Sylvie Gonçalves

NEW THERAPEUTIC STRATEGIES FOR OSTEOARTHRITIS: INJECTIVE CELL THERAPY

Dissertation of Pharmaceutical Biotechnology Master Degree under orientation of Professor Ph.D. Alexandrina Ferreira Mendes, presented to the Faculty of Pharmacy of University of Coimbra

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Oriented by: Professor Ph.D Alexandrina Ferreira Mendes

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ABSTRACT

Osteoarthritis is a chronic joint disorder characterized by the progressive degeneration of the articular components with an increasing incidence. Its aethiology is multifactorial, including several risk factors, namely ageing, obesity, genetic predisposition, female sex and trauma. Its current therapy relies on the alleviation of symptoms that does not prevent or even slow down its progression.

With the need for a therapy able to stop the progression of the disease, several strategies have been attempted. Particularly, cell therapy has been suggested to be able to reverse some of the mechanisms responsible for the symptoms and pathophysiology of osteoarthritis. Both non-stem cells and stem cells may be used in cell therapy, which consists in the delivery of the aforementioned cells hoping to replace or repair the damaged tissue.

This thesis aims at critically reviewing available data concerning the use of injective cell therapies to promote cartilage repair in osteoarthritis affected joints. For this, a brief review of osteoarthritis and its current treatment will be presented in the introduction, followed by the identification and characterization of the cell types used or proposed to be used for articular cartilage repair. Then, the pre-clinical studies that analysed the efficacy of injective cell-based therapies for osteoarthritis will be presented. Finally, the clinical trials performed in the last 10 years that studied the efficacy and safety of the intra-articular injection of MSCs on osteoarthritis will be presented and discussed.

Key words: injective cell therapy, osteoarthritis, MSCs, clinical trials

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LIST OF ABBREVIATIONS

ACI	Autologous Chondrocyte Implantation
ACLT	Anterior cruciate ligament transection
ADL	Activities of daily living
AD-MSCs	Adipose-derived mesenchymal stem cells
BMAC	Bone marrow aspirate concentrate
BM-MSCs	Bone marrow-derived mesenchymal stem cells
BMI	Body Mass Index
BMPs	Bone morphogenetic proteins
BMP-2	Bone morphogenetic protein 2
BMP-4	Bone morphogenetic protein 4
BMP-6	Bone morphogenetic protein 6
BMP-7	Bone morphogenetic protein 7
BMP	Bone morphogenetic protein
C-MSCs	Cartilage-derived mesenchymal stem cells
$CaCl_2$	Calcium chloride
COX-2	Cyclooxygenase-2
DC	Dendritic cells
DJD	Degenerative joint disease
ECM	Extracellular Matrix
EGF	Epidermal growth factor
ESCs	Embryonic stem cells
EULAR	European League Against Rheumatism
FAOS	Foot and Ankle Outcome Score
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor-2
FRI	Functional rating index
Sport	Function in sport
GFAP	Growth factor addition/preservation
GvHD	Graft-versus-host disease
HA	Hyaluronic acid or sodium hyaluronan
HAQ	Health Assessment Questionnaire

HGF	Hepatocyte Growth Factor
HHS	Harris hip score
HIF-2α	Hypoxia-Inducible Factor 2α
HLA	Histocompatibility antigen
hMSCs	Human mesenchymal stem cells
HSC	Hematopoietic stem cells
IA	Intra articular
ICM	Inner cell mass
ICRS	International Cartilage Repair Society
IGF-1	Insulin-like Growth Factor
IGF-1	Insulin-like Growth Factor 1
IKDC	International Knee Documentation Committee
IDO	Indoleamine 2,3-dioxygnease
IL	Interleukine
IL-1	Interleukine 1
IL-1β	Interleukine 1β
IL-4	Interleukine 4
IL-6	Interleukine 6
IL-7	Interleukine 7
IL-8	Interleukine 8
IL-10	Interleukine 10
IL-11	Interleukine 11
IL-12	Interleukine 12
IL-1RA	IL-1 receptor antagonist
INF-ϒ	Interferon ϒ
iNOS	Inducible Nitric-Oxide Synthase
iPSCs	Induced Pluripotent Stem Cells
ISCT	International society for cell therapy
KL	Kellgren and Lawrence
KOOS	Knee Injury and Osteoarthritis Outcome Score
LEFS	Lower Extremity Functional scale
LFTJ	Lateral femorotibial joint
MACI	Matrix-induced Chondrocyte Implantation
MEFs	Mouse embryonic fibroblasts

MeSCs	Meniscus-derived mesenchymal stem cells
MFTJ	Medial femorotibial joint
MHC	Major histocompatibility complex
MMPs	Matrix Metalloproteinases
MMP-1	Matrix Metalloproteinase 1
MMP-3	Matrix Metalloproteinase 3
MMP-9	Matrix Metalloproteinase 9
MMP-12	Matrix Metalloproteinase 12
MMP-13	Matrix Metalloproteinase 13
MRI	Magnetic Resonance Imaging
MOCART	Magnetic Resonance Observation of Cartilage Repair Tissue
ms	Milliseconds
MSCs	Mesenchymal stem cells
NK	Natural killer
NPS	Numeric Pain Scale
NSAIDs	Non-steroidal anti-inflammatory
OA	Osteoarthritis
OHS	Oxford Hip Scale
PBMSCs	Peripheral blood mesenchymal stem cells
PCI	Poor cartilage index
PDGF	Platelet-derived growth factor
PGE_2	Prostaglandin E_2
PRP	Platelet-rich plasma
SDF-1	Stromal cell-derived factor-1
SF-36	Short Form Health Survey
S-MSCs	Synovial-derived mesenchymal stem cells
SVF	Stromal Vascular Fraction
TGF-β	Transforming Growth Factor β
TGF-β1	Transforming Growth Factor β1
TGF-β2	Transforming Growth Factor β2
TGF-β3	Transforming Growth Factor β3
TIMPs	Tissue Inhibitor of Metalloproteinases
TJA	Total Joint Arthroplasty
TJR	Total Joint Replacement

TKR	Total Knee Replacement
THR	Total Hip Replacement
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-alfa
TUG	Timed up-and go
VAS	Visual Analog Scale
WBM	Whole Bone Marrow
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WORMS	whole-organ magnetic resonance imaging score

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CHAPTER I

INTRODUCTION

1. INTRODUCTION

1.1. Osteoarthritis

Osteoarthritis (OA), also known as degenerative arthritis (1-3) or degenerative joint disease (DJD) (2, 4), can affect all synovial joints in the human body. Yet, the joints most affected by OA are the knee, hand and hip (5-8). OA is a chronic and progressive joint disorder and represents the most common form of arthritis in the world (1, 4, 9, 10). In fact, this disease is estimated to affect approximately 10% of the world's population aged 60 years or older (10, 11). Moreover, this disease is more common in middle-aged and elderly people (12, 13). It should be emphasized that the prevalence of OA is expected to increase exponentially in the next two decades as a consequence of increasing longevity and obesity in the population (3, 10, 14-16). Thus, due to the high incidence of this health problem, the individual and socioeconomic impact of OA is enormous (5, 8-10, 17). The burden of OA is measured in not only direct (such as non-pharmacological and pharmacological treatments, surgery, side effects of treatments, long-term care and health care provision) and indirect costs (absenteeism, reduced employment, reduced productivity, premature death and early retirement), but also in intangible costs to the individual (including pain, activity limitations, decrease of quality of life, mood, fatigue, sleep and reduced social participation) (17). OA is not only responsible for approximately 2% of all public health expenses in developed countries but also it has huge indirect costs by causing joint pain, loss of function and disability, and, thus, leading to a decrease in productivity (18, 19). It should be noted that other conditions such as depression, neuropathic pain and sleep disorders have been linked with OA, further increasing its economic burden (5, 6). However, the impact of this health problem is still underestimated and its trend is to increase (5, 10, 17, 20-22).

In contradiction with the misconception that OA is simply a process of cartilage attrition or "wear and tear" (23, 24), it is now clear that OA is a complex condition that affects the whole joint (5, 14, 24-27). It is characterized by progressive degeneration of the articular components (which comprise the loss of matrix, fibrillation, formation of fissures and the complete loss of the cartilage surface (12, 28)), subchondral bone remodeling, hypertrophic bone changes, formation of osteophytes, loss of joint space, hypertrophy of the joint capsule, synovial inflammation, and degeneration of ligaments and menisci (2, 6, 14, 24, 29, 30) (Figure

1). Besides, this disease can also affect periarticular muscles, nerves, local fat pads and bursa (24).

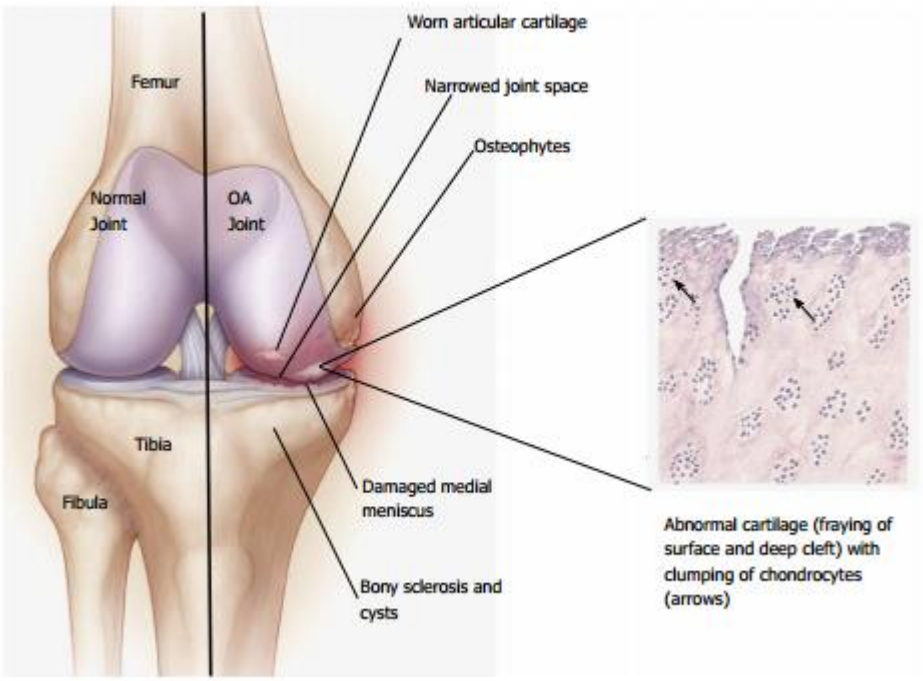

Figure 1: Comparison between a healthy and an OA joint [source (31)].

Articular cartilage is a specialized tissue whose function is the absorption and distribution of forces associated with a high mechanical load as well as the sliding of the joint surfaces with reduced friction (32, 33). Thus, cartilage protects the bone ends acting as a lubricant and a shock absorber (32, 33), This tissue contains a single type of cells, named chondrocytes, which responds to various biochemical (such as growth factors and cytokines) and biomechanical (joint loading) stimuli (32). In accordance with the stimulus, chondrocytes may produce either anabolic or catabolic factors. Anabolic factors, such as bone morphogenetic proteins, promote the formation of new extracellular matrix (ECM), whereas, catabolic factors lead to increased synthesis of matrix metalloproteinases (MMPs) and aggrecanases, which degrade the ECM allowing cartilage turnover and renewal (16, 24, 32). Therefore, OA results from the imbalance between anabolic (the matrix-producing) and catabolic (matrix-degrading) states of cartilage-resident chondrocytes (25, 34). It is generally acknowledged that the articular cartilage has poor capacity for self-repair due to its avascular and alymphatic nature and to the limited proliferation capacity of chondrocytes (4, 23, 32, 33, 35, 36). Occasionally articular defects may occur during life caused by acute trauma (32). The fibrocartilaginous tissue formed in large defects, which is biochemically and biomechanically

different from normal hyaline cartilage, will degenerate over time (32, 37-40). Consequently, the limited capacity of cartilage repair contributes to the progression of OA (23, 32, 38, 39).

The pathogenesis of OA has been linked to inflammation of the synovial membrane (5, 25). This inflammation, which can be caused by the response of synovial macrophages to cartilage matrix debris and catabolic mediators, is one of the main contributors to cartilage matrix destruction (25, 31, 41). These macrophages release pro-inflammatory cytokines (such as interleukine 1β (IL-1β) and tumor necrosis factor (TNF)) and pro-matrix metalloproteinases (25). So the immune system has a crucial role for the development of OA by producing pro-inflammatory cytokines, (such as Interleukin-1 (IL-1), TNF), MMPs (MMP-1, MMP-3, MMP-9, MMP-12, MMP-13) and aggrecanases-1 (3, 42). In addition, hypoxia-inducible factor 2 α (HIF-2α) is one of the most important catabolic transcription factor which induce the expression of catabolic factors but also stimulates chondrocyte hypertrophy (10, 43). It should be noted that IL-1 is the most powerful inducer of proteolytic enzymes, such as MMPs and aggrecanases (16). It is worth mentioning that synovitis is detectable in both early and advanced stages of OA (25).

