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Adipose-derived stromal vascular fraction (SVF) for the treatment of osteoarthritis of the knee, functional outcome and anatomic recovery of the cartilage: a case report

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Abstract

Background: **Osteoarthritis (OA) is a degenerative debilitating disease characterized by progressive erosion of the articular cartilage. Current treatment approaches are reduced to control the associated symptoms, leaving the degenerative changes to progress until a joint replacement becomes mandatory. Therefore, disease-modifying therapeutic alternatives are warranted to improve the patient's quality of life as well as to ameliorate the high economic impact this disease pose on the healthcare system.**
Objective: **We present a case study of a 42-year-old male with stage 3-4 (clinical) and grade 3 and 4 (radiological) OA, to support the evidence of a positive effect of intra-articularly injected Mesenchymal Stem Cells (MSC)-containing adipose-derived stromal vascular fraction (SVF) on both clinical and articular cartilage structural outcomes.**
Materials and Methods: **To obtain the SVF, the patient underwent liposuction and the lipoaspirate was enzymatically processed. The resulting SVF was locally injected into the knee joint. Resultant symptomatology was assessed using the KOOS (Knee Osteoarthritis Objective Score) and the structural response was documented using ultrasonography, both evaluated at various time-points.**
Results: **We observed the largest clinical improvement (~130% in the KOOS score) in the first 6 weeks, stabilized at 24 weeks, and persisted at 20 month. Notable, ultrasonographic changes included progressive widening of joint spaces, elimination of effusions and thickening of articular cartilage that started as early as 6 weeks and persisted throughout the evaluation period.**
Conclusions: **Cell-based therapy for advanced knee OA using MSC-containing adipose-derived SVF was subjectively and objectively positive for the patient. The KOOS and ultrasonographic measurements were simple and aligned with the patient's course response, providing additional evidence of a positive effect both clinically and structurally. In addition, the results here highlight the importance of consecutive measurements with an inexpensive approach to appreciate the biological process of such therapy for OA and to establish a response curve.**

Introduction

Articular cartilage, due to its structural features, has demonstrated a poor regenerative capacity after injury. Although chondrocytes capable of producing a reparative matrix exist in articular cartilage, they exhibit a very low proliferative capacity with a limited synthetic capacity[1]. Therefore, articular cartilage injuries have serious clinical implications as they often result in fibrillation, degradation and erosion, with a parallel compromise of the subchondral bone, ending in degenerative osteoarthritis (OA).

Surgical approaches such as cartilage debridement and bone marrow stimulation (i.e., micro-fracture) are intended to improve the characteristics of the articular surface. However, a long-lasting clinical improvement has not yet fully achieved, with patients "relapsing" in their symptoms and eventually requiring a definitive joint replacement[2].

The concept of using autologous mesenchymal stem cells (MSCs) as part of a regenerative-based approach to treat cartilage injuries has increasingly received more attention in the last years[3,4]. Currently, there are more than a dozen of different protocols using autologous MSCs derived from bone marrow and other sources such as adipose tissue and synovium[5]. The adipose tissue is a rich source of MSC, as it is a highly vascularized tissue. Enzymatically-released SVF contains a heterogeneous population of cells, including MSC, hemopoietic progenitors, pericytes, endothelial progenitors and other cell types. In addition to their chondrogenic differentiation capabilities, there is ample consensus that MSCs have a wide variety of trophic properties including anti-inflammatory and immunomodulatory effects, exerted by the production of a wide range of signaling paracrine growth factors[6,7]. Consequently, MSC may have a two-fold activity in OA: in addition to help rebuild cartilage structurally by virtue of their differentiation potential into chondrocytes, the secreted factors can halt the pathologic vicious cycle between inflammation and cartilage destruction.

Here, we report a case of a patient with an advanced knee OA that benefited both clinically and structurally from an intra-articular injection of autologous processed SVF. The improvement was evident as early as 6 weeks after the procedure, with a prolonged effect noticeable until 20 months. This case, along with additional clinical trials can provide solid evidence for the overall benefit of this regenerative medicine-based approach in treating OA patients.

