1. RESPONSE

A. Clinical Response: Clinical response demonstrates increasing evidence of cartilage regeneration, anti-inflammatory properties, and function improvement. In addition to physical examinations prior to stem cell procedure and 6 months post-procedure, laboratory test results serve as evidence of repair process. Recognized lab tests for monitoring Orthopedic conditions include:

- Complete physical exam (including vital signs of sitting blood pressure, temperature, and heart rate
- Visual Analog Scale
- Patient questionnaire (WOMAC for knee or hip, WOOS for shoulder, AOS for Ankle)
- Laboratory determinations (urinalysis, hematology and biochemistry)

2. PRELIMINARIES

A. Background: The most common joint issues are due to arthritis and injuries. Osteoarthritis (OA), the most common form of arthritis and often called degenerative joint disease, appears when the protective cartilage, hard and slippery tissue, on the ends of your bones wears down over time. This causes an inflammation at the joint(s) leading to an eventual loss of function. OA can occur at any joint in the body, but is most commonly found in the hands, knees, hips, and spine. This affliction it often seen among older people, but those younger, who have undergone joint injuries are more likely to also develop osteoarthritis. The most common joint injuries include but are not limited to sprains (stretching or tearing of ligaments), cartilage fraying or tearing, and fractures (joint can break as a result of a fall or some other trauma). An estimated twenty-seven million men and women age 25 and older have osteoarthritis in the United States. It is seen more commonly in men before the age of 45 and women after 45.

B. Types of the Condition: There is not much difference in what the following types of OA feel like to a patient, the difference is in what causes these two subsets:

- Primary Osteoarthritis: This is most commonly diagnosed form of osteoarthritis. Often considered “wear and tear” or “bone on bone”, this type of OA is associated with aging and diagnosed around the age of 55.
- Secondary Osteoarthritis: This type of OA is due to a specific event (weight, injury, genetics, inactivity, etc.) and often seen in younger patients.

C. Causes:

- Aging
- Injury (articular cartilage, bone fracture, ligament tears)
- Obesity: extra weight bears down on the joints, causing a quicker wear down.
- Inactivity: The less activity, the weaker the muscles and tendons around that joint will be. This can cause instability and misalignment of joints.
- Genetics: There has been a genetic link found for women and OA of the hands. There could be a genetic defect in the joint cartilage.
- Joints not properly formed
- Inflammation from other diseases
- Stress on the joints from certain jobs and activities
D. **Treatment:** Whether a patient has primary or secondary osteoarthritis, the treatments are relatively the same. Those with secondary OA should take extra precautions and avoid the cause of their OA. The following treatments for OA should be managed with a combination of rest, regular exercise, vitamin and mineral supplements, and medication.

- Acetaminophen (Tylenol) for pain without inflammation
- NSAIDs (aspirin or ibuprofen) for pain with inflammation
- Cyclooxygenase-2 inhibitors: same function as NSAIDs with less stomach irritation
- Topical analgesic products or the nutritional supplement combination of glucosamine and chondroitin
- Corticosteroid medications/injections (prednisone or cortisone) to reduce inflammation
- Hyaluronic acid injections: naturally occurring substance similar to the fluid inside joints

### 3. **POTENTIAL BENEFITS OF STEM CELL TREATMENT**

Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells with multi-lineage potential to differentiate into cell types of mesodermal origin. Due to this attribute, MSCs can differentiate into chondrocytes, which are later replaced by bone. This attribute repairs the subchondral bone without any loss of articular cartilage at the surface. MSCs have been shown to therapeutically alter the progression of OA by down-modulating the release and expression of the main OA inflammatory factors and chemokines (signaling proteins secreted by cells) directly involved in the progression of the disease. Additionally, there has been significant improvement in joint function through pain reduction and increase of cartilage in the affected joint.

### 4. **TREATMENT & DELIVERY METHOD REQUIRED**

A. **Typical Recommended Treatment:** Adipose Derived Stem Cells combined with PRP (platelet rich plasm)

B. **Typical Delivery Method Required:** Autologous Ad-SVF containing adult stem cells are infused in 5-10 ml of Platelet Rich Plasma and injected intra-articular.

