

# Microfragmented adipose tissue and its initial application in articular disease

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Osteoarthritis (OA) is a degenerative disease of articular cartilage whose main clinical manifestation is joint pain. The database from the China Health and Retirement Longitudinal Study showed that the morbidity of symptomatic OA (knee Kellgren and Lawrence score  $\geq 2$ , with knee pain) was 8.1% in China. With the development of aging in China, the incidence of OA is rising. As there are no blood vessels, nerves, or lymph in articular cartilage, its self-healing ability is poor. There are many types of nonsurgical treatments, such as medications including non-steroidal anti-inflammatory drugs, glucocorticoid, or opioids,<sup>[1,2]</sup> as well as physiotherapy treatments including injecting hyaluronic acid (HA) or platelet-rich plasma (PRP),<sup>[3,4]</sup> to prevent pain and improve joint function. All of these methods can prevent pain temporarily but are unable to interrupt the degeneration of articular cartilage. After extracting fat from the human body using a liposuction device, microfragmented adipose tissue (MAT) can be obtained via an isolation and washing device without digestive enzymes.<sup>[5]</sup> It supports three-dimensional scaffolds, growing factors, and cellular colonies such as mesenchymal stem cells (MSCs). MAT injection can reduce pain in patients with OA, improve joint function, and does not cause adverse events.

## Mechanism of MAT

MAT can be simply and safely collected without adding digestive enzymes and additives. Patients can choose fat-rich areas such as the lower or lateral abdomen as the donor site. After undergoing local anesthesia, Klein solution is injected at the selected area via a hypodermic injection. The skin incision is approximately 2 to 3 mm, and approximately 60 mL of adipose tissue is extracted manually using an injector connected to a disposable liposuction intubation. The MAT preparation tool is a

closed cylindrical system that filled with 0.9% NaCl solution and is free of air. The adipose tissue is placed into a barrel by a blue filter and shaken for approximately 1 min to emulsify it. A flow of 0.9% NaCl solution is maintained during shaking to eliminate blood elements and the residues associated with adipose tissue emulsification. When the fluid becomes clear, the floating MAT is collected using a gray filter outlet connected with an injector under the device. Approximately 20 to 30 mL of MAT can be collected from 60 mL of adipose tissue.<sup>[6]</sup> Throughout this progress, adipose tissue only encounters slight mechanical force, maintaining the interstitial vascular niche and the completeness of the tissue itself.

In recent years, the study of adipose tissue has demonstrated that it contains a kind of multi-lineage progenitor cell that work as MSCs.<sup>[7]</sup> The existence of MSCs can help adipose tissue to be a cell therapeutic product. The traditional way to prepare adipose tissue is to make it enzymatically to obtain a unicellular suspension and then try to isolate adipose cells via centrifugation to collect the remaining stromal vascular fraction (SVF). MSCs can be isolated from the SVF *in vitro*, which is similar in morphology, growth, and epitopes to MSCs from marrow.<sup>[8]</sup> Studies have shown that vascularized tissues normally have the ability to produce MSCs. The pericytes surrounding capillaries and microvessels<sup>[9,10]</sup> and adventitial stromal cells surrounding arteries and veins<sup>[11-13]</sup> were identified to be progenitor cells *in vitro*. There is some evidence supporting this idea, such as both pericytes and adventitial cells express MSC markers *in vivo* and have the ability to promote mesodermal differentiation upon culture.<sup>[14]</sup> Both MSCs and pericytes show similar gene expression.<sup>[15]</sup> Transcript group analysis of a single pericyte, which was purified from human adipose tissue, confirmed the presence of progenitor cells and revealed

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that adventitial stromal cells may be more primitive than pericytes in development.<sup>[16]</sup>