Clinically, patients with OA may experience a spectrum of signs and symptoms that include joint pain at rest or with mobility (such as walking or climbing stairs), pain at night, joint tenderness or rigidity, decreased range of motion and joint deformity as a result of joint space loss and bony enlargement (14, 41, 44, 45). The diagnosis of OA includes a medical history, physical examination, imaging studies and in some case laboratory tests are also performed (7). Of note, the radiographic findings of OA are classified by using the Kellgren and Lawrence (KL) system (7, 46, 47). This system based on radiological features classifies the severity of OA using five grades (7, 46, 47). Grade 0 indicates that no radiographic features of OA are present, whereas grade 4 is defined by large osteophytes, marked joint space narrowing, severe sclerosis of subchondral bone and definite bony deformity (7, 47). In addition, it should be noted that radiographic findings of OA do not always correspond to clinical symptoms (46).

1.1.2. Risk Factors

It is well known that different sets of risk factors can work independently or in combination to create a common pathway to end-stage OA, in other words, different pathways can promote OA (11, 44, 48). Thus, OA is a complex disease with a multifactorial etiology and the known risks factors are the ageing process, obesity, genetic predisposition, sex and injury, among others (14, 46, 48).

Aging is one of the strongest risk factors for OA but its exact mechanism is not known (24, 48). It is generally thought that aging has an adverse effect on the capacity of the joint to protect itself from biomechanical insults, possibly due to changes in the articular cartilage or in increased joint laxity, which can predispose to injury (46, 48). Besides, it has been demonstrated in several studies that ageing is associated with increased incidence of OA (46).

There is some evidence that OA is more severe in women and a higher prevalence of OA is seen in this gender. The reasons for this difference between men and women are not entirely clear. However, the hypothesis that estrogens may play a role in the development of OA was proposed because of the higher prevalence and incidence of OA in women during menopause.On the other hand, results from clinical trials and observational studies on the effect of estrogens on OA have been controversial (46, 48).

Obesity has been associated not only with an increased risk of incident knee OA, but also with an increased risk of incident hip and hand OA (15, 48). Additionally, it was demonstrated that obesity accelerates knee OA progression (48). It is generally acknowledged that adipose tissue secretes inflammatory adipokines (such as adiponectin, leptin and resistin) which have the potential to influence all synovial joint tissues, affecting cartilage homeostasis (27, 48, 49). Thus, the effect of obesity on OA is possibly due to mechanical effects and systemic effects (e.g. metabolic or inflammatory) (48).

Genetic factors unquestionably play a role in the development of OA and the heritable component has been estimated to be 40-65% (7, 48). Also worthy of mention is that the development and progression of OA are more likely due to an interaction among several genes, in combination with further risk factors (7). Accordingly, the effect of a single gene can lead to OA development in only very few cases (7). Moreover, the heritable component is stronger for hand and hip OA in comparison with knee OA (48). Nowadays, some gene mutations/polymorphisms have been associated with OA. For example , GDF5 (10, 31, 46, 48), MCF2L (48), ASPN (46), SMAD3 (46, 50) and chromosome 7q22 (46, 48) have been associated with OA. In addition, pain sensitivity related to OA is possibly due to a genetic

contribution (48). In support of this idea, a functional polymorphism in the COMT and TRPV1 genes were associated with hip OA and knee OA, respectively (48).

Studies have shown that knee injury, such as anterior cruciate ligament injury and meniscal tears, might be an important risk factor for incidence of knee OA (46, 48). Additionally, it was demonstrated that meniscus injury requiring total meniscectomy has a stronger risk of incident knee OA (46). Thus, the knowledge of increased risk of knee OA related to meniscus injury allow preventive behaviors, in younger athletes, such as effort to avoid acute injury. Also, if meniscal tears occurs every possible effort must be made to preserve meniscal tissue (46).

It was shown that repetitive bending required by certain occupations is associated with an increased risk of OA (46, 48). For example, cross-sectional studies have shown that the risk of knee OA is higher among underground coal miners when compared to a control population (7, 31). Thus, in this occupational group, the main risk factor is due to frequent work in the kneeling or squatting position (7, 31, 48). Additionally, elevated prevalence of knee OA was also observed in construction workers, especially floorers (7, 31, 48). Besides, it has been noticed a relation between lifting and prolonged standing and hip OA as well as the association of the occupational use of manual dexterity with hand OA. (48). Therefore, the knowledge of the influence of occupational activities in the risk of OA may contribute to preventive behaviors (46). Even though some patients may not change their occupations, they can adopt other strategies to minimize other potentially modifiable risk factors (46). For instance, avoiding injury as much as possible or attempting weight loss (46).

Routine levels of physical activity is possibly beneficial for the joint through strengthening periarticular muscles that help to stabilize the joint (48). On the other hand, this activity can be prejudicial if it places undue load on the joint, especially one that is already vulnerable (48). However, vigorous level of physical activity associated with increased incident knee or hip OA are contradictory (46, 48). In some studies it was shown that the intense participation in sports is associated with an increased risk of both hip and knee OA, whereas other studies have not found this consistent association (46).

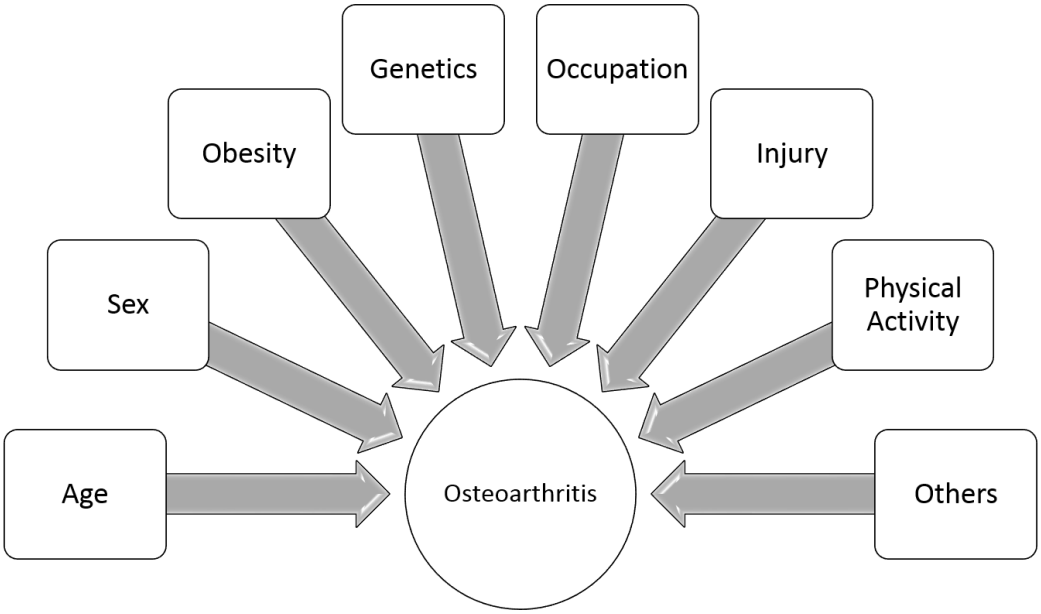

Figure 2: Risk factors of OA.

1.1.3. Current treatment

Currently, OA is an incurable disease since no treatment is available to improve, halt or reverse the process of OA (6, 7, 14, 37). This scarcity of treatment is due to the incomplete understanding of the mechanism by which OA develops and progresses (5, 7). Therefore, current treatment for OA is mainly to alleviate the symptoms and signs of the disease and, if possible, to slow its progression (7, 37). Nowadays, the treatment options for OA comprise conservative treatment and surgical interventions (7, 38).

For the conservative treatment of OA, the European League Against Rheumatism (EULAR) recommends a stepwise therapeutic scheme, in which the severity and distribution of symptoms as well as any possible accompanying illness are taken in consideration (7). The conservative treatment comprises non-pharmacological and pharmacological options (7, 38). The non-pharmacological treatment includes patient education, lifestyle adjustment, weight loss if overweight, physiotherapy and orthopedic aids (7, 44). However, the patient compliance with these treatments is often poor (44). In pharmacotherapy, medications that are usually used to treat OA are analgesics (e.g. paracetamol), non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. specific or non-specific cyclooxygenase-2 (COX-2) inhibitors (44)), opioid analgesics, intra-articular corticosteroid or hyaluronic acid injections and duloxetine.

It should be mentioned that even though paracetamol, also known as acetaminophen, used to be the first-line therapy for mild to moderate pain associated with OA (14, 44), this analgesic is no longer recommended by OA guidelines. In fact, emerging data about potential safety concerns and lack of efficacy resulted in this recommendation (14). Patients with co-morbidities have an increased risk of gastrointestinal complications and when paracetamol is ingested in supratherapeutic doses, not only they have an increased risk of developing multi-organ failure, but also a potential increased risk of cardiovascular events (14). Moreover, a meta-analysis has showed a low-level effect for pain management in OA as well as no benefit over placebo (14). Also worthy of mention is that despite NSAIDs have demonstrated efficacy superior to paracetamol (14), these drugs carry risks of gastrointestinal, renal and cardiovascular toxicities (14, 44). It is well documented that over 16,500 deaths and hospital admissions per year in the USA are due to gastrointestinal toxicity associated with NSAIDs (14). Therefore, due to the potential adverse effects, routine use of NSAIDs is contraindicated in many OA individuals (14). It should be noted that despite COX-2- specific inhibitors (e.g. celecoxib) have lower rates of gastrointestinal toxicities compared to traditional NSAIDs, the risk for cardiovascular events is higher (2, 51). As a result of this, some COX-2-specific inhibitors, such as Vioxx®, Celebrex®, Bextra® were withdrawn from the market or black box labeled (44). On the other hand, topical anti-inflammatories for knee OA have shown increased popularity due to the efficacy of topical anti-inflammatories for knee OA, similar to oral NSAID, with reduction in gastrointestinal adverse reactions (14).

Anti-pain drugs comprise opioids and other centrally acting drugs such as duloxetine (52). It was demonstrated in meta-analysis and systematic reviews that opioids, oral and transdermal, are effective in reducing pain and improving joint function in hip and knee OA. However, the overall benefit of these drugs is small to moderate and, besides that, patients are also more likely to discontinue treatment because of side effects. These medications are associated with cardiovascular and fracture risks. For these reasons, their long-term use is not recommended by most guidelines (14). Nowadays, duloxetine is considered an adjunct or alternative to conventional OA treatments since it is known that OA pain is multi-factorial and, thus, the depression and an important neuropathic pain component are often simultaneously present (14). Duloxetine is a selective norepinephrine and serotonin re-uptake inhibitor which was developed as an anti-depressant (14, 44). In fact, this drug was initially used to treat selected chronic pain conditions, such as fibromyalgia, diabetic peripheral neuropathy and chronic low back pain (44). On the other hand, duloxetine have been

associated with some adverse effects such as nausea, constipation, fatigue, xerostomia and decreased appetite (14).

The treatment of OA with intra-articular injections of corticosteroids has been performed for decades (53). The exact mechanism of this therapy remains unclear, but it is believed to be due to anti-inflammatory effect of the drug (53). It is now well established that these injections provide only a short-term pain relief and improvement in joint OA function (14, 54). On the other hand, intra-articular injections given more frequently (than once every four months) not only increase the risk of infection but also may cause damage in cartilage and joint (14) and subsequently lead to progressive cartilage degeneration (53, 54).

Intra-articular injections of hyaluronic acid (HA), also known as sodium hyaluronate, is now widely used in the treatment of OA (2, 55). HA is a major constituent of the normal synovial fluid, which plays a key role in the maintenance of joint homeostasis (14). Its clinical use was based on the fact that the viscoelasticity of the synovial fluid is reduced due to the lower concentration and molecular weight of endogenous intra-articular HA in patients with OA (2, 14). Thus, the injected HA would not only restore the normal synovial fluid viscoelastic proprieties, but also regulate the synthesis of endogenous HA and inhibit its degradation. In addition, it relieves joint pain (2). However, as a result of rapid clearance and short residence time in the synovial joint, the duration of pain relief is short (2). It should be noted that the duration of analgesic effect varies depending on the molecular weight of HA. That is to say, the higher the molecular weight of HA, the higher its effects are (2). It is important to emphasize that HA provides longer term benefits compared to corticosteroids (2, 20). On the other hand, since HA, as corticosteroids injections, does not halt the progression of OA, the surgical intervention is inevitable (56).

Despite several different surgical treatments, the total joint replacement with a joint prosthesis is performed only as the last resort (18, 19). That is to say, total joint replacement (TJR), also known as total joint arthroplasty (TJA), is indicated when the other treatments used failed and OA has reached advanced stages (3, 16, 45). TJR is most commonly applied to the knee (total knee replacement (TKR)), hip (total hip replacement (THR)) and shoulder joints (57, 58). Even though TJR is generally successful, resulting in improved quality of life by enhancing joint mobility and reducing pain, this procedure is associated with substantial risks of thrombosis, infection, pulmonary embolism, stroke, myocardial infraction and death (3, 5, 10, 45, 59-62). In addition to the foregoing, the life-span of the prosthesis is limited and it may be unable to meet the growing demand from younger and more active patients (3, 23, 45, 61, 63, 64) . Also of note is that these prosthesis revisions have a less favorable outcome in the

health status of the patient and a smaller economic benefit (8). Consequently, the exponential increase in TJR is becoming an inevitable medical and economic problem(5, 8, 63).