C. **Recommended dosing:** Recommended repeat dosing MSC’s combined with PRP every 3 months based on symptoms.

### 5. **POTENTIAL RISKS OF STEM CELL INJECTION(S)**

There are possibilities for unwanted effects related to the local anesthesia, harvesting procedure, and injection of stem cells. Even with the most established protocol, adequate technique, and careful administration; a medical team may encounter uncontrollable events. Although there is no guarantee of perfect results, positive results can be attained. The medical professional provides services in the most responsible, professional and diligent manner, always considering that surgeries imply risks. The risks of complications of adipose tissue harvesting and stem cell infusion are very low. Possible risks include but are not limited to:

- Pain at site of injections
- Bleeding at injection site
- Malaise
- Low-grade fever
- Hot flashes
- Itching at injection site
- Vascular spasm or obstruction
- Bruising
- Nerve or muscle injury
- Allergic reaction
- Dizziness
- Nausea
- Vomiting
- Unknown risks up to and including death

### 6. **FREQUENTLY ASKED QUESTIONS**

1. **What are adults stem cells and how do they work?**
   They are undifferentiated cells found in tissues in the body, responsible for maintaining and repairing surrounding cells. These tissues include but are not limited to adipose tissue (fat tissue) and bone marrow. They have the ability to differentiate into various cell types, which is what makes them a potential treatment to alleviate and improve degenerative conditions. It has been proven that there are 500 times more stem cells stored in adipose tissue verse bone marrow, therefore, adipose derived stem cells are far superior.

2. **What is Adipose-derived stem cells and what makes it unique?**
   Adipose-derived stem cells are stem cells found in the adipose (fat) tissue of the patient. The adipose tissue is an abundant source of mesenchymal stem cells which have shown the most healing potential. Adipose Stem cells are autologous (patients stem cells); therefore, having low risk of immune rejection once the therapy has been completed.
3. How can stem cell therapy help treat Osteoarthritis?
Mesenchymal stem cells are self-renewing, multipotent progenitor cells with multi-lineage potential to differentiate into cell types of mesodermal origin. This property helps in cartilage, ligament, and bone regeneration. MSCs also migrate to sites of inflammation and may reduce the inflammation associated with OA.

4. How will the stem cells be delivered to the patient?
The stromal vascular fraction from the fat (SVF) will be resuspended in 5-10mL Platelet Rich Plasma and injected intra-articularly into the joint.

3. How long do you think it would be before I see some improvement?
The response to the treatment varies from patient to patient. Some patients see a response within the first three months, while many studies have shown patients can improve 6-12 months after a stem cell injection. This is an ongoing research topic and results are not guaranteed.

4. How long does the procedure take?
The procedure will take approximately 2-3 hours.

5. Are there any complications associated with this procedure?
Minimal bruising and soreness can be observed due to the liposuction procedure. This will normally last around 1-2 weeks, depending on the patient. Some patients have experienced a low grade fever. None of these symptoms were long lasting and resolved within 24-72 hours.

6. Does smoking or drinking affect the therapy?
Smoking and the consumption of alcohol has been shown to be harmful to stem cells. We advise that people do not smoke or drink during their treatment, and it must absolutely be avoided the week following the treatment.

7. Will anyone follow up with me after the procedure?
A team member will follow up with you 1 day, 1 week, 3 months, 6 months and 1 year after the procedure. Follow ups help us evaluate the effectiveness of our treatment, and improve treatment protocols. We will be monitoring your progress closely. We are happy to address any issues or questions at anytime.

7. SUPPORTING ARTICLES

Intra-articular injection in the knee of adipose derived stromal cells (stromal vascular fraction) and platelet rich plasma for osteoarthritis.

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Abstract

BACKGROUND: Stromal vascular fraction (SVF) can easily be obtained from a mini-lipoaspirate procedure of fat tissue and platelet rich plasma (PRP) can be obtained from peripheral blood. We evaluated the safety and preliminary efficacy of administering SVF and PRP intra-articularly into patients with osteoarthritis grade 1 and 2.

