of action of regenerative treatments. Osteoarthritis has a multifactorial etiology involving mechanical insults that drive inflammation and remodeling of the whole joint, including the cartilage, synovium, fat, and bone^[26]. Under physiological conditions, chondrocytes generate a dense ECM composed of water, type II collagen, glycosaminoglycans (GAGs), and proteoglycans, which confer the shock-absorbing and gliding properties of hyaline cartilage^[27]. The ECM plays a reciprocal role in maintaining chondrocyte homeostasis and phenotypic stability^[28]. Therefore, loss of cartilage integrity has deleterious effects on chondrocytes, which in turn fail to preserve healthy ECM, leading to progressive degeneration of cartilage tissue.

Loss of ECM integrity following a mechanical insult leads to the production of pro-inflammatory cytokines by chondrocytes and the synovium, including interleukin (IL)-1 β and tumor necrosis factor-alpha (TNF- α)^[26], as well as the mechanosensitive induction of protease and chemokine expression^[28,29]. Activation of nuclear factor-kappaB (NF- κ B) signaling pathways by inflammatory cytokines upregulates the expression of cartilage-degrading enzymes by chondrocytes, including matrix

metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin-1 motifs^[26].

Activation of NF- κ B pathways by IL-1 β has also been shown to increase the production of chemokines by chondrocytes, including C-C motif chemokine ligand (CCL)-2^[30], which play an important role in osteoarthritis pathogenesis^[31]. CCL-2 is considered a key factor in initiating and propagating chronic inflammation in osteoarthritis^[32-34], and its deletion is considered chondroprotective. It is responsible for recruiting pro-inflammatory, M1, macrophages to the site of injury, and may act in an autocrine fashion leading to upregulated expression of CCL-2 itself, chondrocyte apoptosis, and greater MMP expression *via* the MAPK pathway^[35], another principal pathway involved in osteoarthritis pathogenesis alongside NF- κ B signaling^[26].

Additional contributors to disease progression include the dysregulated expression of molecules involved in the homeostasis of healthy cartilage. These include transforming growth factor (TGF)- β and NOTCH^[36,37]. Sustained high levels of TGF- β expression in osteoarthritis contribute to chondrocyte hypertrophy, bone remodeling, and osteophyte formation^[36]. Pathological NOTCH signaling acts *via* the NF- κ B and MAPK pathways to upregulate protease and chemokine production^[37]. Both molecules are thought to activate pathways that converge on the upregulation of Runx2, a transcription factor that modulates MMP expression in chondrocytes^[38]. Similarly, alterations in the physiological expression profiles of microRNAs (miRNAs), which are responsible for post-transcriptional alterations in gene expression, have been implicated in disease process^[39,40]. The reduced expression of miRNAs responsible for promoting chondrocyte differentiation, inhibiting inflammation, and downregulating the expression of proteinases is coupled with an increase in the expression of pro-inflammatory and pro-apoptotic miRNAs.

The proximity of the articular cartilage to the underlying subchondral bone and their reciprocal interactions are important for understanding the pathogenesis of osteoarthritis^[41]. The osteochondral unit is composed of articular hyaline cartilage and subchondral bone, separated by a zone of calcified cartilage, the tidemark^[26].

Subchondral bone and cartilage interact both biomechanically and biochemically^[42]. Altered transmission of loads between the cartilage and the underlying bone leads to pathological bone remodeling, which is associated with thickening of the cortical subchondral bone and thinning of the deeper cancellous bone, changes that precede cartilage degradation^[26]. Increased RANKL expression in osteocytes in areas of reduced mechanical loading promotes osteoclastogenesis and bone resorption during early osteoarthritis^[43]. Greater osteoclastic activity is associated with greater TGF- β 1-induced bone formation by osteoblasts, which act reciprocally on osteoclasts to create a positive feedback loop that drives bone remodeling^[43]. The increased stiffness of the subchondral bone in osteoarthritis exerts a greater load on the overlying cartilage, contributing to secondary cartilage damage^[42].

As a result of remodeling, the tidemark is disrupted and calcification advances, contributing to cartilage thinning^[26]. This effect is exacerbated by pathological angiogenesis^[26,44]. Invasion of the overlying cartilage by new blood vessels is facilitated by the reduced integrity of the tidemark and cartilage ECM^[45]. Biochemical mediators include vascular endothelial growth factor secreted by chondrocytes, in contrast to anti-angiogenic factors secreted under physiological conditions^[45]. Osteoclast precursors within the osteoarthritic subchondral bone also drive this process by secreting excessive levels of platelet-derived growth factor-BB, which stimulate angiogenesis^[44].