The need for novel OA treatment strategies is urgent especially in younger patients who would likely require one or more revisions of their TJR, as well as to decrease the socio-economic burden of this disease (3, 5, 8, 20, 45, 62-65). Therefore, therapeutic strategies that can halt or at least slow down disease progression and promote joint repair or regeneration are currently the focus of intense research. Several strategies have been studied for the treatment of OA, such as biological and gene therapies (27, 66). It is worth noting that cell therapy is a form of biological therapy and can be combined with gene therapy (33, 38, 44). However, this thesis will solely focus on injective cell therapy.

1.2. Cell Therapy

Cell therapy is one of the main areas of research for the treatment of OA (26). In fact, it has been suggested that cell therapy can be able to reverse many of the mechanisms that cause the pathophysiology of OA (67). This therapy consists in the delivery of cells in order to replace or repair damaged organs or tissues (36). Clinical studies of cell therapy involve the evaluation of multiple factors, which comprise cell source, isolation, proliferation and differentiation as well as immunological risk management and eligibility of the donor (36). The ideal cell sourcing, in an aging population, is the one that is easy to obtain with a low risk of complications and has a high cell yield with good proliferation and differentiation potentials that are not affected by ageing (37).

Cell therapy can be subdivided into non-stem cell therapy (autologous and allogeneic tissue-specific cells) or stem cell therapy which comprises embryonic stem cells, adult multipotent stem cells and induced pluripotent stems cells (55, 68). It is noteworthy that stem cell therapies for OA can be performed by intra-articular injection or via surgical arthrotomy with cell transplantation at the site of the injury (38, 65).

However, for cell therapy to be accepted into available therapies by practitioners and regulators, it is essential to find the best cell sources for each regenerative medicine applications and understand its complete mechanism of action (5, 69). Nowadays, it is known that stem cell therapies have two major mechanisms of action. The first one consists in the replacement of damaged cells by engraftment of cells into the injured tissue, whereas the second mechanism is based on the trophic effect of these cells, which influence the microenvironment for the stimulation of the endogenous self-healing process (36, 37). Other challenges which prevent the approval of this therapy to the clinical use include safety concerns, undesirable post-administration cell differentiation and cell migration, potency characterization, uncontrolled proliferation or tumorigenicity, immunogenicity and undesirable cross-interactions with adjacent tissues. In addition, their administration regimens adapted to each disease stage have to be considered (36). It is noteworthy that OA is not a life-threatening disease and, for this reason, the safety is an indispensable prerequisite for translational application of stem cell therapies (70).

1.2.1. Stem Cell Therapy

1.2.1.1. Embryonic Stem Cells

Embryonic Stem Cells (ESCs) are pluripotent cells derived from the inner cell mass (ICM) of the blastocyst (30, 33, 36). These cells are noteworthy for their capacities to self-renew, proliferate and maintain pluripotency for a long term (53, 71). It is worth mentioning that the pluripotency is defined as the ability to differentiate into any type of cell from the three germ layers, i.e., endoderm, mesoderm and ectoderm lineages (9, 30). However, ESCs are not able to develop into a complete organism, by failing to give rise to the placenta and other tissues that are essential for fetal development (72). Self-renewal is the ability of stem cells to divide for an indefinite period of time and maintain themselves as undifferentiated cells (72). Therefore, these cells when correctly induced, can differentiate into any type of cell of the body and also maintain their identity even if they proliferate infinitely *in vitro* (73). In spite of the potential use for clinical application to cure many degenerative and genetic diseases, these cells cannot be used for ethical reasons due to the destruction of embryos (32, 74), and safety issues, such as immune rejection and formation of teratomas (33, 36, 65, 72, 73). Besides, for the implementation of ESCs in cell therapies it is vital to development reproducible and robust differentiation protocols (36)

1.2.1.2. Induced Pluripotent Stem Cells

The first time that somatic cells were reprogrammed was in 2006 by Yamanaka and Takahashi (36, 68, 72). In that study, the researchers reprogrammed mouse embryonic fibroblasts (MEFs) by ectopic expression of Oct4, Sox2, Klf4 and c-Myc (75). In 2007, Yamanaka and co-workers demonstrated also the reprogramming of human cells (76). As recognition for his work on induced pluripotent stem cells (iPSCs), Yamanaka was awarded the Nobel Prize in 2012 (69).

On the one hand, these cells are advantageous due to their capacities to self-renewal and pluripotency (36, 74), similar to ESCs, without ethical issues (32). IPSCs can also allow the development of personalized treatments because these cells are developed from a patient's own somatic cells (36). Additionally, these cells can be used not only for regenerative medicine but also for drug discovery, toxicity testing (74, 77) and pathophysiological studies (77, 78). On the other hand, the main obstacles to clinical use of iPSCs are the risks of teratoma formation and tumorigenesis by a possible integration of retroviral vectors that deliver a set of genes required for somatic cells reprogramming (38). Besides, the process of

reprogramming cells is very expansive and labor-intensive (68). It should be noted that the set of transcription factors used to reprogram somatic cell is oncogenic (79). Therefore, since the discovery of iPSCs many studies have been performed to increase the efficiency (69, 74, 80) and kinetic of reprogramming (74, 80) as well as reducing the number of oncogenes in this process by using either other combinations of transcription factors or small molecule compounds (such as chemicals inhibitors and signaling molecules) (74, 80). In support of this idea, the safer generation of iPSCs is possible by using adenoviruses, bacterial plasmids, episomal vectors and piggyBac transposons, instead of retro- or lentivirus vectors (74). However, despite the application of these cells being promising for articular cartilage repair, up to now, they have just been used in preclinical models (32, 81). These cells have demonstrated to be able to improve cartilage repair when implanted at the defect site without any teratoma or tumor formation (32, 81). Yet, the efficacy and safety of iPSCs need to be much more investigated (32, 82). One reason for this is the difficulty to obtain a uniform differentiated cell population. A non-uniform differentiation to the cell of interest not only limits the effectiveness of the therapy, but also increases the risk of teratoma formation by undifferentiated cells in the population (77).

1.2.1.3. Mesenchymal Stem Cells

Alexander Friedenstein and co-workers were the first to describe mesenchymal stem cells (MSCs) in1966 apud (31, 68, 83). They characterized these cells as an adherent, fibroblast-like population within the stromal compartment of the bone marrow apud (84). In 1970, Caplan and colleagues provided the first evidence of the differentiation potential of MSCs and in 1991 they introduced the term "mesenchymal stem cells" apud (68). Since then, the field of MSCs investigation increased in many biomedical applications (85). The cell therapy with MSCs is particularly attractive because they have ideal characteristics for regenerative medicine, such as immunodulatory function, homing potential to damaged tissues, differentiation potential, inhibition of apoptosis and scarring, stimulation of angiogenesis, easy availability, proliferation and self-renew potential (33, 86). These characteristics can be used for therapeutic applications to treat several diseases, such as autoimmune disease, severe steroid-refractory graft-versus-host disease (GvHD), Crohn's disease, diabetes mellitus, acute myocardial infarction, stroke, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury and OA, among others (6, 68). Actually, human clinical trials have already been

performed with allogeneic MSCs to treat a number of conditions, for example GvHD, Crohn's disease, myocardial infarcts, stroke, spinal injury, cartilage and meniscus repair (87).

Nevertheless, it should be noted that the translation to clinical applications of these cells requires proof-of-concept studies, as well as the complete understanding of their biological characteristics and function (88). In other words, the challenges reside in knowing the adequate number of cells in the tissues undergoing repair, long-term safety and the durability of the benefit (9). Besides, the usage of these cells is well accepted by society (37).

MSCs have as principal physiologic functions the homeostasis, renew and maintenance of the cell population (84). Additionally, MSCs repair tissue which was damaged by injury, such as disease and trauma, or by apoptosis (84, 87, 89). It is well documented that all of the cells in the body have a life span that varies from 20 minutes to many years depending on the type of cell (89). Therefore, MSCs permit not only the replacement of expired cells, but also, the replacement of abnormal cells or that could be non-functional (87, 89), providing physiological balance in the organism (89). However, it should be noted that this mechanism seems to decrease with ageing (87), due to the fact that not only the numbers of bone marrow-derived mesenchymal stem cells decrease (90), but also their life span, self-renew and proliferation capacity decrease (23, 37, 89, 91). The effect of age on differentiation potential is still controversial because some investigators defend that the capacity of MSC to differentiate is independent of age (23, 87, 91), whereas others defend that the differentiation potential declines with ageing (16, 37). On the other hand, some studies have demonstrated that the quantity, phenotype, and differentiation potential of MSCs are also influenced by the disease state, such as OA (5). One of these studies was performed in 2002 by Murphy and co-workers, who demonstrated that MSCs from patients with severe OA have reduced proliferative capacity as well as chondrogenic and adipogenic activity. In addition, they observed that the osteogenic activity was increased. Thus, despite the potential role of MSCs in the development of OA (91), it is possible to use these cells isolated from patients with OA for the regeneration of cartilage (16). Since it was demonstrated that, independently of their age or the etiology of their disease, these cells are present in sufficient numbers with adequate chondrogenic differentiation potential (16).

Nowadays it is known that MSCs can be harvested from several human tissues, such as bone marrow (31, 55, 67, 83, 92, 93), adipose tissue (31, 55, 67, 83, 92, 93), infrapatellar fat pad (83, 86), skeletal muscle (31, 55, 92, 93), synovial membrane (55, 83, 86), synovial fluid (55, 83, 86), cartilage (94), meniscus (43, 95), periosteum (55, 83, 86, 92), peripheral blood

(38, 55), umbilical cord tissue and umbilical blood (55, 67, 83), endometrium (55), amniotic fluid (38, 55), placenta (55, 67, 93), trabecular bone (31, 71, 83, 86), dental pulp (67), deciduous teeth (31, 71, 86), dermis (83, 86, 93), Wharton's jelly (38, 84, 93), lung, liver and spleen (93).

It is well known that MSCs have the capacity to differentiate into cells of the mesodermal lineage, in other words, they can differentiate into bone, cartilage, adipose tissue, bone marrow stroma, muscle, tendon and ligament, and other connective tissues (28, 47, 89). The mechanism of differentiation of MSCs involves multi-step cell lineages, which allows a lot of checks and balances to insure that short lapses in whole physiology or traumatic events do not result in aberrant end-stage phenotypes (89). The differentiation is controlled by bioactive factors existent in the local micro-environment or supplied in the culture environment of ex vivo cultivated cells (89). It should be noted that these cells have also demonstrated to be able to differentiate into endo- and ectodermal lineages (33, 93, 94). This ability is known as MSC plasticity or transdifferentiation (33).

Nevertheless, it is important to emphasize that despite MSCs from different niches have similar phenotypic characteristics, they exhibit different propensities in differentiation and proliferation potentials, as well as different surface marker profiles (57, 65, 68, 82, 83).

In this regard, despite the exhaustive research on MSCs, the profile of surface markers remains unknown as other characteristics (31, 33, 86). According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT), the human MSCs must fill a minimal set of standard criteria. First of all, in standard culture conditions they must present plastic adherence (38, 65, 83, 85, 86). Secondly, ≥ 95% of the MSCs population must be positive for stromal cell markers CD73, CD90 and CD105, while they must lack the expression of the hematopoietic markers CD34, CD45, CD11b or CD14, CD19 or CD79α, and histocompatibility antigen (HLA) class II (6, 38, 65, 67, 83, 85, 86). Finally, in standard *in vitro* differentiating conditions these cells must have the ability to differentiate into cells of the mesodermal lineage. In other words, they must differentiate into chondrocytes, osteoblasts and adipocytes (38, 65, 83, 85, 86). However, some investigators believe that not all MSCs fall under these criteria. Also, they defend that other cells can co-express CD73, CD90 and CD105 (6, 33). Furthermore, it has already been demonstrated that MSCs isolated from different tissue types within the knee joint express other markers. For instance, synovium-derived cells express CD90, CD105, CD147 and CD44 and meniscus-derived cells express CD90, CD44, CD105, CD 147, CD166 and CD271 (5, 14, 43). In addition, the marker CD 44 is expressed in bone marrow-derived mesenchymal stem cells,

cartilage-derived mesenchymal stem cells and adipose-derived mesenchymal stem cells (94). For all the reasons above, the profile of surface markers on MSCs is still a controversial issue (16, 31, 33, 87).

It is well documented that MSCs exhibit homing potential to the sites of injury and inflammation (67). This migration is explained by the SDF-1/CXCR4 pathway (43, 67). Briefly, MSCs express several chemokine receptors (such as CCR1, CCR7, CCR9, CXCR3, CXCR4, CXCR5 and CX3CR1 (33)) that enable their migration in response to the chemokine produced by the injury sites (33). Thus, the migration of MSCs is due not only to the upregulation of stromal cell-derived factor-1 (SDF-1) in the damaged site, but also to the expression of its cognate receptor CXCR4 in migratory cells (43). In support of this idea, it was demonstrated that in the absence of the SDF-1 signal, the migration of MSCs was impaired (67). In this regard, SDF-1 is a promising candidate as a homing-inducing factor (68).