Quantitative study of MAT and SVF by laser flow cytometry showed that the MAT retained more pericytes but fewer adventitial stromal cells, which proved that arteries and veins surrounded by the adventitia would be lost after mechanical dissociation and microvessels persisted in the MAT. Immunofluorescence for pericyte markers showed that pericytes expressing NG2 or platelet-derived growth factor receptor- $\beta$  (PDGFR- $\beta$ ) were resistant and worked as peri-endothelial cells in microvessels after being mechanically segmented, which proved that the perivascular niche was preserved intact.<sup>[6]</sup> Moreover, pericytes could also be involved in tissue repair through the secretion of growth factors and cytokines. Compared with the SVF, the MAT contains more angiogenic growth factors, such as angiogenin, endoglin, dipeptidyl peptidase IV, hepatocyte growth factor, placenta growth factor, and other cytokines such as adiponectin, CD14, CD31, insulin-like growth factor binding protein 2, and complement D.<sup>[6,17]</sup> These secretions cannot only help to repair tissues but also have anti-inflammatory effects. In mice with sepsis, inflammation could be significantly reduced after MAT injection.<sup>[18]</sup> In addition, according to the comparative studies of Nava *et al*,<sup>[17]</sup> among 17 human MATs and adipose tissues, MATs maintained the secretion of active cytokines for 28 days, though the MSC contents and cytokine activity from syngeneic adipose tissue decreased rapidly within a week. Evidence of this is that both MAT and adipose tissue media could reduce the migration of U937 monocytes at the early stage; however, the adipose tissue would lose its effect while the MAT medium could persist after 14 days. To sum up, MAT maintains the intact stromal vascular niche, can help to repair damaged areas, and reduces inflammatory responses through the secretory ability of the niches.

### Animal Model Exploration

When researchers conducted basic experiments to explore the mechanism and indications of MAT, they also identified that MAT could be used for treating cartilage damage. As there are no vessels in articular cartilage and material transport mainly depends on diffusion, it is feasible to repair cartilage through the injection of MAT. To evaluate the safety, feasibility, and clinical effects of MAT injection into articular cartilage, Zeira *et al*<sup>[19]</sup> chose 130 dogs with spontaneous OA for single joint MAT injection. Dog model is probably the closest to a gold standard animal model for OA currently available.<sup>[20]</sup> Follow-up was performed for 2 weeks, 1 month, 3 months, and 6 months after treatment, including examining radiographic results, the Helsinki chronic pain index (HCPI), and a modified orthopedic score (OS) that can assess lameness.<sup>[21]</sup> The OS results showed that 88% of dogs improved within 6 months, 11% showed no changes, and 1% deteriorated. The HCPI results demonstrated that 63% of dogs were apparently improved within 6 months, 29% were obviously improved, 6% were slightly improved, and 2% deteriorated. The radiographic results of dogs with improved condition showed that cartilage lesions were filled and joint fluid was slightly reduced.

According to the follow-up results, MAT injection can reduce pain and improve joint function. No treatment-related complications were observed. Notably, the reduction in pain and decreased dysfunction observed by MAT injection was similar to the experimental results from PRP injection.<sup>[22]</sup> Because there is no large-scale PRP research focused on dogs and PRP requires multiple injections while MAT only requires one injection to promote long-term effects, there is no complete control research currently available. In future research focused on cartilage damage repair, a comparative study of MAT, HA, and PRP is necessary.

### Initial Clinical Application

Rotator cuff injury and chronic shoulder pain may result in severe restrictions of shoulder movement, which can greatly affect one's quality of life. Striano *et al*<sup>[23]</sup> followed up with 20 subjects after MAT injection. Each of them had more than a 1-year history of chronic shoulder pain and could not raise their arms in excess of 90°. Their shoulder movement limitations made it difficult for the patients to dress themselves. The patients were also not treated with physiotherapy, cortisone or PRP injections within 60 days. Follow-up was performed at the 1st and 5th weeks and the 3rd, 6th, and 12th months after injection, and the assessments included the numerical pain scale (NPS) and the American Shoulder and Elbow Surgeons Score (ASES). NPS had improved significantly after treatment compared with the baseline and continued to benefit the patients over the next 12 months. The ASES improved linearly in the first three months and continued to benefit the patients throughout the entire 12 months. No adverse events were reported during this follow-up period. The possible reasons of no adverse events may be as follows: MAT contained some anti-inflammatory cytokines, the MAT preparation filtered out substances associated with inflammatory reactions, and MAT from a patient's own adipose tissue would not lead any immune response.<sup>[5]</sup> Although the number of subjects was small, this study showed that MAT injection could effectively reduce the pain in patients. The follow-up will last for approximately 2 years to observe the long-term effects of MAT injection for the treatment of shoulder pain.