Although hyaline cartilage is aneural, pain is a hallmark of osteoarthritis. The bone is richly innervated by sensory nerves running alongside the vasculature^[46]. This may explain why cystic bone lesions are independently associated with pain severity^[47]. Furthermore, vessels extending into the overlying cartilage are accompanied by neuronal ingrowth, which contributes to pain associated with the disease^[47].

Also relevant to the disease process is the biochemical crosstalk between cells within the subchondral bone and the overlying cartilage^[41,42,45]. Although disruption of the tidemark weakens the barrier between the bone and cartilage, calcified cartilage is permeable to small molecules, even in healthy knee joints^[42]. Therefore, it is likely that soluble factors crossing from the bone to articular cartilage and *vice versa* induce

pathological changes in osteoarthritis. It has been shown that the co-culture of chondrocytes with osteoblasts from sclerotic bone induces a hypertrophic chondrocyte phenotype, expressing reduced levels of proteoglycans and elevated levels of MMPs^[48]. TGF- β 1 is one chemical mediator which has been implicated in the crosstalk between subchondral bone and cartilage, resulting in an increased chondrocyte apoptotic rate^[43]. Similarly, the transmission of inflammatory cytokines produced by chondrocytes in osteoarthritis act to increase osteoclastogenesis and consequent bone resorption^[43].

ASCS: MECHANISM OF ACTION

The International Society for Cellular Therapy outlines a three-part definition of MSC characterization^[49]. MSCs display plastic adherence and chondrogenic, osteogenic, and adipogenic differentiation *in vitro* and are characterized by the expression of specific cluster of differentiation (CD) markers, including CD73, CD90, and CD105, as well as the absence of CD14, CD19, CD45, and HLA-DR. We identified an additional panel of cell surface markers that should be tested for accurate characterization of ASCs, allowing for consistent identification between studies^[50].

Initially termed mesenchymal “stem” cells, this is now recognized as a misnomer^[51]. Despite the multipotency of MSCs *in vitro*, the concept that their regenerative function of MSCs is reliant on their differentiation into resident tissues *in vivo* has been disregarded. MSCs are present in all vascularized tissues and arise from perivascular pericytes^[51]. Rather than regenerating tissues by direct differentiation, the regenerative capacity of MSCs *in vivo* is thought to rely on their ability to migrate to sites of injury and secrete bioactive molecules with immunomodulatory and regenerative functions in response to local cues. One such molecule is prostaglandin-E2 (PGE2), which is produced by MSCs in response to inflammatory cytokines, such as TNF- α ^[52]. PGE2 converts pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages. MSCs also induce a transition from TH1 to TH2 lymphocytes, increase IL-10 and IL-4 levels, and decrease interferon gamma production, which has been implicated in their ability to promote cartilage repair^[52].

The low reported survival rate of MSCs implanted into knee joints^[17] and their tendency to migrate to the synovium and menisci, not solely to the damaged cartilage itself^[53], further strengthens the doubt over their role in direct differentiation into neocartilage. Instead, regeneration is likely promoted by ASCs *via* immunomodulatory mechanisms and secretion of chondroprotective biomolecules^[52,54,55].

We previously investigated the effect of co-culturing infrapatellar fat pad-derived ASCs and chondrocytes *in vitro* to investigate changes in chondrogenic differentiation in comparison with ASC monocultures^[56]. Although there are doubts about the ability of MSCs to differentiate into resident tissues *in vivo*^[51], our findings suggest that chondrocyte-ASC crosstalk may be involved in the pro-reparative function of ASCs. We found that a high relative ratio of chondrocytes to ASC resulted in greater expression levels of chondrogenic genes, as detected using polymerase chain reaction, including COL2A1, L-SOX5, SOX6, SOX9, ACAN, and COMP. Our findings suggest that chondrocyte-ASC crosstalk is relevant to the upregulation of chondrogenic genes. The underlying mechanisms may include bidirectional paracrine signaling and cell-to-cell contact (juxtacrine). Although we suspect that the upregulated expression in this case represents chondrogenic differentiation of ASCs *in vitro*, it is possible that this reflects the reciprocal activity of ASCs in maintaining chondrocyte differentiation. Cell sorting techniques to separate the two cell types following co-culture would have allowed the analysis of the gene expression of each cell type.