Also worthy of mention is that when MSCs reach the sites of injury and inflammation, they can differentiate into the tissue cells or secrete a broad spectrum of bioactive molecules that have regenerative and/or immunoregulatory activities (33, 47). The complex and multifaceted effects that result from the secretory activity of MSCs provide a regenerative microenvironment to limit the area of damage and to mount a self-regulated regenerative response. The bioactive factors secreted by MSCs have been shown to inhibit scarring (fibrosis) and apoptosis, stimulate angiogenesis and enhance the mitosis of tissue-intrinsic stem or progenitor cells (47, 87). This regenerative microenvironment is known as trophic activity (47, 87).

Another very important and interesting characteristic of MSCs is the immunosuppressive activity. The first *in vivo* study that demonstrated the immunosuppressive effect of MSCs was performed in 2002 by Bartholomew and co-worker apud (86, 93, 96). In this study they administered allogeneic MSCs to baboons to prolong skin-graft survival apud (86, 93, 96). However, the most noteworthy result was obtained by Le Blanc and colleagues apud (96). In their study, they used MSCs to treat severe steroid-refractory GvHD apud (93, 96). Acute GvHD disappeared in six out of eight patients treated with MSCs. Also, the survival rate was better for patients treated with MSCs than for patients treated without MSCs apud (86, 97).

The observed immunosuppressive activity of MSCs is due to the inhibition of components of the immune system, as T and B lymphocytes, memory T cells, Natural Killer (NK) cells and Dendritic cells (DC) (86, 96). The immunomodulation by MSCs occurs in

response to pro-inflammatory cytokines such as tumor necrosis factor-alfa (TNF-α), interferon-gamma (INF-ϒ) and IL-1 (14, 93, 98), which lead MSCs to secrete several chemokine (such as indoleamine 2,3-dioxygnease (IDO) and SDF-1), anti-inflammatory cytokines (including IL-10, IL-1 receptor antagonist (IL-1RA), IL-4, IL-6, IL-7, IL-8, IL-11) and growth factors [as transforming growth factor β (TGF-β), hepatocyte growth factor (HGF) and bone morphogenetic protein-2 (BMP-2)] and prostaglandin E_2 (PGE_2) (6, 86, 97).

More specifically with regard to the immunosuppressive activity, MSCs suppress the activation, proliferation and cytokine secretion of helper T cells, cytotoxic T cells as well as T memory cells by inhibiting TNF-α, INF-ϒ and IL-1 production and, consequently, increasing IL-10 and IL-4 levels (33, 86, 87). Moreover, MSCs also increase the proportion of regulatory T cells, which have suppressor activity, through the production of IDO, PGE_2 and BMP-2 (93, 97). Also, MSCs inhibit the proliferation, maturation and immunoglobulin production of B cells (96).

Besides, MSCs can inhibit the proliferation, cytokine secretion (INF-ϒ), and cytotoxicity of NK cells (86) through soluble factor such as transforming growth factor-β1 (TGF-β1) and PGE_2 (93). Interestingly, several studies have demonstrated that IL-2-activated NK cells can lyse allogeneic and autologous MSCs (93). This cytotoxicity against MSCs is mainly due to activating NK cell receptors, NKp30, NKG2D and DNAM-1. Therefore, MSCs express ligands (ULBPs, PVR and Nectin-2) for these activating NK receptors to escape NK cells (93).

In the case of DCs, MSCs inhibit the maturation, activation, proliferation, cytokine secretion (TNFα, IL-12) and antigen presenting cell function (33, 96). It should be noted that MSCs reduce the formation of DCs from monocytes so, the activation of T cells is indirectly reduced (97). Furthermore, the cytokine secretion profile of DCs, T cells, and NK cells are altered by MSCs to induce anti-inflammatory cytokines (86). Finally, MSCs can also induce the production of anti-inflammatory cytokines, such as IL-10 (84, 99, 100) and IL-12p40 (99, 100), in macrophages by a PGE_2-dependent mechanism (84). It should be emphasized that some mechanisms of immunosuppression are constitutively expressed, such as the production of PGE_2 (33). In contrast, others are not constitutively expressed, for instance, human MSCs express IDO only when stimulated by INF-ϒ (33, 97). It is also worth mentioning that the proliferation of T cells is affected by the expression of IDO. In other words, IDO depletes the cellular microenvironment of the essential amino acid, tryptophan, which is vital for T cell proliferation (33).

It is generally acknowledged that all cells in the body express major histocompatibility complex (MHC) molecules that allow the immune system to distinguish what is self from what is nonself or foreign (97). Thus, allogeneic cells are rejected by the immune system in absence of immune suppression or tolerogenic mechanisms (97). However, it is now well established as a result of the low expression of MHC class I molecules and no expression of MHC class II molecules on MSCs' surface that they are hypo- or non-immunogenic and can evade the immune system (62, 67, 86). It is well documented that chondrocytes, osteocytes and adipocytes differentiated from MSCs keep the non-immunogenic nature (62, 67, 86, 97). Nonetheless, stimulation with INF-ϒ will increase both MHC class molecules in these cells (97). For all the reasons above, especially for their trophic and immunomodulatory characteristics, these cells have been intensively investigated in a variety of clinical indications, including OA (62, 101, 102).

1.2.1.3.1. Bone marrow-derived mesenchymal stem cells

According to the literature, bone marrow-derived mesenchymal stem cells (BM-MSCs) were the first type of MSCs to be identified and are the best characterized stem population, making them the closest to clinical translation (68, 83). The application of MSCs can be done as a cell suspension expanded by culture or simply as a bone marrow concentrate (BMAC) (83). As aforementioned, BM-MSCs, as others MSCs, have several characteristics that make them an attractive population of cells for cartilage repair, such as the migration of these cells to the sites of injury and inflammation, self-renewing ability, differentiation potential, the potential to modulate local microenvironment by anti-inflammatory and immunosuppressive functions (67) as well as secretion of several bioactive soluble factors with regenerative proprieties (33, 67). These characteristics not only allow protection of cartilage but also facilitate regeneration by progenitor cells *in situ* (67). Additionally, these properties allow the use of BM-MSCs in both autologous and allogeneic cell therapy (67). Moreover, these cells can be easily expanded in culture for many generations, while still retaining their capacity to differentiate when exposed to appropriate signals (28).

On the other hand, these cells have some drawbacks, such as the low proportion of these cells in the bone marrow (0.01-0.001% of the nucleated cells) (93, 94) and the decline in marrow activity and quantity with age (68). In addition to its variation according to patient's age, the number of MSCs in bone marrow also varies depending on the localization of the harvest and patient's gender (103). Furthermore, the invasive procedure used to collect bone

marrow (68) is painful and is associated with risks of wound infections as well as donor site morbidity (37). Therefore, these limitations have motivated investigation of alternative sources of multipotent stem cells (68). Despite these limitations, cell-based therapies using BM-MSCs for cartilage repair are an active area of research. Nevertheless, it should be noted that the surgery for the harvest of BM-MSCs is less invasive with lower morbidity and hospitalization costs than chondrocyte harvest for autologous chondrocyte implantation (ACI) (6). ACI with or without a scaffold matrix is a surgical cell therapy used, in the last two decades, for repair of small articular cartilage lesions, thus excluding patients with OA (39, 68, 70, 84). Also, in comparison with chondrocytes, MSCs not only integrate better in the damaged tissue but also it was demonstrated that they can regenerate the cartilage and the underlying subchondral bone (40).

1.2.1.3.2. Adipose-derived mesenchymal stem cells

It is well known that the adipose tissue is an energy reservoir and plays a crucial role in metabolic disorders (99). In addition, it was demonstrated that this complex endocrine organ contains MSCs [adipose-derived mesenchymal stem cells (AD-MSCs)] (98, 99). According to the literature, these cells are one of the most promising stem cell population identified up to now (71). In fact, these cells have been used in several fields of regenerative medicine and they have been demonstrated to be safe and effective in preclinical and clinical studies (71). Additionally, AD-MSCs can be used as research tools and as cellular therapy. As with bone marrow, the application of MSCs can be done as a cell suspension expanded by culture or freshly isolated stromal vascular fraction (SVF) obtained by enzymatic digestion of adipose tissue (55, 104, 105). AD-MSCs share some characteristics with BM-MSCs, such as differentiation potential and immunosuppressive properties (98, 99). However, these cells have some advantages when compared with BM-MSCs (106). For example, AD-MSCs are acquired by a simple, repeatable, and less invasive procedure named liposuction (94, 106). Additionally, these cells not only are easily available and are collected in larger numbers with minimal morbidity and discomfort (33, 71, 83, 94) (in adipose tissue, AD-MSCs represent approximately 10% of all nucleated cells (55)), but also, are easily expanded in culture with a higher number of passages before senescence (65, 106). In addition, like other MSCs, they can differentiate into cells of the mesodermal lineages (68, 83, 94) as well as into ectoderm (68, 71, 94) and endoderm lineages (71). Besides, comparing with BM-MSCs, the quality of AD-MSCs is less influenced by aging or disease of patients (106). However, it was demonstrated

that the number of AD-MSCs decreases with obesity (55). In fact, it was shown that AD-MSCs from obese patients have reduced proliferation capacity, differentiation potential and greater cell senescence (23). It should be noted that the number of MSCs in adipose tissue varies depending on the localization of the harvest (55). Further, in contrast to BM-MSCs, AD-MSCs are smaller, have a different profile of surface markers and gene expression (65). It is well documented that AD-MSCs secrete chondrogenic factors, such as bone morphogenetic protein 4 (BMP-4), TGF-β1, anti-fibrotic and anti-apoptotic growth factors (26). It was also reported that these cells have a lower chondrogenic potential in comparison with BM-MSCs. However, this limitation may be overcome by using a combination of bone morphogenetic proteins - BMPs (such as BMP-6 (65, 86)) and transforming growth factor- β2 (TGF-β2) or by the combination of TGF-β2 and insulin-like growth factor I (IGF-I) (83). Also, it is well documented that AD-MSCs reduced hypertrophy and de-differentiation of chondrocytes, decrease thickening of synovium, promote cartilage protection and delay the development and progression of OA (106). Of note, many characteristics of AD-MSCs differ with the location where they are collected from, for example AD-MSCs harvested from superficial abdominal regions are significantly more resistant to apoptosis than other AD-MSCs (71).

1.2.1.3.3. Mesenchymal stem cells in the diarthrodial joints.

It was shown that MSCs are present in most tissues of diarthrodial joints, such as synovial membrane, cartilage, meniscus, bone marrow, fat pad and ligament (anterior cruciate) (5). In 2001, De Bari and colleagues were the first to described joint resident MSCs in adult human synovial membrane (5). Since then investigators have paid special attention to synovial-derived MSCs (S-MSCs) as an alternative cell line for cartilage repair (33, 83). MSCs derived from the synovial membrane can be successfully harvested by two ways: arthroscopically, which has a low degree of invasiveness and causes minimal complications at the donor site due to its high regenerative ability (65, 92) and from synovial fluid, which however, yields a very small number of S-MSCs (82). In comparison with other MSCs, S-MSCs have higher proliferative and chondrogenic potentials, particularly when incubated with BMP-2 (65) (Table 1). Besides, in contrast to BM-MSCs, these cells demonstrated in preclinical studies less osteogenic capacity (83).

Infrapatellar fat pad-derived mesenchymal stem cells can be used as an alternative source of MSCs for cartilage repair (37). It was demonstrated that these cells maintain their potential to differentiate into cells of mesodermal lineage, by using appropriate media, even in

the later stages of life (37). Another advantage of these cells is that they are harvested in a larger number than BM-MSCs, decreasing the cost and time for culture expansion, as well as associated risk of contamination (37). In addition to the foregoing, it was demonstrated that AD-MSCs from infrapatellar fat pad have superior chondrogenic potential in comparison with AD-MSCs from subcutaneous adipose tissue, which seems to have a superior osteogenic commitment (81).

Cartilage-derived MSCs (C-MSCs) share numerous properties with BM-MSCs such as profile of surface makers, self-renewal, proliferation and differentiation capacities (94). However, when compared with BM-MSCs and AD-MSCs, these cells have the highest capacity for chondrogenesis based on the expression of collagen II, the major collagen in articular cartilage matrix (32), and the formation of cartilage matrix (94). Therefore, C-MSCs can be an alternative for cartilage tissue engineering (94).

Despite Meniscus-derived MSCs (MeSCs) have been less extensively investigated than other MSCs, it was shown that these cells share some characteristics with BM-MSCs (5). For example, both express the same set of typical cell surface markers (5) and have similar non-immunogenic and immunosuppressive properties (95). It was also demonstrated that MeSCs displayed a higher level of COL II expression and showed higher clonogenicity in comparison with BM-MSCs and S-MSCs (43). Besides, MeSCs possess robust chondrogenic activity (5). On the other hand, the transplantation of autologous MeSCs is limited due to a small cell numbers, but this limitation can be overcome by using allogenic MeSCs (95).

1.2.1.3.4. Other mesenchymal stem cells

Periosteum-derived MSCs have demonstrated to have considerable chondrogenic and osteogenic potentials (83, 92). Besides, they have been successfully employed to repair cartilage defects *in vivo* (65). However, the use of periosteum-derived MSCs is limited by the complexity of the surgical procedure of extraction and the reduced availability (65). Additionally, in 2001, De Bari and colleagues demonstrated that human periosteum-derived MSCs from donor younger than 30 years exhibit spontaneous chondrogenic activity in culture, whereas cells from older donors and cells from young donors that had been extensively cultured do not exhibit this activity (5, 98).