Striano *et al*<sup>[24]</sup> injected MAT in a 59-year-old male patient who suffered from severe knee pain and had undergone a few failed treatments including arthroscopic meniscus surgery. Magnetic resonance imaging (MRI) showed that he suffered from OA, a medial meniscus tear and patellomalacia. Approximately six months after the MAT injection, his VAS score was increased from 8 to 0 and MRI showed that the articular cartilage had expanded the joint space from 0.75 to 1.50 mm. The MRI results showed that MAT could provide support, buffer and fill the soft tissue and had a latent healing ability. To study the long-term clinical effects of MAT injection on diseased joints, Franceschini *et al*<sup>[25]</sup> followed a subject for 30 months after injection. The subject, a 33-year-old man, had knee injuries from falling while skiing. MRI showed a tear of the anterior cruciate ligament and two cartilage injuries. The man underwent surgeries focused on the microfracture and reconstruction of the anterior cruciate

ligament by autografting the tendon, leading to the function of ligament being restored. However, his knee pain was persistent and unbearable. Arthroscopic examination after 10 months showed that the cartilage area had not effectively increased, after which arthroscopic debridement and autologous PRP injection were performed. The patient chose to have a MAT injection due to the lingering pain and severe movement limitations. The patient's pain improved after 10 days, and the pain was completely gone in the 6th week. The clinical situation was stable in the 12th and 30th months, and the pain disappeared completely so the patient could perform physical activities without limit. Even though it is a single case report, MAT injection could reduce pain, restored joint function for up to 30 months and had no adverse events, while the microfracture surgery and autogenous PRP were ineffective, suggesting the long-term effects of MAT injection as an option that is worthwhile for patients.

Cattaneo *et al*<sup>[26]</sup> selected 38 patients with knee OA and performed MAT injections. Their conditions included knee cartilage disease grade >II (International Cartilage Research Society classification), persistent knee pain, failed HA and PRP injections and failed hormone therapy for almost 12 months. Thirty-eight patients suffered from normative arthroscopic cartilage curettage and received MAT injections and 14 patients received meniscectomy as a necessary additional surgery. At 1, 3, 6, and 12 months after surgery, they were assessed using physical examinations and the knee injury and osteoarthritis outcome score. The 12-month results showed that 92% of the patients improved and that 100% of them were satisfied with the treatment. The most important result was that there were no adverse reactions or complications during follow-up. As previous studies have shown that meniscectomy could accelerate OA tendencies,<sup>[25]</sup> the situation for those 14 patients seemed inspiring. The experiments described above show the safety and effectiveness of the MAT injection, though the persuasion is still insufficient due to the lack of contrasting techniques.

## Conclusions

As an innovative technology, MAT injection has been certified by the Food and Drug Administration (FDA) in the United States, Conformite Europeenne (CE) Mark in Europe and Therapeutic Goods Administration from Australia. It is simple, cost-effective and safe, and can achieve great effectiveness with only one injection. A great number of basic studies and clinical trials focused on MAT injections in patients with articular cartilage injury are currently underway. According to the follow-up results (including unpublished data), MAT injection can reduce pain in patients, improve joint function, and does not cause adverse events.<sup>[27]</sup> Even in patients who have undergone many failed treatments such as arthroscopic meniscus surgery, pain reduction by MAT injection is apparent.<sup>[24]</sup> However, the mechanism by which MSCs and their cytokines act with chondrocytes is unclear, the number of subjects in clinical trials is small, the treatment assessment system is not uniform, the follow-up time is too short to observe long-term effects and the lack of control trial makes it difficult to persuade practitioners to treat patients with MAT. We hope that longer-term follow-up and an

increased number of randomized controlled trials will lead to clearer conclusions and make the MAT injection a new option as a non-operative orthopedic treatment.

## Conflicts of interest

None.

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