Case Report

A 42-year-old male athlete (Division I NCAA basketball coach) presented with a 20-year history of right knee pain, after being diagnosed with torn lateral meniscus and frayed tibial plateau cartilage. The patient underwent two (2) initial arthroscopic knee surgeries, with a third one ten (10) years later involving the removal of torn meniscal tissue and loose cartilage bodies within the joint. Currently diagnosed with stage 3-4 (clinical) and grade 3 and 4 (radiological) right knee osteoarthritis (OA) involving mainly the lateral compartment, his symptoms evolved to a limited flexion and extension, use of daily analgesics, chronic effusions that occasionally required drainage, difficulty ambulating without limping, inability to drive more than an hour without pain, altogether significantly limiting his daily activities including basketball coaching.

Facing an advanced OA of the right knee, and in an effort to delay a unicompartmental or a total knee arthroplasty, the patient became aware of a regenerative medicine-based treatment, based on the intra-articular injection of adipose-derived stromal vascular fraction (SVF)-containing mesenchymal stem cells (MSC). On August 19, 2013 the patient underwent a lipoaspirate under general anesthesia to obtain the adipose-derived SVF. After processing the adipose tissue and immediately after the cell count, 6.8 x 10^7 viable SVF cells resuspended in 5 cc of Ringer's Lactate were injected into the right knee joint using real-time ultrasound guidance. Using an in-plane approach, the needle was visualized entering the suprapatellar synovial bursa, where an effusion was evident (Figure 1) and

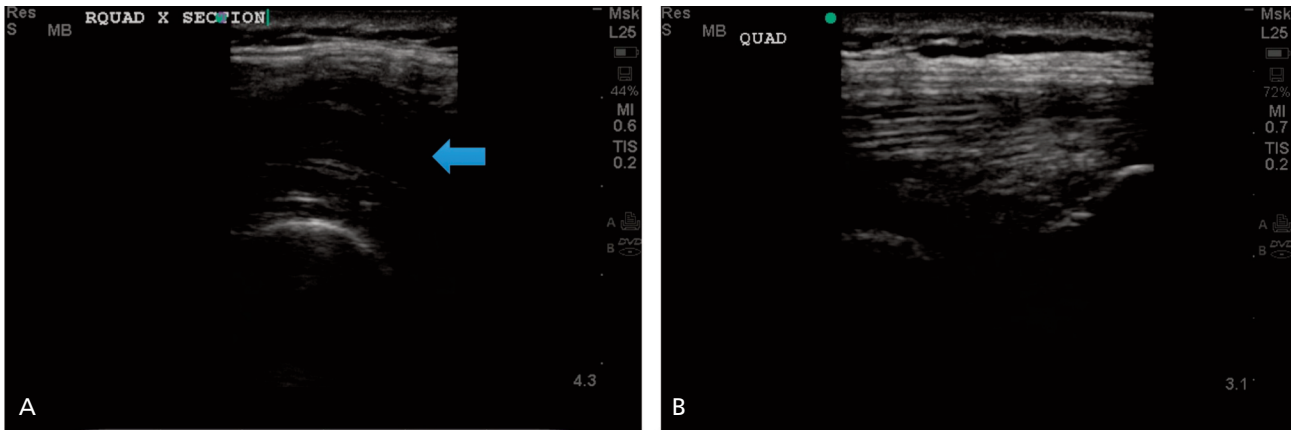

Figure 1. Ultrasound of the right knee at baseline *(A)* and 24 weeks post-procedure *(B)*, evidencing a suprapatellar bursa effusion (black area at 2 cm depth, blue arrow in *A*) present before the procedure. The effusion is not evident in further controls *(B)*.

Table 1. Clinical evaluation (KOOS scores) performed over time, up to 20 months. Values range from 100 (asymptomatic) to 0 (extremely symptomatic). A progressive clinical improvement is readily observable.

Koos Category	Pretreatment	3 weeks	6 weeks	12 weeks	24 weeks	20 months
Symptoms (n = 7)	29	54	64	61	71	67
Pain (n = 9)	67	75	81	83	92	97
Activities of daily living (n = 17)	90	100	96	98	98	96
Sports (n = 5)	55	60	80	80	80	75
Quality of life (n = 4)	44	50	69	62	75	66
Total score	**285**	**339**	**390**	**384**	**416**	**401**

drained. Subsequently, as the SVF was injected, further expansion of the synovial bursa underneath the quadriceps tendon was observed and documented. In addition, the area around a discrete calcification within the iliotibial (IT) band was injected with 0.5 cc of the cell suspension. The patient tolerated the procedure without any secondary effect, both short and long term.