Umbilical cord blood-derived mesenchymal stem cells are another promising source of MSC for cartilage repair (31, 32, 53). These cells were described as less mature than BM-

or AD-MSCs and consequently they exhibit a larger potential in regenerative medicine, even if the stem cell population is heterogeneous (31, 32). Also, in theory, the use of this source of MSCs is ethically acceptable and economical since the umbilical cord tissue would otherwise be discarded during the process of childbirth (81). The benefits of using umbilical cord blood-derived MSCs are the non-invasive procedure to collect stem cells, the abundant supply of MSCs and the possibility of using these cells in allogenic cell therapy (31, 81). When they are used for allogeneic stem cell therapy, these cells are harvested from donated human umbilical cord tissue after a normal and healthy births and the submission of the mother to infectious diseases tests as well as the screening of her medical history (31). Nowadays, several preclinical or clinical trials with MSCs from umbilical cords have been performed in the field of cartilage repair (32). In comparison with BM-MSCs or AD-MSCs, umbilical cord blood-derived stem cells have a lower isolation yield, but expansion is more efficient (65). Moreover, these cells, as periosteum-derived MSCs, can be induced to chondrogenic differentiation by TGF-β (65).

Another attractive alternative for cartilage repair are muscle-derived stem cells due to their availability (32, 65). In fact, the skeletal muscle is the largest organ in the body and its harvest is a minimally invasive procedure (32). Like other MSCs, they can differentiate into cells of the mesodermal lineages (65). It was also demonstrated that these cells improve the repair of cartilage defects *in vivo* (32, 65). However, their capacity of differentiation and cartilage regeneration are sex-dependent. It was shown that male muscle-derived stem cells have a higher capacity for chondrogenic differentiation and cartilage regeneration (65). In addition to the foregoing, it should be noted that muscle-derived stem cells have smaller chondrogenic and osteogenic potentials in comparison with BM-MSCs, S-MSCS and periosteum-derived MSCs (92) (Table 1). It was demonstrated that the use of TGF-β1 is important not only for inducing the chondrogenesis of these cells, but also for maintaining their chondrogenic phenotype (32).

Peripheral blood MSCs (PBMSCs) have also been investigated as an alternative source of MSCs (11, 65). These cells are advantageous due to easy harvest with no significant donor site morbidity (55). Besides, they display similar *in vitro* chondrogenic potential to BM-MSCs (55). However, it is worth mentioning that the peripheral blood MSCs number is very low. Therefore, patient stimulation is required in order to increase their number (11, 83). Moreover, these cells cannot be easily isolated and the knowledge about them remains very limited (83).

1.2.1.3.5. Comparison of human stem cells derived from different mesenchymal tissues

According to a comparative study of MSCS, it was concluded that S-MSCs not only have higher proliferative potential, but also have a greater differentiation potential for chondrogenesis, adipogenesis and osteogenesis. To put it briefly, S-MSCs have the best potential for chondrogenesis, followed by BM-MSCs and periosteum-derived MSCs, in terms of osteogenesis, S-MSCs, BM-MSCs and periosteum-derived MSCs are superior. Finally, the adipogenesis ability is superior in S-MSCs and AD-MSCs. (92) (Table 1).

Table 1: Comparison of the major characteristics of human MSCs of different origins.

	Proliferation	*Osteogenic*	*Adipogenic*	*Chondrogenic*
BM-MSC	++	+++	++	++
AD-MSC	+	++	+++	+
S-MSC	+++	+++	+++	+++
Periosteum MSC	++	+++	+	++
Muscle-MSC	++	++	+	+

This table summarizes the results of a comparative study (92) evaluating MSCs from bone marrow, adipose tissue, synovium, periosteum and muscle. The differences between these cells were represented by "+++", "++" and "+". "+++" means that MSCs have a higher ability. "++" means that MSCs have a moderate ability. "+" means that MSCs have the least ability.

Abbreviations: AD-MSCs, adipose-derived mesenchymal stem cells; BM-MSC, bone marrow-derived mesenchymal stem cells; S-MSCs, synovial-derived mesenchymal stem cells

1.2.1.3.6. Chondroinductive agents

Ashton and co-workers were the first to report the chondrogenesis of MSCs, in 1980 (37). In 1998, Johnstone and colleagues were the first to describe a defined medium for *in vitro* chondrogenesis of MSCs (37). They used micromass cultures supplemented with TGF-β and dexamethasone (37). According to the literature, nowadays, TGF-β (such as TGF-β1, TGF-β2, TGF-β3), BMPs (such as BMP-2, BMP-4, BMP-6 and BMP-7), fibroblast growth factor 2 (FGF-2), HA and dexamethasone are being used as chondroinductive agents (12, 23, 28, 29, 40, 62, 86, 107).

1.2.1.3.7. Pre-clinical studies

The direct intra-articular injection of MSCs was proposed by Murphy and co-workers in 2003. In this study, OA was induced in goats by anterior cruciate ligament transection (ACLT) and medial meniscectomy. After six weeks, HA alone or autologous MSC in a dilute solution of HA were injected into the knee joints. In the control animals, which received HA alone, OA developed as expected, with fibrillation and erosion of large areas of the articular cartilage, accompanied by the formation of osteophyte and alterations in the trabecular organization of the subchondral bone. In MSC-treated joints, meniscus regeneration and reduction of the cartilage destruction were observed. The authors also concluded that the beneficial effect of MSCs on OA progression and on cartilage protection was due to a paracrine effect and not the direct structural contribution of MSCs (28). Since then, several studies have been focused on the intra-articular injection of MSCs on animal models of OA, summarized in Table 2.

On the whole, these studies have shown the beneficial effect of MSC on cartilage morphology and histology (Table 2). Moreover, these studies also allowed the elucidation of some points of the function of MSCs in OA. For example, the homing ability was confirmed by using labeled MSCs as well as it was demonstrated, for the first time, that intra-articular injection of MSC enhanced regeneration through the SDF-1/CXCR4 pathway (26, 29, 43, 108). The bio-distribution of MSCs in medial meniscus and synovium reinforce the hypothesis that the beneficial effect of MSCs on OA progression and cartilage protection is due to a trophic mechanism (26). It was also demonstrated that these cells are able to suppress the immune response and are non-immunogenic (29, 47, 95). In addition, MSCs inhibits the progression of OA by reducing the expression of TNF-α, IL-1β, MMP-1and HIF-2α in cartilage tissue (26, 43, 99). In the studies performed by Lee et al. and Toghraie et al., it was concluded that MSCs require time to differentiate and proliferate, since the best results were achieved when the period of time of MSCs in the joints was longer (4, 40, 47). Moreover, MSCs were more beneficial when injected in the early stages of OA (29, 108). Nonetheless, studies with larger numbers of animals conducted for a longer periods of time are essential to provide more evidence of the effectiveness and safety of this therapeutic approach in OA (4, 40, 47, 108).

Also worthy of mention is the fact that some of these studies have used as vehicle HA (28, 29, 40, 108). It is known that HA have chondroinductive and chondroprotective properties but also facilitates the migration, proliferation, differentiation and the adherence of

MSCs at the site of injury (29, 40). However, despite the fact that HA has an important role in the process of cartilage regeneration when administrated with MSCs, on its own, it produces an inadequate biomechanical tissue (40). Some studies have demonstrated that the quality of the repair tissue in animals treated with HA alone was inferior, possibly because the number of endogenous MSCs recruited is insufficient (40, 108). Besides, it was shown to deteriorate with time (40)

Table 2: Summary of some studies of injective MSC-based therapy in pre-clinical experimental models of OA.

Study	Disease Model	Animal Species	Cell Source	Outcomes
Murphy et al. 2003 (28)	ACLT + meniscectomy for 6 weeks	Goat	Autologous bone marrow	BM-MSCs stimulated regeneration of meniscal tissue and reduced the degeneration of the articular cartilage, osteophyte formation, and subchondral sclerosis.
Lee et al. 2007 **(40)**	Partial-thickness cartilage defect model	Porcine	Autologous bone marrow	BM-MSCs stimulated regeneration of the articular cartilage with better results at 12 weeks. Improvement in the quality of the repair tissue was seen in the MSC treated group.
Mokbel et al. 2011 **(108)**	Chemical induction of OA	Donkey	Autologous bone marrow	BM-MSC retarded the progression of OA and stimulated regeneration. MSCs was more beneficial when injected in the early stage of OA.
Toghraie et al. 2011 (47)	ACLT	Rabbit	Allogeneic Infrapatellar fat pad	MSCs reduced the degeneration of cartilage, osteophyte formation, and subchondral sclerosis. The quality of cartilage was better in cell-treated at 20 weeks.
Diekman et al. 2012 **(109)**	Traumatic OA (knee fracture)	Mouse	Allogeneic bone marrow	OA was prevented OA by the delivery of BM-MSCs after fracture. Reduction of cytokine level in serum and synovial fluid by BM-MSCs.
Faqeh et al. 2012 (12)	ACLT + menisectomy	Sheep	Autologous bone marrow	Chondrogenic-induced BM-MSCs had better results than BM-MSCs alone, especially in meniscus regeneration. Chondrogenic-induced BM-MSCs group demonstrated good cartilage histoarchitecture comparable to normal knee joint cartilage.

Table 2: Continued

Study	Disease Model	Animal Species	Cell Source	Outcomes
Horie et al. 2012 **(110)**	Hemi-meniscectomy	Rat	Commercial human (BM-MSCs)/ Allogeneic rat bone marrow	Rapid reduction of hMSCs. hMSCs stimulated meniscal regeneration and retarded osteoarthritis progression.
Sato et al. 2012 (29)	Spontaneous OA	Guinea pig	Commercial human MSCs	MSCs stimulated partial regeneration of articular cartilage and retarded the progression of OA.
Ter Huurne et al. 2012 **(99)**	Collagenase-induced OA	Mouse	Autologous adipose	AD-MSCs inhibited synovial lining thickening, enthesophyte formation and promoted cartilage protection by the reduction of IL-1β expression in the synovium and increase of the levels of expression of TIMPs.
Toghraie et al. 2012 (4)	ACLT	Rabbit	Allogeneic Adipose	Reduction of degeneration of cartilage with better results at 20 weeks in the AD-MSC-treated group.
Desando et al. 2013 (26)	ACLT for 8 weeks	Rabbit	Allogeneic adipose	AD-MSCs stimulated the regeneration of cartilage and meniscus. The progression of OA was inhibited by AD-MSCs through the reduction of TNF-α and MMP-1 expression in the synovial membrane and menisci.
Weiliang Shen et al. 2013 **(95)**	Meniscectomy	Rabbit	Allogeneic meniscus	MeSCs stimulated the regeneration of meniscus and delayed the progression of OA.
Weiliang Shen et al. 2014 (43)	Meniscectomy	Rat	Human meniscus	MeSCs stimulated the regeneration of meniscus and delayed the progression of OA by the inhibition of HIF-2α. SDF-1/CXCR4 promote the trafficking of MeSCs to the meniscus injury site.

Abbreviations: ACLT, anterior cruciate ligament transection; AD-MSCs, adipose-derived mesenchymal stem cells; BM-MSCs, bone marrow-derived mesenchymal stem cells; HIF-2α, hypoxia-inducible factor 2α; hMSCs, human mesenchymal stem cell; IL-1β, interleukine 1β; MeSCs, meniscus-derived mesenchymal stem cells; MMP-1, matrix metalloproteinase 1; OA, osteoarthritis; TIMPs, tissue inhibitor metalloproteinases; TNF-α, tumor necrosis factor α

CHAPTER II

OBJECTIVE AND METHODOLOGY

2. OBJECTIVE AND METHODOLOGY

This dissertation aims at identifying and critically reviewing available data concerning the use of injective cell therapies to promote cartilage repair or regeneration in OA-affected joints. For this, a Pubmed search was conducted to identify relevant clinical studies published in the last 10 years. Moreover, the clinicaltrials.gov was also searched to identify completed clinical trials. Nevertheless, some clinical trials have not presented results until now. The table 8 summarizes the clinical trials without published results. This way it is possible to know the main characteristics of these clinical trials.

For the bibliographic search the key words used were: "osteoarthritis and mesenchymal stem cells", "injective therapy and osteoarthritis", "cell therapy and osteoarthritis", "clinical trials and osteoarthritis"

CHAPTER III

CLINICAL TRIALS OF INJECTIVE CELL THERAPIES FOR ARTICULAR CARTILAGE REPAIR OR REGENERATION: ANALYSIS AND DISCUSSION OF PUBLISHED STUDIES

3. ANALYSIS AND DISCUSSION OF PUBLISHED CLINICAL STUDIES

MSCs have been widely used in clinical trials for articular cartilage repair using both injective and surgical treatments (65). Despite the great variety of cells identified and characterized as possible candidates for articular cartilage repair, only BM-MSCs, AD-MSCs and PBMSCs have been used in injective therapy. For example, S-MSCs, one of the most promising stem cells type due to its higher chondrogenic and proliferative potentials, have already shown their capacity to improve cartilage repair *in vivo* (65). However, up to now no clinical trials using these cells have been performed (6, 83).