METHODS: A total of ten patients underwent a local tumescent liposuction procedure to remove approximately 100 ml of fat tissue from the abdomen. SVF was isolated using an enzyme digestion and resuspended in PRP for intra-articular injection in the knee. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score and six-minute walk distance (6MWD) were used to evaluate clinical effects and included measure of patient’s subjective assessment of pain, joint mobility, and physical disability. WOMAC score, 6MWD and laboratory tests were repeated at 3 and 6 months and 1, 1.5 and 2 years. XRAY and MRI were completed at 1 year.
RESULTS: The average total WOMAC score was 64 at baseline and significantly reduced to 52 at 3 months, 46 at 6 months, 42 at 1 year, 38 at 1.5 years, and 41 at 2 years. Patients walked an average of 1310 feet at baseline and demonstrated a statistically significant improvement at 3 and 6 months and 1, 1.5, and 2 years post treatment. Cartilage thickness as determined by MRI improved by at least 0.2 mm in six patients, was unchanged in two patients and decreased by at least 0.2 mm in two patients.

CONCLUSIONS: Overall, all of the patients were pleased with the treatment results. They reported a reduction in pain levels, especially after 3 months. More importantly, the procedure demonstrated a strong safety profile with no severe adverse events or complications reported. Trial registration NCT03089762; Name of registry: http://www.clinicaltrials.gov.

KEYWORDS: Adipose derived stromal/stem cells (ADSCs); Adipose tissue; Cell therapy; Connective tissue; Osteoarthritis; Platelet rich plasma (PRP); Stem cells; Stromal vascular fraction (SVF)

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Culture-expanded allogenic adipose tissue-derived stem cells attenuated cartilage degeneration in an experimental rat osteoarthritis model.

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Abstract
Mesenchymal stem cell (MSC)-based cell therapy is a promising avenue for osteoarthritis (OA) treatment. In the present study, we evaluated the efficacy of intra-articular injections of culture-expanded allogenic adipose tissue-derived stem cells (ADSCs) for the treatment of anterior cruciate ligament transection (ACLT) induced rat OA model. The paracrine effects of major histocompatibility complex (MHC)-unmatched ADSCs on chondrocytes were investigated in vitro. Rats were divided into an OA group that underwent ACLT surgery and a sham-operated group that did not undergo ACLT surgery. Four weeks after surgery mild OA was induced in the OA group. Subsequently, the OA rats were randomly divided into ADSC and control groups. A single dose of 1 × 106 ADSCs suspended in 60 μL phosphate-buffered saline (PBS) was intra-articularly injected into the rats of the ADSC group. The control group received only 60 μL PBS. OA progression was evaluated macroscopically and histologically at 8 and 12 weeks after surgery. ADSC treatment did not cause any adverse local or systemic reactions. The degeneration of articular cartilage was significantly weaker in the ADSC group compared to that in the control group at both 8 and 12 weeks. Chondrocytes were co-cultured with MHC-unmatched ADSCs in trans-wells to assess the paracrine effects of ADSCs on chondrocytes. Co-culture with ADSCs counteracted the IL-1β-induced mRNA upregulation of the extracellular matrix-degrading enzymes MMP-3 and MMP-13 and the pro-inflammatory cytokines TNF-α and IL-6 in chondrocytes. Importantly, ADSCs increased the expression of the anti-inflammatory cytokine IL-10 in chondrocytes. The results of this study indicated that the intra-articular injection of culture-expanded allogenic ADSCs attenuated cartilage degeneration in an experimental rat OA model without inducing any adverse reactions. MHC-unmatched ADSCs protected chondrocytes from inflammatory factor-induced damage. The paracrine effects of ADSCs on OA chondrocytes are at least part of the mechanism by which ADSCs exert their therapeutic activity.

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Potential use of mesenchymal stem cells in human meniscal repair: current insights.

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3 National Leading Research Laboratory, Department of Biological Sciences, Myongji University, Yongin, Gyeonggido, Republic of Korea.
Abstract
The menisci of the human knee play an important role in maintaining normal functions to provide stability and nutrition to the articular cartilage, and to absorb shock. Once injured, these important structures have very limited natural healing potential. Unfortunately, the traditional arthroscopic meniscectomy performed on these damaged menisci may predispose the joint toward early development of osteoarthritis. Although a very limited number of studies are available, mesenchymal stem cells (MSCs) have been investigated as an alternative therapeutic modality to repair human knee meniscal tears. This review summarizes the results of published applications of MSCs in human patients, which showed that the patients who received MSCs (autologous adipose tissue-derived stem cells or culture-expanded bone marrow-derived stem cells) presented symptomatic improvements, along with magnetic resonance imaging evidences of the meniscal repair.