Other studies investigating co-cultures of ASCs and chondrocytes have analyzed the expression profiles of these two cell populations individually^[54,57]. These results provide further evidence that ASCs secrete chondroprotective molecules that promote cartilage repair and reduce the expression of proinflammatory cytokines and chemokines in chondrocytes. Hepatocyte growth factor produced by ASCs downregulates TGF- β expression and fibrotic markers (collagen I and III) in chondrocytes, whereas PGE2 decreases the expression of pro-inflammatory molecules, including IL-1 β , TNF- α , and CCL-2.

The immunomodulatory function of MSCs has been widely reported^[52] and is likely to play a key role in the treatment of osteoarthritis. Aggarwal and Pittenger^[58] co-cultured human MSCs with subpopulations of immune cells to investigate alterations in their cytokine expression profiles. They found that MSCs reduced the expression of proinflammatory cytokines and induced anti-inflammatory phenotypic changes in various immune cells. MSCs themselves were found to display increased expression of PGE2 in co-culture, implying that PGE2 was responsible for promoting anti-inflammatory changes and that its expression was mediated by crosstalk between immune cells and MSCs.

The concept of reciprocal interactions between MSCs and immune cells has been demonstrated repeatedly^[59], including the induction of PGE2 expression using TNF- α , which in turn leads to an anti-inflammatory switch by immune cells. Pro-inflammatory M1 macrophages that produce IL-1 β , TGF- β , and TNF- α drive osteoarthritis, whereas the anti-inflammatory, pro-reparative (M2) phenotype that produces IL-10 is considered chondroprotective^[17,53].

MiRNAs are additional bioactive molecules implicated in the immunomodulatory functions of ASCs^[60]. In response to the synovial fluid obtained from human patients with osteoarthritis, ASCs demonstrate altered expression of miRNAs embedded in extracellular vesicles, which act on chondrocytes and macrophages to increase the expression of chondrogenic genes and promote the M2 phenotype, respectively. This provides additional evidence that MSCs secrete bioactive molecules in response to local injurious stimuli.

Although studies on the effectiveness of ASCs in treating osteoarthritis primarily address their ability to regenerate cartilage, there is emerging evidence of their ability to promote bone repair^[61]. A study investigating the effect of ASCs on osteoclastogenesis, both *in vitro* and in a mouse model, demonstrated that RANKL-induced osteoclastogenesis was suppressed following ASC treatment^[62]. As osteoclasts are derived from the myeloid lineage, the anti-inflammatory function of MSCs may also be applicable to the modulation of osteoclast activity^[61]. The mechanisms involved may

include cell-cell contact or paracrine effects. For example, secretion of osteoprotegerin by MSCs, a decoy receptor that competitively binds to RANKL, reduces osteoclastogenesis^[61]. Therefore, as proposed for cartilage repair, evidence for the potential reparative role of ASCs in damaged bone suggests that the tissue may be regenerated through a combination of anti-inflammatory mechanisms and protection from further tissue destruction.

In addition to their local action, ASCs injected into human osteoarthritic knee joints mediated a systemic immune regulatory function^[63]. Three months after the intraarticular injection of ASCs, increased peripheral regulatory T cells and decreased circulating classical monocytes were observed in fresh peripheral blood. Systemic effects of MSCs on immune cells have also been demonstrated in other diseases. In addition to interactions in colocalized areas, there is evidence for the role of MSCs in inducing the migration of macrophages to sites of inflammation, including atherosclerotic plaques and infections^[64,65]. Interestingly, CCL-2 expression in MSCs was responsible for this effect. Therefore, although elevated CCL-2 levels are considered proinflammatory, its production by MSCs in the context of osteoarthritis could be explained by CCL-2's beneficial role in the initial recruitment of M1 macrophages to the site of injury. Through reciprocal interactions with MSCs, they may induce a switch to M2 pro-reparative macrophages.

ADIPOSE-DERIVED REGENERATIVE THERAPIES FOR KNEE OSTEOARTHRITIS: IMPORTANT DISTINCTIONS

An array of heterogeneous clinical studies using different permutations of adipose-derived therapies^[66] has demonstrated growing interest in harnessing the pro-reparative properties of adipose tissue for cartilage regeneration. Given the differences in the preparation, composition, and physical properties of different adipose-derived therapies, it is pertinent to avoid conflating these and make an early distinction between their properties and, therefore, their therapeutic potential.