Interestingly, Platelet-rich plasma (PRP) and HA have been used as an adjuvant in several clinical studies presented in the following tables. PRP can be easily obtained by centrifuging patient's blood with anticoagulant citrate dextrose solution (2, 20, 58, 111). This non-immunogenic blood product contains a higher concentration of platelets than baseline values (2, 37, 111, 112), despite the platelets count can vary depending on the donor's age, gender and health (62). PRP contains a variety of growth factors (such as TGF-β, epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), among others), cytokines, chemokines and many other mediators (20, 58, 113, 114). Growth factors induce chemotaxis, cell migration, angiogenesis, proliferation, differentiation and matrix production (2). Therefore, PRP provides biological mediators that are crucial to natural repair (64, 111). In clinical studies, it was proved that PRP injections, in patients with knee osteoarthritis, is a safe method and has the potential to reduce pain, improve function and quality of life (37, 55, 112). In addition, the combination of PRP with MSCs in intra-articular injections increase the expression of collagen type II and reduces apoptosis of chondrocytes (62). Also worthy of note is that PRP used in clinical studies is activated and the activation of PRP is done by adding calcium chloride, thrombin, or collagen that will allow the release of growth factors (2, 58, 111). In the clinical studies presented in Tables 3, 4 and 6, PRP has been used to facilitate growth and differentiation of MSCs (37, 64, 111, 112, 114). As already discussed, HA provides an environment that facilitate the migration, proliferation, differentiation and the adherence of MSCs at the site of injury (29, 40, 58). However, it should be emphasized that HA has a high affinity for cartilage injuries and its use is common in the clinical treatment of knee OA (5, 58). Therefore,

due to its common use in the treatment of OA, HA is an "active" control used in clinical studies as a comparator (115).

3.1 Evaluation parameters

Evaluation of the effect of the therapy in patients is performed by several clinical assessments, which can be divided into 2 main categories: methods that allow the evaluation of the perception of the therapy by the patients and methods that allow the evaluation of structural effects and cartilage repair. Even though the use of uniform outcome parameters would facilitate the comparison between treatments evaluated in clinical studies, there is no consensus on which parameters to use (82). That is to say, despite both parameters are essential for the evaluation of patients and their combination would be more complementary, doubts still exist about which one should be the main outcome: the clinical parameters, the structural parameters or the combination of both parameters (82). For instance, although clinical parameters are undoubtedly an important outcome, the patient's perspective can be affected by the placebo effect of the treatment (42, 82). In addition, the clinical improvement does not necessarily correspond to the regeneration or repair of cartilage lesions (42, 82).

3.1.1. Structural parameters:

For the evaluation of cartilage regeneration, it is common to use the following techniques: x- ray (42, 116), ultrasonography (116, 117), Magnetic Resonance Imaging (MRI) (13, 111, 115, 118, 119), Magnetic Resonance Imaging quantitative T2-mapping (18, 19, 41, 120), whole-organ Magnetic Resonance Imaging score (WORMS) (112), Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) (118, 121), arthroscopic examination and histological analysis of biopsies (82, 100). It should be noted that this diversity of parameters presents advantages and drawbacks that will be determinant for its choice. It is worth noting that some of these parameters exhibit results more precise. For instance, the arthroscopy and histological analysis of biopsies allow better assessment of the cartilage regeneration, but these methods are too invasive (82, 100). Therefore, depending on the type of parameter selected the degree and size of cartilage damage and the quality of the repair tissue eventually formed may be evaluated more or less accurately.

3.1.2. Subjective or clinical parameters

For evaluation of the patients' perception regarding the therapeutic efficacy, questionnaires are commonly used in clinical trials. Some examples are the Short Form Health Survey (SF-36) (19, 65, 120), Visual Analog Scale of pain (VAS) (65, 120, 122, 123), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (13, 65, 106, 120, 122, 123), Foot and Ankle Outcome Score (FAOS) (13), Harris Hip Score (HHS) (13), International Knee Documentation Committee score (IKDC) (63), Numeric Pain Scale (NPS) (60, 63, 124), Lower Extremity Functional scale (LEFS) (60, 63), Lysholm knee scale (37, 65, 100, 112, 113, 125), Knee Injury and Osteoarthritis Outcome Score (KOOS) (35, 61, 65, 117), Oxford Hip Scale (OHS) (124), Lequesne index (19, 41, 120), functional rating index (FRI) (126), Tegner activity scale (127, 128) and Health Assessment Questionnaire (HAQ) (129). Depending on the questionnaire used, the pain severity, pain frequency, the difficulty in performing activities of daily living, sport activities, joint function, range of motion, symptoms and quality of life would be evaluated. It should be emphasized that some questionnaires are composed by several categories mentioned above. For example, IKDC questionnaire is used for the detection of changes in symptoms, joint function and sport activities (63).

The variety of outcomes and methods of clinical and structural assessment used in different clinical studies, as well as the differences in the number of cells administrated per injection and the frequency of MSC administration, the total duration of the studies and the intermediate time points for assessment of response, among other variables, make comparisons between studies difficult and therefore, conclusions on the efficacy of this therapy hard to establish. This situation makes the need for standardization of clinical trial methodology quite obvious and also emphasizes the importance of systematic evaluations of clinical trials already performed. Attempting to contribute to this goal, the following tables summarize the clinical trials for evaluation of injective MSC-based therapies for OA identified using the methodology described in chapter II.

3.2. Clinical studies

Table 3: Clinical studies conducted using injective BM-MSC for cartilage repair in patients with OA

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Centeno et al. 2006 (130)	Severe Hip OA	(2) IA injection of 4-5x10^6 autologous BM-MSCs in combination with PRP and HA	None	1 patient, who was a candidate for bipolar hip replacement	3 months	Serial of MRIs showed partial articular cartilage surface neocortex regeneration. A significant improvement in range of motion. At 12 weeks of follow-up, the functional rating index questionnaire demonstrated improvement in travel, recreation, standing and sitting tolerance, as well as in the patient's walking distance.
Centeno et al. 2008 (131)	Knee OA	IA injection of 4.56x10^7 autologous expanded BM-MSCs (from the iliac crest)	None	1 patient	3 months	Analysis of MRI demonstrated that the volume of the meniscus increase. VAS score decreased from 3.33 to 0.13. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee promotes regeneration of meniscus cartilage and reduction of pain.
Centeno et al. 2008 (107)	Knee OA	IA injection of 2.24x10^7 autologous expanded BM-MSCs (from the iliac crest)	None	1 patient, who was a candidate for total knee arthroplasty	6 months	Analysis of MRI demonstrated that the volume of the meniscus and cartilage increase. Improvement of range of motion and VAS pain score. VAS scores decreased by 95%. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee promotes regeneration of cartilage, reduction of pain and improvement of joint mobility.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Centeno et al. 2010 (119)	Chronic or degenerative joint disease	IA injection of an average of $19.8x10^6$ autologous expanded BM-MSCs	None	227 patients, in which 213 patients were injected into peripheral joints	24 months	Fourteen patients were lost during follow-up. Analysis of MRI failed to demonstrate any evidence of tumor formation or ectopic tissue formation at the re-implant sites. Mild to moderate complications were reported; seven patients had complications related to the injection and three related to stem cell. All of them were transient or were remedied with simple therapeutic measures. One patient was diagnosed with cancer after the MSC procedure, however, it was unrelated to the therapy. Intra-articular injection of autologous expanded BM-MSCs are a safe method.
Centeno et al. 2011 (59)	Chronic or degenerative joint disease	IA injection of autologous expanded BM-MSCs	None	339 patients, in which 135 patients were injected into knee OA	36 months	Analysis of MRI failed to demonstrate any evidence of tumor formation or ectopic tissue formation at the re-implant sites. Mild to moderate complications were rarely reported, eleven patients had complications related to the injection and three related to stem cell. All of them were transient or were remedied with simple therapeutic measures. Two patients were diagnosed with cancer after the MSC procedure, however, it was unrelated to the therapy. The knee OA group reported reduction of pain. Intra-articular injection of autologous expanded BM-MSCs are a safe method and therapeutically beneficial by reducing pain.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Davatchi et al. 2011 (42) **NCT00550524**	Knee OA, moderate to severe	IA injection of 8-9x10^6 autologous expanded BM-MSCs	None	4 patients	12 months	The x-ray analysis no demonstrated improvement. Improvement of VAS pain score and the number of stairs they could climb. The walking time improved for 3 out of 4 patients. The improvement of the range of motion was minor. Intra-articular injection of autologous expanded BM-MSCs into OA knees are a safe method without any complication.
Emadedin et al. 2012 (122) **Phase I**	Knee OA Advanced OA (KL IV)	IA injection of 20-24x10^6 autologous expanded BM-MSCs (from iliac crest)	None	6 patients	12 months	Analysis of MRI demonstrated an increase in cartilage thickness, extension of the repair tissue over the subchondral bone and the subchondral edema was reduced in 3 of the 6 patients. Improvement of range of motion, the walking distance and VAS pain score were noticed at 6 months post-MSC injection. However, after that, the patients' pain appeared to be slightly increased and patients' walking abilities slightly decreased. The total WOMAC score was reduced. Intra-articular injection of autologous expanded BM-MSCs into OA knees are a safe method with no local or systemic adverse events.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Lee et al. 2012 (132)	Knee OA	Group A: Arthroscopic microfracture and IA injection of autologous expanded BM-MSC (from iliac crest) in combination with HA Group B: BM-MSC cells sheets were implanted onto the defect beneath a sutured periosteal patch.	35 patients	35 patients	24.5 months	No serious adverse events were reported. Significant improvement in IKDC, Lysholm, VAS and SF-36 scores in both groups at the final follow-up. However, the injective group had better clinical results in IKDC and Lysholm scores. Analysis of MRI demonstrated significant reduction in the subchondral edema and good fill and integration of the neo-cartilage in the injective group. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee is as good as the surgical procedure but with the advantage of being minimally invasive.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Orozco et al. 2013 (19) **NCT01183728** **Phase I/II**	Knee OA, KL II-IV	IA injection of $4x10^7$ autologous expanded BM-MSCs (from iliac crest)	None	12 patients	12 months	Only minor adverse effects were reported; 50% of patients had complications related to the injection, i.e., pain and discomfort in the knee injected. This situation was remedied with ibuprofen. MRI quantitative T2 mapping showed a significant reduction of poor cartilage area and improvement of cartilage quality in 11 out of 12 patients. Improvement in VAS, WOMAC and Lequesne indices. The pain relief was rapid, with more than 50% of the total improvement was achieved 3 months after MSC injection. The improvement of pain associated with sports activities was better than the pain relief during daily activity. At the end of follow-up, the SF-36 Quality of Life questionnaire revealed a very slight impact of MSC therapy. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee are a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial by reducing pain and promoting regeneration of cartilage.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study Group	Follow-up	Outcomes/Results
Wong et al. 2013 **(133)**	Knee OA with genu varum	Group A: IA injection of $1.46x10^7$ autologous expanded BM-MSCs (from iliac crest) in combination with HA after HTO and microfacture Group B: IA injection of HA following HTO and microfracture (2) IA injection of HA in both groups after the first treatment	28 patients	28 patients	24 months	Improvement of Tegner, Lysholm, IKDC and MOCART scores were significantly better in the cell treatment than the control group. In the MSC group, 9 patients had complete cartilage coverage of their lesions, 10 patients the cartilage coverage was greater than 50% and in 61% of patients the integration of the regenerated cartilage was complete. In the control group, only 4 patients had cartilage coverage greater than 50% and 86% of patients demonstrated incomplete integration. Intra-articular injection of autologous expanded BM-MSCs in conjunction with HA into an osteoarthritic knee are a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial by improving clinical and MOCART outcomes.
Centeno and Freeman 2014 (134)	Hand OA	Group A: IA injections of $5.76x10^6$ autologous expanded BM-MSCs (from iliac crests) Group B: Untreated patients	4 patients	6 patients	12 months	In the treatment group, it was reported improvement in symptoms (VAS) and range of motion, whereas in the untreated control group, it was reported that their symptoms got worsen with time. No complications were reported in the treatment group. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic hand is therapeutically beneficial by improving range of motion and symptoms related to the OA.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Vangsness et al. 2014 (115) **NCT00225095**	Knee OA and Partial medial meniscectomy	<u>Group A:</u> IA injection of $50x10^6$ allogeneic BM-MSC in combination with HA <u>Group B:</u> IA injection of $150x10^6$ allogeneic BM-MSC in combination with HA <u>Group C:</u> IA injection of HA	19 patients	36 patients	24 months	Analysis of MRI demonstrated a significant increase in meniscal volume in 24% of patients in group A and 6% in group B. An increase in meniscal volume was not seen in any patients in the control group. No evidence of ectopic tissue formation was observed in the MRIs. It was noted that patients who received MSCs had a significant pain relief, with better results in group A, in comparison with the control group. Mild to moderate complications were reported, most of them being mild. Severe complications were also being reported but none of which were related to the therapy. Intra-articular injection of allogeneic BM-MSCs (from 18-30-year-old donors) into OA knees are a safe method with no ectopic formations. It is therapeutically beneficial by reducing pain and promoting partial meniscus regeneration.
Emadedin et al. 2015 (13) **NCT01436058**	Ankle joint OA, moderate to severe (KL III, IV)	IA injection of autologous expanded BM-MSC (from iliac crest)	None	6 patients	30 months	Analysis of MRI demonstrated that intra-articular injection of MSCs caused cartilage repair and the cartilage thickness increased, as well as the subchondral edema was reduced in 4 of the 6 patients at six months after treatment. No evidence of tumor or neoplastic changes were observed in the MRIs during 30 months of follow-up. Improvement of the walking distance, VAS, total WOMAC and sub-scores, FAOS scores after the MSCs transplantation. However, VAS scores increased after 12 months. Intra-articular injection of autologous expanded BM-MSCs into OA ankles are a safe method with no severe adverse effect and therapeutically beneficial by reducing pain and improving function of ankle.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Emadedin et al. 2015 (13) **NCT01207661**	Knee OA, moderate to severe (KL III, IV)	IA injection of autologous expanded BM-MSCs (from iliac crest)	None	6 patients	30 months	Analysis of MRI demonstrated that intra-articular injection of MSCs caused cartilage repair and increased cartilage thickness, as well as the subchondral edema was reduced in 3 of the 6 patients. No evidence of tumor or neoplastic changes were observed in the MRIs during 30 months of follow-up. Improvement of the walking distance, VAS and total WOMAC and sub-scores after the MSCs transplantation. However, VAS scores increased after 12 months. Intra-articular injection of autologous expanded BM-MSCs into OA knees are a safe method with no severe adverse events and therapeutically beneficial by relieving pain and improving knee function.
Emadedin et al. 2015 (13) **NCT01499056**	Hip OA, moderate to severe (KL III, IV)	A single IA injection of autologous expanded BM-MSC (from iliac crest)	None	6 patients	30 months	One patient was lost during follow-up because of fractures in the inferior limb caused by an accident. Analysis of MRI shows that intra-articular injection of MSCs caused cartilage repair and increased cartilage thickness. The articular cartilage repair was seen in 3 of the five patients. No evidence of tumor or neoplastic changes were observed in the MRIs during 30 months of follow-up. Improvement of the walking distance, VAS, total WOMAC and sub-scores, HHS scores after the MSCs transplantation. However, VAS scores increased after 12 months. Intra-articular injection of autologous expanded BM-MSCs into OA hip are a safe method with no severe adverse events and therapeutically beneficial.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Soler Rich et al. 2015 (41)	Knee OA, KL II-III	IA injection of $4x10^7$ autologous expanded BM-MSCs (from iliac crest)	None	50 patients	12 months	Only minor adverse effects were reported; 50% of patients had complications related to the injection, i.e., pain and discomfort in the knee injected. This situation was remedied with ibuprofen. MRI quantitative T2 mapping showed an improvement in cartilage quality, reduction of PCI in 37 out of 50 patients. 10 patients had the same PCI and in 3 patients the value was worse at 12 months after MSC injection. Significant improvement in VAS, WOMAC and Lequesne indices at the end of follow-up. The improvement of pain associated with daily activities was 60% and in sport activities was 63%. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee is therapeutically beneficial by reducing pain and improving cartilage quality and with no serious adverse events.
Mehrabani et al. 2016 (21)	Severe Knee OA, KL IV	IA injection of $3.6x10^7$ autologous expanded BM-MSCs (from iliac crests)	None	1 patient	12 months	No local or systemic adverse effects were reported. Analysis of MRI demonstrated an increase in cartilage thickness and an extension of the repair tissue over the subchondral bone. Improvement in WOMAC, VAS pain, walking distance, number of stairs she could climb and functional status of knee. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee are a feasible and safe method with no adverse events. In addition to this, it is therapeutically beneficial by improving quality of life and promoting cartilage healing.

