A SINGLE-BLIND, RANDOMIZED, CONTROLLED STUDY OF A SINGLE, INTRA-ARTICULAR INJECTION OF AUTOLOGOUS MICROFRACTURED ADIPOSE TISSUE (aMAT) IN PATIENTS WITH OSTEOARTHRITIS (OA) OF THE KNEE
STUDY SYNOPSIS

INTRODUCTION
The societal impact of degenerative diseases such as articular cartilage pathology and osteoarthritis (OA) is steadily increasing, because of the continued rise in the mean age of the active population [1,2]. Unfortunately, articular cartilage lesions, with their inherent limited healing potential, are hard to treat and remain a challenging problem for orthopedic surgeons and all physicians. The regeneration capacity of cartilage is limited because of its isolation from systemic regulation and its lack of vessels and nerve supply [3-5]. Unlike most tissues, none of the inflammatory processes is available for its repair, and chondrocytes cannot migrate from an intact healthy site to the site of injury [3,4]. Biomechanical, metabolic, and biologic changes, as well as trauma and isolated chondral lesions, may lead to the loss of tissue homeostasis, resulting in accelerated degeneration of the articular surface and leading to end-stage arthritis. OA has a major impact on functioning and independence and ranks among the top 10 causes of disability worldwide [6]. With the population aging, the prevalence of OA is increasing, and its consequences are having a significant impact on society. Thus, one of the goals of modern medicine is to extend the quality of life and years of athletic activity of the population affected by cartilage lesions and OA.

A variety of noninvasive solutions has been proposed for pain treatment, improvement in function and disability, and ultimately, modification of the course of severe cartilage lesions and OA, with variable success rates [7]. Pharmacologic management usually begins with analgesia and anti-inflammatory agents [8]; the large apparent variation in individual response to each drug, the absence of clear clinical data regarding the therapeutic potency, and the potential side effects represent limits for their administration [9]. Topical agents have only been proven useful for short-term use for mild to moderate pain in mild joint degeneration [10]. Intra-articular injections of corticosteroids, as indicated by a few studies, are only of short-term benefit for pain and function [11]. Furthermore, some evidence indicates that they are not able to change the natural history of the disease and may have negative consequences on knee structures [12]. Glucosamine and chondroitin sulfate have not been clearly shown to be effective either, and they cannot be considered ideal agents for the treatment of pain from chronic severe cartilage degeneration or OA [13]. Among the available pharmacologic solutions, despite contradictory findings and controversies regarding its effective usefulness, intra-articular hyaluronic acid (HA) is widely applied in clinical practice, with good results reported in many studies [14-18]. The current clinical solutions suffer from significant limitations, such as safety and effectiveness, and they are not able to completely restore the patient’s mobility and quality of life.

With recent increase of interest in field of regenerative medicine, many studies about regeneration of articular cartilage using stem cells are actively underway. Initial method was mainly culture, for differentiation and proliferation of mesenchymal stem cells, but its disadvantages such as complexity and high cost of the procedure were also existed [19,20]. Recently, therefore, the use of autologous mesenchymal stem cells (MSCs) was introduced as a new and promising method. Numerous studies have been performed on intra-articular injection of mesenchymal stem cells, bone marrow concentrate (BMC), and adipose derived stem cells (ADSCs), reporting improvement of symptoms and function [21, 22, 23, 24, 25]. Among the
different sources, the abundance and easiness of adipose tissue (AT) make it a promising alternative with for regenerative purposes, with no need for culture-expansion nor extensive manipulation, thanks to the most recent achievements of biotechnologies. AT processed in such way may act through a two different mechanism: 1) the mechanical activity of AT as an intra-articular lubricant 2) the trophic and paracrine activity of ADSCs contained in aMAT, that has anti-inflammatory properties and might promote cartilage regeneration, in the end.

OBJECTIVES
The present study proposes to compare performances and safety of intra-articular injections of aMAT with those of a control group (PRP injections) [21, 22, 23, 26] for the treatment of symptomatic OA of the knee.

The end-points will be determined evaluating the performances of the treatment group in terms of improvement of the symptomatology, functional recovery and radiological appearance, compared to the control group.