ASCs have gained popularity over traditionally studied BMSCs because of their ease of isolation and high proliferative ability^[17]. In a recent systematic review, we found that isolated ASCs are a successful and safe therapy for the treatment of focal cartilage defects of the knee that risk progression to osteoarthritis^[67]. Similarly, ASCs have shown promise for the treatment of established osteoarthritis^[68] and are superior to BMSCs in this field^[13].

SVF is a related therapy produced by the enzymatic digestion of adipose tissue. The composition of various cell types, including ASCs, immune cells, pericytes, fibroblasts, and endothelial cells, is thought to offer a higher therapeutic potential because of the complementary action of different cell populations^[69]. SVF has demonstrated therapeutic benefits in patients with established osteoarthritis^[19,69]. However, SVF is not strictly an ASC treatment. Although meta-analyses have demonstrated the promise of both ASCs and SVF as adipose-derived therapies^[67,68], it is more appropriate to consider that these distinct treatments should be investigated separately or in comparison to one another.

MFAT was developed to disrupt the adipose tissue while maintaining its *in vivo* composition^[22]. Unlike SVF, this technique involves mechanical, rather than enzymatic, disruption. This has the advantage of maintaining an adipose ECM composed of collagen, laminin, fibronectin, and GAGs, which can improve graft fixation and cell retention within the site of delivery^[70]. Again, although MFAT contains a fraction of ASCs, it is not strictly an ASC therapy because of the lack of cell isolation and culture expansion, preserved ECM, and additional immune cells^[71].

Numerous studies have demonstrated the efficacy of MFAT in alleviating osteoarthritis symptoms in recent years, but few have evaluated its treatment in randomized controlled trials (RCTs)^[72-74]. More RCTs are needed to provide more evidence regarding MFAT^[15]. Wu *et al*^[24] expanded on emerging evidence by reporting the outcomes of their prospective, multicenter, randomized trial, recruiting a larger, more heterogeneous population than those in previous studies. Intraarticular delivery of MFAT, performed alongside various other arthroscopic treatments for osteoarthritis,

was compared with that of an intraarticular HA control. Patients with Kellgren-Lawrence grade 2-3 osteoarthritis of the knee (*i.e.*, moderate disease) that had been symptomatic for over six months and failed to respond to conservative measures were included. Follow-up was conducted over 24 months. Outcome measures included changes in various PROMs, occurrence of adverse events, and magnetic resonance imaging (MRI) evidence of osteoarthritis resolution.

No serious adverse events were observed in either group. While there was no significant difference in the PROMs at baseline, the MFAT performed significantly better at all follow-up time points. Both groups demonstrated improvement relative to baseline, which was possibly related to the arthroscopic intervention. Notably, in the treatment group, the total Western Ontario and McMaster Universities Osteoarthritis index declined between 12 and 24 months. This might indicate a limited duration of treatment efficacy; however, a longer follow-up period is necessary to draw any conclusions. While the MFAT group demonstrated MRI improvements relative to baseline ($P < 0.05$), this was not significant compared to the control group at 24 months. Discrepancies between clinical and imaging outcomes following adipose-derived therapies have been noted in other studies and may require more than 24 months to correlate^[15]. Again, a longer follow-up period or more sensitive imaging techniques may be needed to determine the true extent of morphological improvements.

The strength of this study lies in its generalizability. The multi-centre, adequately powered design, and use of intention-to-treat analysis confer external validity. This is likely reflected by the fact that the improvements observed were more modest than those in other trials. Although unavoidable, both the patients and surgeons were not blinded, possibly introducing a degree of bias. In addition, the repeated use of PROMs tools at various follow-up points may have introduced a response bias.

In conclusion, Wu *et al*^[24] demonstrated the efficacy of MFAT in treating moderately advanced knee osteoarthritis, in accordance with the existing literature. The ability to offer a minimally invasive therapy that provides sustained functional improvements at the years follow-up is encouraging. Further follow-up data will be important in

determining the longevity of cartilage repair by MFAT and whether this correlates with the evidence of healing seen on imaging.

PROPERTIES OF DIFFERENT ADIPOSE-DERIVED THERAPIES

It is important to consider how SVF and MFAT function as therapies that are distinct from isolated ASCs. Unpassaged (p0) stromal cells comprise a more heterogeneous cell population, whereas culture passages select for plastic-adherent ASCs^[75]. ASCs in culture may also undergo genetic alterations, depending on the culture conditions^[76] which may alter their behavior *in vivo*. Furthermore, given that MSC function relies on immunomodulatory mechanisms and bidirectional signaling with native cells, it is expected that their delivery within an adipose niche composed of various cell types, including immune cells, will have an impact on their therapeutic function.