Table 3: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Soler et al. (2016) (18) **Phase I/II**	Knee OA, KL II-III	IA injection of $40x10^6$ autologous expanded BM-MSCs (from iliac crests)	None	15 patients	48 months	A few adverse effects were reported, most of them being mild. Only one patient had a serious adverse event, ovary cyst exertion, but it was unrelated to the therapy. At 12 months after cell therapy, it was observed a significant improvement in VAS (for pain in daily activity and on exertion), SF-36, WOMAC, Lequesne and HAQ scores. In all patients, MRI quantitative T2 mapping not only showed signs of cartilage regeneration but also showed no progression of OA in previously healthy areas at 12 months after cell injection. In four years, only one patient required total knee replacement and another patient required surgery by suffering from an acute meniscus lesion. Intra-articular injection of autologous expanded BM-MSCs into an osteoarthritic knee is a safe method with a low rate of adverse events. In addition to this, it is therapeutically beneficial by improving the quality of life, relieving pain and improving regeneration of cartilage.

Abbreviations: BM-MSCs, bone marrow-derived mesenchymal stem cells; FAOS, foot and ankle outcome score; HA, hyaluronic acid; HAQ, health assessment questionnaire ; HHS, harris hip score ; HTO, high tibial osteotomy; IA, intra-articular; IKDC, international cartilage repair society; KL, kellgren lawrence; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; MOCART, magnetic resonance observation of cartilage repair tissue; OA, osteoarthritis; PCI, poor cartilage index; PRP, platelet-rich plasm; SF-36, short form health survey; VAS, visual analogue scale; WOMAC, western ontario and McMaster Universities Osteoarthritis Index.

In the clinical studies presented in Table 3, intra-articular injection of BM-MSCs was applied in several joints: knee, hip, ankle and hand. The majority of these studies used autologous MSCs, i.e., BM-MSCs from and injected in the same patient. It should be noted that only two studies used allogeneic BM-MSCs (obtained from normal healthy volunteers) which will be discussed further below. BM-MSCs were used alone (15 studies) or combined with HA (1 study), activated PRP and HA (1 study), or HA following surgery methods (2 studies). The surgeries performed were high tibial osteotomy (HTO) combined with microfracture and arthroscopic microfracture. The results of these studies, in general, suggest that the intra-articular injection of BM-MSCs promotes the regeneration of cartilage and reduction of pain, as well as improvement in joint mobility and quality of life.

However, it should be emphasized that only 5 out of 19 studies had a control group. The control groups are an important element of study design since it allows to compare and contextualize the changes in both subjective and structural parameters. Thus, it facilitates the determination of the effect of MSCs. In the first study, performed by Lee et al. (132), the authors compared intra-articular injection of autologous expanded BM-MSCs and HA following arthroscopic debridement and microfracture with surgical transplantation of BM-MSCs included in a solid matrix (control group) in patients with knee OA. They concluded that the injective treatment is as good as the surgical procedure, with the advantage of being minimally invasive. In the second study, performed by Wong et al. (133), the authors evaluated the effects of injecting autologous expanded BM-MSCs following knee surgeries in patients with osteoarthritic knees and genu varum. A total of fifty-six patients, who underwent HTO and microfracture, were randomized into two groups: MSCs and control. The MSCs group received autologous expanded BM-MSCs with HA by intra-articular injection, whereas the control group received intra-articular injection of HA. At the final of 24 months of follow-up, the MSCs group was superior to the control group in MOCART and clinical evaluations. Thus, they concluded that the injective treatment with MSCs is safe and effective for OA. In the third study, performed by Centeno and Freeman (134), they compared the intra-articular injection of expanded BM-MSCs with untreated patients (control group). At the end of 12 months of follow-up, the results obtained were encouraging, however, due to the small number of treated subjects and minimally matched control groups as well as the use of only subjective methods, these results must be interpreted with caution. Therefore, these results suggest that this injective treatment with BM-MSCs is safe, because no adverse events were reported, and effective in osteoarthritic hand disease by

improving range of motion and symptoms. However, it is vital to perform more controlled randomized studies with larger groups not only to confirm the results but also to evaluate the cartilage regeneration by structural methods, such as high-field MRI. It should be noted that this study was the only one to be exclusively performed in the OA hand. The fourth study, performed by Vangsness et al. (115), was the first to use allogenic MSCs. A total of fifty-five patients, who underwent a partial medial meniscectomy, were randomized into three groups: Group A, patients received an injection of $50x10^6$ allogeneic BM-MSC in combination with HA; Group B, patients received an injection of $150x10^6$ allogeneic BM-MSC in combination with HA; and Group C, the control group, received an intra-articular injection of HA. During the follow-up they observed no ectopic tissue formation but some adverse events were reported, most of them being mild, such as joint swelling or pain. Patients who received MSCs experienced a significant pain relief and increased cartilage volume compared with those who received the control vehicle. Yet, it should be noted that group A, who received a lower number of cells, obtained better results in both cartilage volume increase and pain relief. Also, it was suggested that the higher dose of allogeneic MSCs can be safely injected into the knee. Last but not least, the fifth study, performed by Vega et al. (120), also evaluates the safety and feasibility of using allogenic MSCs to treat knee OA. For this evaluation, the authors compared intra-articular injection of allogeneic BM-MSCs, with intra-articular injection of HA (control group). At the end of follow-up, they concluded that allogeneic MSCs therapy is feasible, safe and effective by reducing pain and disability, improving quality of life as well as promoting cartilage repair.

The success of these last two studies indicates that allogenic MSCs could be an alternative source for OA. In fact, the application of allogeneic MSC has been successfully performed in several clinical trials for treating various diseases, as aforementioned. Allogenic MSCs are advantageous over autologous MSCs (120, 135, 136). For example, the use of these cells does not require surgery to harvest bone marrow in patients, resulting in less discomfort to the patients as well as the contiguously beginning of the treatment (6). Since in the autologous therapy it is necessary to harvest and cultivate MSCs, the beginning of the treatment is delayed (6). Moreover, the cell expansion of autologous MSCs makes the procedure slow and expensive, whereas the allogeneic cells would be cheaper with higher homogeneity (120). In addition, as a result of MSCs being affected by diseases and age of patients, the allogeneic MSCs have higher quality because the control of donor age and health of the bone marrow donors is performed (57, 86, 135, 136). One possible disadvantage of using allogeneic cells would be the rejection of these cells by the

immune system. However, as already discussed, MSC are immune privileged, immune evasive and inhibit immune responses (86, 120). On the other hand, it should be noted that in the study performed by Vega et al., the authors compared their results with results achieved by Orozco et al. (19) and Jo et al. (106), who used autologous BM-MSCs and AD-MSCs, respectively. They concluded that the efficacy of allogeneic treatment appears to be somewhat smaller than those reported for treatment with autologous MSC. However, direct comparisons are difficult because the other studies were uncontrolled. Therefore, it is important to confirm this observation in future studies designed to directly compare autologous with allogeneic cells in different arms of the same trial (120).

The most meaningful study using BM-MSCs alone was performed by Centeno and collaborators (59). In this study the highest number of patients were enrolled (339 patients) and a longer follow-up (36 months) than most of the studies presented in Table 3. It should be emphasized that the majority of the studies presented in the Table 3 were performed in knee OA, and more studies are needed to evaluate the effectiveness of BM-MSCs in other joints. In general, all studies suggest the safety and efficacy of the injective MSC therapy, although the follow-up periods are insufficient for definitive conclusions to be drawn.

Table 4: Clinical studies conducted using injective BMAC for cartilage repair in patients with OA

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Varma et al. 2010 (137)	Mild to moderate Knee OA	Group A: IA injection of BMAC after to underwent to arthroscopic debridement Group B: Only underwent to arthroscopic debridement	25 patients	25 patients	6 months	Improvement in range of motion, VAS and the overall osteoarthritis outcome scores, especially the quality of life in group A during and at the end of follow-up. Intra-articular injection of BMAC into an osteoarthritic knee promotes reduction of pain, improvement of joint mobility and better quality of life.
Hauser and Orlofsky 2013 (138)	Hip, Knee or Ankle OA	(2-7) IA injection of autologous WBM, in combination with hyperosmotic dextrose	None	7 patients	2-12 months	Improvement in pain intensity, range of motion and quality of life. Five in the seven patients had a complete relief and/or a strong functional improvement. Intra-articular injection of autologous WBM in conjunction with hyperosmotic dextrose into osteoarthritic joints are a safe method with no adverse events. It is therapeutically beneficial by reducing pain, improving range of motion and quality of life of patients.
Centeno et al. 2014 (124)	Hip OA, KL I-IV	IA injection of $5.27x10^6$ autologous BMAC (from iliac crest) in combination with PRP	None	196 patients (216 hips)	12 months	Only 12 patients reported complications. These complications were mild to moderate adverse events and the most reported were pain and swelling (6 out of 12). All of them were transient and/or unrelated to the treatment. Improvement on the OHS and NPS. Younger patients were significantly more likely to report functional improvement than older patients (>55 years). Intra-articular injection of autologous BMAC into an osteoarthritic hip is safe with a low rate of adverse events and no serious adverse events. In addition to this, it is therapeutically beneficial by reducing pain and improving function of the hip.