KEYWORDS: adipose tissue-derived stem cells; articular cartilage; bone marrow-derived stem cells; human knee; meniscal tear; therapeutic modality
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4. Clinical applications of mesenchymal stem cells
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INTRODUCTION
Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells with multilineage potential to differentiate into cell types of mesodermal origin, such as adipocytes, osteocytes, and chondrocytes [1]. While MSCs are most commonly isolated from bone marrow [2], they are also isolated from other tissues including adipose tissue [3,4], placenta [5], amniotic fluid [6], and umbilical cord blood [7,8]. Due to their accessibility and convenient expansion protocols, MSCs have been recognized as promising candidates for cellular therapy. However, growing interest in MSCs has led to
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Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells with multilineage potential to differentiate into cell types of mesodermal origin, such as adipocytes, osteocytes, and chondrocytes. In addition, MSCs can migrate to sites of inflammation and exert potent immunosuppressive and anti-inflammatory effects through interactions between lymphocytes associated with both the innate and adaptive immune system. Along with these unique therapeutic properties, their ease of accessibility and expansion suggest that use of MSCs may be a useful therapeutic approach for various disorders. In the clinical setting, MSCs are being explored in trials of various conditions, including orthopedic injuries, graft versus host disease following bone marrow transplantation, cardiovascular diseases, autoimmune diseases, and liver diseases. Furthermore, genetic modification of MSCs to overexpress antitumor genes has provided prospects for clinical use as anticancer therapy. Here, we highlight the currently reported uses of MSCs in clinical trials and discuss their efficacy as well as their limitations.

5. Stem cells for repair of cartilage and bone: the next challenge in osteoarthritis and rheumatoid arthritis
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Over the last few years, immunotherapy targeting proinflammatory cytokines has been the main goal of research into rheumatoid arthritis (RA), and recently the new anti- (tumour necrosis factor) blocking agents have dramatically improved the course of the disease by stabilising the symptoms.[1] However, this treatment has no effect on regeneration of articular cartilage damaged during the inflammatory process. The next challenge is thus to ensure cartilage repair through cell therapy and tissue engineering (fig 1). Tissue in the body is replaced by two main mechanisms. One is self repair by fully differentiated cells (healing), and the second is replacement with newly differentiated cells derived from stem cells. Recently, the regenerative potential of mesenchymal stem cells (MSCs) has been under intense investigation because of their ability for self renewal and differentiation to reconstitute muscle, cartilage, or bone?

6. Adipose-Derived Mesenchymal Stem Cells Exert Antiinflammatory Effects on Chondrocytes and Synoviocytes From Osteoarthritis Patients Through Prostaglandin E2

Cristina Manferdini,1 Marie Maumus,2 Elena Gabusi,1 Anna Piacentini,1 Giuseppe Filardo,1 Julie-Anne Peyrafitte,3 Christian Jorgensen,4 Philippe Bourin,3 Sandrine Fleury-Cappelless,3 Andrea Facchini,5 Dani le No ël,2 and Gina Lisignoli1

Objective. To examine the effect of different sources of Good Manufacturing Practice clinical grade adipose-derived mesenchymal stem cells (AD-MSCs) on inflammatory factors in osteoarthritic (OA) chondro- cytes and synoviocytes.

Methods. AD-MSCs from infrapatellar Hoffa fat, subcutaneous (SC) hip fat, and SC abdominal fat were cocultured in Transwells with chondrocytes or synovio- cytes. Inflammatory factors (interleukin-1 [IL-1], tumor necrosis factor-1, IL-6, CXCL1/growth-related oncogene-1, CXCL8/IL-8, CCL2/monocyte chemotactic protein 1, CCL3/macrophage inflammatory protein 1, and CCL5/RANTES) were evaluated by quantitative reverse transcription–polymerase chain reaction or multiplex bead–based immunoassay. The role of different immunomodulators was analyzed.