Desando *et al*^[53] investigated how cell migration patterns differ between ASC, SVF, and MFAT in a rabbit model of knee osteoarthritis. Isolated ASCs and those in the SVF showed higher tropism for the synovium and menisci than for the cartilage. Conversely, MFAT showed the highest tropism for the cartilage on day seven, followed by the synovium on day 30. The authors suggested that the collagen network in MFAT permitted greater survival of cells embedded within the hypoxic environment of the osteoarthritic cartilage and protection from lytic enzymes. The migration pattern may also be explained by the structure of MFAT, which acts as a reservoir of cells until the collagen degrades, a concept that has been supported *in vitro* and is associated with a prolonged release of anti-inflammatory cytokines^[77]. Furthermore, while all three therapies showed strong positivity for MSC markers, MFAT contained a greater abundance of blood vessels, preadipocytes, and monocytes, as well as more CD163+ (M2) macrophages, than SVF. MSCs from MFAT also demonstrated greater co-localization with CD163+ cells within the knee joint.

Other authors have commented on how the preparation of MFAT may improve its efficiency compared to other adipose-derived therapies. Vezzani *et al*^[78] showed that mechanical disruption of adipose tissue maintains ASCs within the perivascular

environment observed *in vivo*, unlike the enzymatic digestion process involved in SVF. This was associated with significantly higher levels of secreted growth factors and cytokines that promote tissue regeneration, which may be explained by the improved function of ASCs when retained within their usual niche and the altered gene expression induced by enzymatic tissue digestion.

FUTURE CHALLENGES

As previously mentioned, ASCs have gained popularity owing to their ease of isolation, abundance in adipose tissue, anti-inflammatory properties, and proliferative potential^[17]. Furthermore, the intraarticular delivery of adipose-derived therapies, including SVF and MFAT, is conducive to relatively noninvasive treatment techniques^[79]. However, various challenges exist in translating adipose-derived regenerative therapies from trial settings to clinical practice.

One challenge is our incomplete understanding of how to stratify patients to predict successful treatment outcomes. For example, ASCs obtained from morbidly obese patients show reduced proliferation rates and chondrogenic differentiation capacity^[80,81]. Furthermore, studies have often been conducted using cells obtained from healthy donors. The proliferative capacity of MSCs decreases with age, which may affect the applicability of the experimental evidence in elderly patients with advanced osteoarthritis^[80,81]. Tailoring MSC therapy to particular patient groups is also complicated because the phenotype of osteoarthritis differs depending on its etiology^[82].

Safety concerns raised regarding ASC therapies include the potential for immunoreactivity with animal-derived products used for culture expansion and the theoretical possibility of tumorigenesis, as the genetic instability of ASCs increases following long-term culture^[83]. However, a meta-analysis of 11 trials using MSC therapies demonstrated no increase in the incidence of complications compared to control therapies^[84]. Finally, regulatory restrictions necessitating minimal manipulation of the transplanted tissue may act as a barrier to the clinical implementation of adipose-

derived therapies, including SVF, which requires the enzymatic digestion of adipose tissue^[83]. The preparation of MFAT, which involves the mechanical disruption of tissue and no cell culture^[24], may overcome several safety and regulatory concerns.

CONCLUSION

Osteoarthritis is a progressive disease that results from the degeneration of hyaline cartilage. The lack of available disease-modifying therapies has led to ample research on therapies with the potential to regenerate articular cartilage. ASCs are a promising cell therapy that has demonstrated efficacy in human trials and are likely to function through the production of immunomodulatory and chondroprotective biomolecules in response to local inflammatory stimuli.

Adipose tissue is gaining popularity as a source of ASCs because of the ease of harvesting and high proliferative capacity of ASCs compared to other MSC sources. SVF and MFAT are other adipose-derived regenerative therapies which retain a heterogeneous population of cell types including, but not limited to ASCs. Both have successfully alleviated osteoarthritis symptoms in humans. The lack of enzymatic processing of adipose tissue required to produce MFAT, unlike SVF, is likely to confer additional beneficial properties that might potentiate the mechanism of action of MSCs. This may explain the encouraging results of MFAT treatment observed across multiple trials in recent years. The success of MFAT in treating osteoarthritis, as evidenced by both symptomatic and radiological improvements, is certainly encouraging and warrants continued investigation to determine the optimal treatment approach and longevity of cartilage repair.

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