The patient reported pain relief with flexion of his knee within 2 hours of the procedure. As early as 48 hours later, the patient reported an improvement in his symptoms with regular activities such as short walks, sleeping without pain, with a visible difference in the size of his knee due to a reduction in swelling. A clinical assessment using the KOOS score was performed pre-op (baseline), and at 3, 6, 12 and 24 weeks, and 20 months (Table 1). The scores revealed significant changes in each category throughout the assessment, with most changes observed during the first 6 weeks, stabilized thereafter until the end of the study (20 months). The final delta (total at 20 months *vs.* pre-op) demonstrated an improvement of 134%.

Ultrasound imaging of the knee was repeated during some of the follow-ups (6 and 24 weeks and 20 months), using the same protocol and performed by the same operator. All evaluations (Table 2) suggested a progressive increase in both the joint space and tibial plateau cartilage thickness of the lateral compartment of the right knee, accompanied by the absence of joint effusions. In addition, a decreased shadowing (less dense) of the IT band calcification correlates with a decrease in point tenderness reported by the patient. These changes were maintained until the end of the analysis and could be related to his clinical improvement (Figure 2).

Materials and methods

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national, Universidad Nacional Autonóma de Nicaragua, León) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the participant included in the case study and the patient provided consent to publish the case study in all formats.

Obtaining of adipose-derived stromal vascular fraction (SVF)

Under short general anesthesia, 108 cc of dry fat were harvested from subcutaneous fat directly into a sterile processing canister (GID SVF-1, Louisville, CO, USA). The lipoaspirate was washed three times to remove red cells and fat oil. Appro-

Table 2. Ultrasound Measurements of the Right knee over time documenting a structural recovery on the lateral tibial articular cartilage and the absence of joint effusions.

	Lateral joint space	Lateral articular cartilage	Medial joint space	Medial articular cartilage	Supra patelar bursa	Infra patelar bursa
Baseline	0.38 cm	0.06 cm	1.09 cm	0.20 cm	Medium effusion	Small effusion
6 weeks post	1.01 cm	0.17 cm	1.03 cm	0.24 cm	No effusion	No effusion
24 weeks post	0.95 cm	0.18 cm	1.09 cm	0.23 cm	No effusion	No effusion
20 months post	1.05 cm	0.25 cm	1.08 cm	0.25 cm	No effusion	No effusion

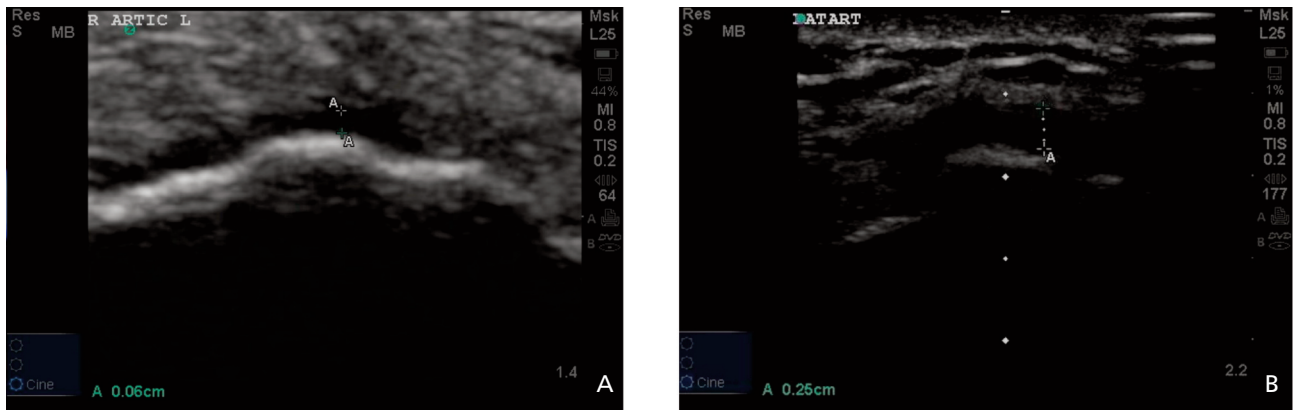

Figure 2. Ultrasound evaluation of the lateral tibial plateau articular cartilage thickness at baseline *(A)* and 20 months post-procedure *(B)*. A significant difference (~4 times) in the cartilage thickness is evident, from 0.06 cm at the beginning and 0.25 cm at the end of the evaluation period.