Results. All the inflammatory factors analyzed were down-modulated at the messenger RNA or protein level independently by all 3 AD-MSC sources or by allogeneic AD-MSCs used in coculture with chondrocytes or synoviocytes. Inflammatory factor down-modulation was observed only when AD-MSCs were cocultured with chondrocytes or synoviocytes that pro-duced high levels of inflammatory factors, but no effect was observed in cells that produced low levels of those factors, thus highlighting a dependence of the AD-MSC effect on existing inflammation. The immunomodula- tors IL-10, IL-1 receptor antagonist, fibroblast growth factor 2, indoleamine 2,3-dioxygenase 1, and galectin 1 were not involved in AD-MSC effects, whereas the cyclooxygenase 2 (COX-2)/prostaglandin E2 (PGE2) pathway exerted a role in the mechanism of antiinflam- matory AD-MSC action.

Conclusion. The antiinflammatory effects of AD-MSCs are probably not dependent on AD-MSC adipose tissue sources and donors but rather on the inflam- matory status of OA chondrocytes and synoviocytes. AD-MSCs seem to be able to sense and respond to the local environment. Even though a combination of dif-ferent molecules may be involved in AD-MSC effects, the COX-2/PGE2 pathway may play a role, suggesting that AD-MSCs may be useful for therapies in osteo- articular diseases.

7. Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study

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Abstract—Osteoarthritis is one of the most common diseases, and it affects 12% of the population around the world. Although the disease is chronic, it significantly reduces the patient’s quality of life. At present, stem cell therapy is considered to be an efficient approach for treating this condition. Mesenchymal stem cells (MSCs) show the most potential for stem cell therapy of osteoarthritis. In fact, MSCs can differentiate into certain mesodermal tissues such as cartilage and bone. Therefore, in the present study, we applied adipose tissue–derived MSCs to os- teoarthritis treatment. This study aimed to evaluate the clinical efficiency of autologous adipose tissue–derived MSC transplantation in patients with confirmed osteoarthritis at grade II and III. Adipose tissue was isolated from the belly, and used for extraction of the stromal vascular fraction (SVF). The SVF was mixed with activated plate-let-rich plasma before injection.
The clinical efficiencies were evaluated by the pain score (VAS), Lysholm score, and MRI findings. We performed the procedure in 21 cases from 2012 to 2013. All 21 patients showed improved joint function after 8.5 months. The pain score decreased from 7.6±0.5 before injection to 3.5±0.7 at 3 months and 1.5±0.5 at 6 months after injection. The Lysholm score increased from 61±11 before injection to 82±8.1 after injection. Significant improvements were noted in MRI findings, with increased thickness of the cartilage layer. Moreover, there were no side-effects or complications related to microorganism infection, graft rejection, or tumorigenesis. These results provide a new opportunity for osteoarthritis treatment. Level of evidence: IV.

8. Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis

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Abstract: Stromal vascular fraction (SVF), containing large amount of stem cells and other regenerative cells, can be easily obtained from loose connective tissue that is associated with adipose tissue. Here we evaluated safety and clinical efficacy of freshly isolated autologous SVF cells in a case control study in patients with grade 2-4 degenerative osteoarthritis (OA). A total of 1128 patients underwent standard liposuction under local anesthesia and SVF cells were isolated and prepared for application into 1-4 large joints. A total of 1866 joints, mainly knee and hip joints, were treated with a single dose of SVF cells. 1114 patients were followed for 12.1-54.3 months (median 17.2 months) for safety and efficacy. Modified KOOS/HOOS Clinical Score was used to evaluate clinical effect and was based on pain, non-steroid analgesic usage, limping, extent of joint movement, and stiffness evaluation before and at 3, 6, and 12 months after the treatment. No serious side effects, systemic infection or cancer was associated with SVF cell therapy. Most patients gradually improved 3-12 months after the treatment. At least 75% Score improvement was noticed in 63% of patients and at least 50% Score improvement was documented in 91% of patients 12 months after SVF cell therapy. Obesity and higher grade of OA were associated with slower healing. In conclusion, here we report a novel and promising treatment approach for patients with degenerative OA that is safe, cost-effective, and relying only on autologous cells.

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