STUDY DESIGN
The clinical trial is a prospective, randomized, controlled, two-arm, single-blind study, involving 80 patients affected by symptomatic OA of the knee joint. Eligible subjects will be randomly allocated to one of the two treatment groups, with a 1:1 randomization ratio. Respectively, 40 patients in the treatment group, treated by a single intra-articular injection of aMAT, and 40 patients in the control group, treated with single intra-articular injection of PRP.

In the early phase, subjects suffering from symptomatic knee OA will be identified by symptoms and radiological findings. Patient’s defect will be evaluated with pre-treatment MRI and X-Ray and each patient enrolled must meet all the entry criteria for the trial. Before enrolment, each subject should declare his voluntary participation to the study by informed consent signature.

The patients enrolled in the study will be treated according to the study protocol and followed after treatment with periodic visits and diagnostic imaging examinations, as Magnetic Resonance Imaging at twelve and twenty-four months of follow-up and X-Ray, at three, six months, twelve, and twenty-four months of follow-up. During each follow-up visit, the Case Report Form (CRF) will be filled in the specific section. Within the CRF, at each follow-up section, commonly used and specific scores will be assigned for the established end-points (IKDC form, KOOS, Tegner Score, EQ-5D, VAS Pain Score, MRI Mocart Scoring System, WORMS MRI score, and ICRS Cartilage Repair Assessment).

The study will be conducted after approval by the ethics committee board of the center and possibly by the national competent authorities.

Patients selected will be randomized to undergo one of the two groups, as prescribed by the randomization list.

Treatment protocol
Investigators and all the personnel involved will be properly instructed to the surgical protocol and study procedures.

In all patients of the treatment group, adipose tissue will be obtained from abdominal region or buttocks and aMAT, containing ADSCs, will be extracted with Lipogems® technological process. Lipogems® is a disposable, sterile medical device, class IIa, CE-marked, for point of care closed
circulating tissue processing of autologous (same patient) lipoaspirates. The result is a homogeneous micro-fragmented tissue with preserved vascular stromal cellular components, with a significant reduction in oil residues and blood components. The procedure is entirely based on mechanical forces, with no need of any enzyme or added reagents. Therefore, this technique is equivalent to a tissue “separation” procedure with “minimal manipulation”, according to the European Community Regulations.

Patients randomly assigned to the control group will be injected with PRP. In patients randomly assigned to treatment group, the obtained aMAT, containing an optimum number of ADSCs will be injected.

All treated patients should follow a common standard post-injective and rehabilitation program. Therefore for each patient 1, 3, 6, 12, and 24 months post-operative follow-up visits will be carried out. All pre- and post-treatment MRI (12 and 24 months) and X-Ray exams (6, 12, and 24 months) will be carried out, for each patient, in the clinic of treatment during the follow-up visit and blind evaluated by two senior radiologists.

**Inclusion Criteria**
1. Patients provided written informed consent;
2. Patients aged between 18 and 75 years;
3. Knee symptomatic OA (Kellgren-Lawrence grade 1-4)
4. Failure of conservative treatment for at least 3 months;
5. Patients agreed to actively participate in the rehabilitation protocol and follow-up program;
6. Male or female patients;
7. Women of childbearing age had to use a proven method to prevent pregnancy, before the surgical treatment.

**Exclusion Criteria**
1. Patients incapable to understanding and will;
2. Patients participating in previous, concurrent or not, trials (ongoing or completed within 3 months);
3. Patients surgically treated for the same defect within one year;
4. Patients affected by malignancy;
5. Patients affected by metabolic or thyroid disorders;
6. Patients used to alcohol or drug (medication) abuse;
7. Patients affected by synovitis;
8. Varus or valgus misalignment exceeding 15°;
9. Body Mass Index > 40;
10. Patients with trauma within 6 months pre-operative.
**Table 1-Flow-chart**

<table>
<thead>
<tr>
<th>Inclusion Exclusion Criteria evaluation</th>
<th>Pre-surgical Visit V1</th>
<th>Surgery V2</th>
<th>1 months f-up visit V3</th>
<th>3 months f-up visit V4</th>
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* only in case of post-operative arthroscopy and biopsy sample.
BIBLIOGRAPHY