ximately 125 ml of Lactated Ringer's solution was added to the adipose tissue with collagenase enzyme (Worthington CLS-1, Lakewood, NJ, USA) at a concentration of 200 CDU/ml of total volume. The mixture was dissociated for 40 minutes in an incubated shaker table at 38ºC and 150 RPM. After dissociation, the mixture was centrifuged for 10 minutes at 800x gravity. The resulting concentrated SVF at the bottom of the device was removed using a 6-inch needle. Ten microliters (µl) of SVF were taken from the final suspension and submitted for differential staining. Two samples were then passed through an image cytometer (ADAM MC, Portsmouth, NH, USA) for cell counting and viability assessment. The viable cell count per gram of fat obtained was 184,000.

Evaluation

Postoperative evaluation was carried out using two methodologies. (1) Clinical symptomatology was assessed semi-quantitatively using the KOOS (Knee Osteoarthritis Objective Score) pre-op (baseline), and at 3, 6, 12 and 24 weeks, and 20 months post-procedure; (2) Anatomic response was documented using ultrasonography pre-op (baseline) and at 6 and 24 weeks and 20 months.

Knee Osteoarthritis Objective Score (KOOS)

The KOOS was applied as follows: it consists of five categories of questions, each one receiving a numerical value of 0 to 4. The categories and the number of questions in each are: pain (9), symptoms (7), activities of daily living (17), sports and recreation (5), and quality of life (4). Each category is quantified as an adjusted score using the following steps. First, the values for each question are summed and an average obtained. Second, this value is multiplied by 100 and divided by 4 (the maximum possible average score). Third, this value is subtracted from 100. *Adjusted score = 100 – [(average score x 100)/4]*

The values of each category are independent. Absolute values are 100 (no symptoms) and 0 (extreme symptoms). Although a total point score can be used, it is not statistically valid to average out the subcategories.

Ultrasonography

Point of care ultrasound examinations of the patient's affected knee joint was performed immediately prior to the lipoaspirate. Using a linear, high frequency 10-5 MHz probe, images were recorded, and measurements of the right knee joint were taken. The protocol included measurements of the medial collateral ligament/medial joint space, quadriceps femoris tendon and suprapatellar synovial bursa, patellar ligament and infrapatellar fat pad, lateral collateral ligament/lateral joint space, and both medial and lateral tibial plateau articular cartilages.

Discussion

Articular cartilage repair after injury constitutes a significant challenge from both basic science and clinical perspectives. The former based on the complex biology of the tissue, while the latter involves the lack of a healing response that leads to a progressive/multi-symptomatic degenerative disease. Consequently, various approaches have been developed over the years attempting to locally provide the necessary resources to start a truly regenerative effect. These approaches encompass: first, *ex vivo* techniques such as Tissue Engineering in which

chondrogenic cells are combined with molecular queues loaded into specific scaffolds; and second, *in vivo* protocols aimed at recruiting chondrogenic cells to the lesion, as well as to improve the local conditions (e.g., visco-supplementation) to ameliorate the associated symptoms. Even though some of these approaches provide some temporal relief, while others are still in development and seem promising, overall they have not been successful controlling the progressive cartilage damage.

The presence of osteoblastic progenitors inside the bone marrow was first identified by Friedenstein[8]. Later, Caplan and collaborators described their mesenchymal origin and introduced the mesengenic process concept based on the multi-progenitor capacity of the cell, thus naming it MSC[9,10]. In the recent years, a more comprehensive appreciation of the potential therapeutic capabilities of MSC has been undercovered. They have been largely studied in the context of their regenerative capacity in injured tissues due to their immunomodulatory and trophic effects[7]. In parallel, MSC intimate relationship with the vascular system has been also described[11-14]. Microvasculature vessels are constructed from two cell types: endothelial cells and pericytes[15,16]. Endothelial cells represent one of two possible developmental pathways for the primitive hemangioblast, being the other pathway the hematopoietic line[11,17-19]. Pericytes, on the other hand, are found in association with all vessels of the body[20], are developmentally related to neural crest cells[21], and are precursors for MSC[12]. Pericytes both in bone marrow and adipose tissue share a similar phenotype (i.e., CD signature), and indeed, the pericyte is the cell of origin of white fat[22].

Quarto in 2001 reported the first successful use of cultured MSC for orthopaedic applications[23]. Later, other examples of repair of long bone defects using cultured bone marrow-derived MSC have been reported[24]. Regarding cartilage injury, and specifically in OA patients, various pilot studies and cases have been reported as successful in terms of both clinical[25-27] and structural improvement[28,29]. The potential for success with bone marrow stem cell therapy is marred by the challenges of its historically variant performance and the need for cell expansion in order to achieve an adequate number of cells enough for clinical application. The existence of MSC in the adipose tissue as part of the SVF, reported in 2001 by Zuk et al[30], offered a new source with about 1000 times more cells per gram of tissue than with bone marrow[31]. The basic biology and clinical behavior of adipose-derived MSC have been widely described[32-34]. Despite a reduced chondrogenic differentiation of adipose-derived MSC compared to their bone marrow-derived counterpart, the clinical effects can be comparable thus suggesting that the paracrine trophic activity of MSC constitutes a significant portion of the overall clinical activity in cartilage repair.

The case described here (a grade 3-4 OA) supports the use of intra-articular injection of adipose-derived SVF, containing MSC. Technically, the GID technique produced a dose of 6.8 x 10^7 living mononuclear cells in less than 60 minutes, constituting an easy, effective and rapid way to obtain therapeutic SVF. The rapid recovery and long-lasting amelioration of the clinical symptoms (assessed by KOOS), as well as the progressive structural changes of the cartilage (assessed by ultrasound), provide solid support for the clinical efficacy of this approach. The methodologies of the KOOS and ultrasonography were easy to perform and well aligned with the patient's clinical course. In fact, there was compatibility between the values computed by the KOOS and physical measurements of the joint spaces and articular cartilage. These findings are consistent with observations in other similarly treated patients where regression of symptoms accompanied increases in cartilage thickness.

The KOOS is a self-administered test consisting of a series of questions divided into 5 categories reflecting both symptoms and activity levels, to evaluate injuries of the knee that can lead to post-traumatic OA (PTOA). It can be used for limited periods of time or as part of a long-term study. Structures at risk that are sensitive to KOOS are the anterior cruciate ligament, the meniscus, and the articular cartilage, applicable to degenerative OA[35-37]. The clinical utility of KOOS is that it permits monitoring of patient response to medications, physical therapy regimens, and surgical interventions. An advantage of KOOS is that its categories include physical function as well as activities of daily living, including recreation, thus it encompasses a wide range of occupational and sports activities.

The historical standard musculoskeletal (MSK) imaging modality for assessing articular cartilage and ligamentous structures in the knee is magnetic resonance imaging (MRI). The MRI is a proven and effective imaging modality; however, it is expensive and time-consuming. As a consequence, MRI can be seen an inadequate and cost prohibitive tool for screening or serial musculoskeletal examina-

tions, especially in underdeveloped economies. As an alternative, MSK ultrasound has a growing presence in medicine as an efficient and cost-effective imaging alternative, with the added benefit of its ability to be used at the point of care. There have been several studies showing that ultrasound imaging is more cost effective than MRI for some soft tissue structures[38-40]. This modality has been used effectively in the evaluation of soft tissues, muscles, tendons, and ligaments and meniscal tears[41,42]. It has been gaining some popularity for assessing articular cartilage abnormalities[43,44], although it is still underutilized. The time, safety, and cost implications of incorporating MSK ultrasound into the orthopaedic imaging practice as an adjunct and sometimes substitute to MRI are significant. In addition, ultrasound has been shown to be superior to traditional anatomic methods for needle placement into large intra-articular spaces[45]. Subluxation of the medial meniscus into the adjacent collateral ligament, effusions, and joint space narrowing are readily documented by ultrasound[46-48].

Conclusions

This case highlights the importance of obtaining multiple consecutive clinical and imaging measurements to better understand the biological process and, therefore, establish a response curve to adipose-derived SVF-containing MSC during cartilage repair. This case provides the foundations for a bigger clinical trial aimed at certifying the therapeutic benefit of such regenerative medicine-based approach.

Authors' declaration of personal interests

Michael H. Carstens and Diego Correa have served as consultants for the GID Group. No other individuals have reported interest.

Declaration of funding interests

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