Table 4: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study Group	Follow-up	Outcomes/Results
Centeno et al. 2014 (60)	Knee OA, KL I-IV	Group A: IA injection of BMAC in combination with PRP Group B: IA injection of BMAC in combination with adipose tissue and PRP	None	Group A: 518 patients (616 knees) Group B: 163 patients (224 knees)	12 months	Both groups had reported adverse effects. Pain and swelling were the most frequent adverse events reported. Two patients were diagnosed with cancer after the MSC procedure, however, it was unrelated to the therapy. Patients with higher BMI were more likely to report functional improvement than patients with lower BMI. Both groups had significant improvements in LEFS and NPS score. Although, the group A reported a greater improvement rating, the differences between groups were not significant. Intra-articular injection of autologous BMAC into an osteoarthritic knee is safe with a low rate of adverse events and therapeutically beneficial by reducing pain and improving function of the knee. The addition of adipose tissue does not provide an obvious benefit.
Kim et al. 2014 (139)	Knee OA, KL I-IV	IA injection of autologous BMAC with adipose tissue	None	41 patients (75 knees)	6-12 months	Improvement in VAS, IKCD, SF-36, KOOS and Lysholm Knee scores. However, in patients with KL IV, the effect of BMAC with adipose tissue was poorer in all clinical measures. Intra-articular injection of BMAC with adipose tissue was more effective in patients with early to moderate stage of OA. Intra-articular injection of autologous BMAC into an osteoarthritic knee is therapeutically beneficial by reducing pain and improving function of knee as well as improving clinical results.

Table 4: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Duration of follow-up	Outcomes/Results
Centeno et al. 2015 (63)	Knee OA, KL I- IV	Group A: IA injection of ≤$4x10^8$ autologous BMAC Group B: IA injection of >$4x10^8$ autologous BMAC	None	Group A: 188 patients (224 knees) Group B: 170 patients (185 knees)	> 12 months	Improvement in NPS, LEFS and IKDC scales in both groups, however, patients who received higher concentration of cells had better pain outcome (NPS). No significant difference in functional outcomes (LEFS and IKDC) between these two groups. Intra-articular injection of autologous BMAC into OA knees are therapeutically beneficial by reducing pain and improving function of knee.
Oliver et al. 2015 (56)	Knee OA, KL II-IV	IA injection of autologous BMAC (from iliac crest) in combination with adipose tissue	None	70 patients	12 months	Only transient pain and swelling were reported. Improvement in all KOOS scores. Intra-articular injection of autologous BMAC in conjunction with adipose tissue into osteoarthritic knee is a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial by improving the quality of life.

Abbreviations: BMAC, bone marrow aspirate concentrate; BMI, body mass index IA, intra-articular; IKDC, international cartilage repair society; LEFS, lower extremity functional scale; KL, kellgren lawrence; KOOS, knee injury and osteoarthritis outcome score; MSCs, mesenchymal stem cells; NPS, numeric pain scale; OA, osteoarthritis; OHS, oxford hip scale; PRP, platelet-rich plasm; SF-36, short form health survey; VAS, visual analogue scale; WBM, whole bone marrow.

Bone marrow aspirate concentrates (BMAC) have been used in some clinical studies. It consists in bone marrow, which can be easily collected from many anatomical locations (such as iliac crest, tibia, and femur), concentrated in an FDA compliant device (6, 56). BMAC contains MSCs, hematopoietic stem cells (HSC), platelets, T-lymphocytes, B-lymphocytes, monocytes, macrophages and epithelial progenitor cells (63). These constituents produce growth factors (such as, PDGF, TGF-β, BMP-2 and BMP-7), chemokines and cytokines that together will create a microenvironment favorable to the proliferation and differentiation of MSCs, as well as cartilage repair (56, 63, 103). BMAC is one of the few forms of delivering stem cells (minimally manipulated) and growth factors currently approved by the United States Food and Drug Administration (FDA) (103). In the clinical studies presented in Table 4, BMAC was used alone or combined with PRP, adipose tissue, surgery (arthroscopic debridement) or PRP and adipose tissue. BMAC seems to improve the quality of life and function and to reduce pain when used for knee and hip cartilage repair. This therapy was more effective in patients with early to moderate OA stages and in younger patients. In summary, the injective treatment with autologous BMAC can be considered a feasible, safe (with a low rate of adverse events) and effective treatment for knee and hip OA (56, 60, 63, 124, 137, 139). However, the limited follow-up periods do not allow definite conclusions both on safety and efficacy.

In comparison with the studies presented in the Table 3, the studies in this table have larger groups of patients. However, 7 out of 8 studies do not have a control group and they do not use structural parameters. As a result of using exclusively subjective parameters, part of these results may be due to placebo effect.

Table 5: Clinical studies conducted using injective AD-MSCs for cartilage repair in patients with OA

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Jo et al 2014 (106) **Phase I/II**	Knee OA (KL III, IV)	**Phase I:** <u>Group A:</u> IA injection of $1.0x10^7$ autologous expanded AD-MSCs <u>Group B:</u> IA injection of $5.0x10^7$ autologous expanded AD-MSCs <u>Group C:</u> IA injection of $1.0x10^8$ autologous expanded AD-MSCs **Phase II:** IA injection of $1.0x10^8$ autologous expanded AD-MSCs	None	18 patients (9 patients in Phase I and 9 in phase II)	6 months	Analysis of MRI demonstrated significant decrease of the size of cartilage defect, a gradual regeneration of articular cartilage and increased volume of cartilage in the medial femoral and tibial condyles in high-dose group. However, the original defect was not completely covered by the cartilage regenerated. Arthroscopic and histological measures revealed a decrease of articular cartilage defects by regeneration of hyaline-like articular cartilage in the high dose group. Significant improvement in WOMAC, VAS pain, KSS Knee scores in the high-dose groups. However, no patients in the low- and mild-dose groups improve their scores. A few adverse effects were reported. Only one patient in the low-dose group had a serious adverse event, urinary stone. None of these adverse events were related to the therapy. No patients were withdrawn from the study because adverse event. Intra-articular injection of autologous expanded AD-MSCs (from the abdominal subcutaneous) into OA knees are a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial in the high-dose group by improving function and pain of the knee joint, and reducing cartilage defect through regeneration of cartilage.

Table 5: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Pers et al. 2016 (22) **NCT01585857** **Phase I**	Severe Knee OA, KL III-IV	<u>Group A:</u> IA injection of $2x10^6$ autologous expanded AD-MSCs <u>Group B:</u> IA injection of $1x10^7$ autologous expanded AD-MSCs <u>Group C:</u> IA injection of $5x10^7$ autologous expanded AD-MSCs	None	18 patients (six patients in each group)	6 months	Minor complications were reported and the most reported were pain and swelling. These complications were transient or treated with common analgesics. Only one patient reported a severe adverse events but unrelated with the stem cell therapy. Only one patient underwent to TKA after MSC injection. Improvement in pain levels and WOMAC scores in all three groups, however, only in the group A, the results were statistically significant at the end of follow-up. Arthroscopic and histological measures revealed regeneration. Intra-articular injection of autologous AD-MSCs into an osteoarthritic knee is a safe method with no serious adverse events and it is therapeutically beneficial by reducing pain and improving cartilage regeneration.

Abbreviations: AD-MSCs, adipose-derived mesenchymal stem cells; IA, intra-articular; KL, kellgren lawrence; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; OA, osteoarthritis; TKA, total knee replacement; VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Table 5 summarizes clinical studies using AD-MSCs. Only 2 studies were identified and both used autologous expanded AD-MSCs. In these studies, the authors evaluated the effects of different concentrations of stem cells in patients with knee OA. The first study, performed by Jo et al. (106), was the first dose-dependent study, which consists in a phase I/II clinical trial. In phase I, 9 patients were randomized into three groups: Group A, low-dose group, received an injection of $1.0x10^7$ autologous expanded AD-MSCs; Group B, mild-dose group, patients received an injection of $5x10^7$ autologous expanded AD-MSCs; and Group C, high-dose group, received an injection of $1.0x10^8$ autologous expanded AD-MSCs. In phase II, 9 patients received the highest amount of stem cells ($1.0x10^8$). At the end of 6 months of follow-up, the authors observed no adverse events related with the therapy. In addition, they concluded that the higher dosage of AD-MSCs is more efficacious than lower concentrations of stem cells. The intra-articular injection of $1.0x10^8$ AD-MSCs promoted regeneration of cartilage and reduction of pain, as well as improvement in joint mobility. It should be noted that this study does not have a control group and future studies need to be performed in larger controlled and randomized clinical trials. The second study, performed by Pers et al. (22), consists in a phase I clinical trial. A total of 18 patients were divided into low dose ($2x10^6$), mild-dose ($10x10^6$) and high-dose ($50x10^6$) groups with 6 patients each. After 6 months of follow-up, the authors observed no adverse events related with the therapy. In addition, they concluded that patients treated with the lowest dose of AD-MSCs exhibited the best response to treatment. Also, this group showed a significant improvement in pain and WOMAC scores compared with those receiving higher doses. These results contrast with the results achieved by Jo et al., thus, the possibility raised by Pers et al. was the level of inflammation in the lowest dose group. Once, patients treated with the low-dose AD-MSCs had higher level of inflammation (reflected by the highest level of pain at baseline) and this microenvironment might have primed the injected AD-MSCs to exert their immunomodulatory role more efficiently than in the groups where the inflammation was lower. It should be noted that this study does not have a control group and future studies, with larger groups and controlled long-term, are now mandatory to confirm if this cell therapy has a clinical and structural benefit in OA.

Both studies evaluated subjective and structural parameters, making these studies more robust. Moreover, the structural parameters used, the arthroscopy and histological analysis of biopsies, are the most precise methods. Another positive point of these studies is that they used AD-MSCs alone (without PRP or HA), thus showing the effectiveness of AD-MSCs in regenerating

cartilage. However, they obtained different results. In addition to this, another study, performed by Vangsness et al. (115), who used allogeneic BM-MSCs with HA had the same results obtained by Pers et al. (22). Despite the hypothesis raised by Pers et al. (22), the real reasons to lowest dose of MSCs having better outcomes is unclear. Therefore, more studies are necessary not only to understand which factors influence the response to the dose but also to determine the most adequate dose.

Table 6: Clinical studies conducted using injective AD-MSCs in form of SVF for cartilage repair in patients with OA

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Pak 2011 (114)	Knee OA	IA injection of autologous adipose-derived SVF (from the abdominal area) in combination with HA, PRP, $CaCl_2$ and a nanogram dose of dexamethasone	None	2 patients, who were candidates for TKA	3 months	The MRI analysis demonstrated a significant increase in meniscus cartilage volume and cartilage thickness. Improvements in range of motion, VAS pain score and FRI. Intra-articular injection of autologous adipose-derived SVF (in conjunction with HA, PRP, $CaCL_2$ and dexamethasone) into an osteoarthritic knee promotes regeneration of meniscus cartilage and it is therapeutically beneficial by improving physical therapy outcomes, subjective pain and functional status.
Koh and Choi 2012 (37)	Knee OA	<u>Group A:</u> IA injection of autologous adipose-derived SVF (from infrapatellar fat pad) combined with PRP after the arthroscopic debridement <u>Group B:</u> IA injection of PRP after arthroscopic debridement (2) IA injection of PRP in both groups after the first treatment	25 patients	25 patients	12-18 months	Only minor adverse effects were reported. Intra-articular injection of MSCs was more effective in younger patients and in patients with earlier stage of OA. Similar clinical results in Lysholm, Tegner activity scale and VAS scores at the final follow-up in both groups, but the degree of improvement was greater in the adipose-derived SVF group once preoperative clinical scores and ICRS grade were significantly worst in the study group. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee are a safe method with no serious adverse events. It is therapeutically beneficial in OA by reducing pain and improving the function of knee OA.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study Group	Follow-up	Outcomes/Results
Koh et al. 2013 (112)	Knee OA, KL III, IV ICRS grade, 3 and 4	IA injection of $0.3x10^6$-$2.7x10^6$ autologous adipose-derived SVF (from infrapatellar fat pad) in combination with PRP following arthroscopic, debridement (2) IA injection of PRP	None	18 patients	24-26 months	Only minor adverse effects were reported. Intra-articular injection of MSCs was more effective in patients with earlier stage of OA and in patients who received a larger numbers of cells. Improvement in Lysholm, WOMAC, VAS and WORMS scores at the final follow-up. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee are a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial by reducing pain and improving the function of knee OA in patients being treated.
Koh et al. 2014 (125)	Knee OA, KL I-III	<u>Group A:</u> IA injection of $4.11x10^6$ autologous adipose-derived SVF (from buttocks) in combination with PRP before underwent HTO <u>Group B:</u> IA injection of PRP before underwent HTO	23 patients	21 patients	24 months	Significant improvement in the VAS pain score and two KOOS subscales, pain and symptoms. These improvements were significantly better in the cell treatment than the control group at the last follow-up. Similar improvement in Lysholm score at the final follow-up in both groups, Arthroscopic evaluation revealed that 50% of the patients in the MSC group had a partial or even fibrocartilage coverage, whereas only 10% of the patients in the control group. Intra-articular injection of autologous adipose-derived SVF into OA knees in conjunction with HTO are therapeutically beneficial by improving all of the KOOS subscales, the VAS pain score and cartilage healing.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Pak et al. 2013 (111)	OA various joints	IA injection of autologous adipose-derived SVF (from abdomen) in combination with PRP, HA and $CaCl_2$	None	91 patients (100 joints)	26.7 months	Some complications were reported; 37 joints had pain and swelling in joints treated; tendonitis and tenosynovitis was reported mainly by elderly patients; 1 patient had a localized rash around the injected site and another patient had a hemorrhagic stroke. Significant improvement in VAS scores (50-60%) in patients with hip OA and knee OA at the final of 3 months. The improvement in the low back and ankle was minor. Analysis of MRI failed to demonstrate any evidence of neoplastic formation at the re-implant sites in 27 joints analyzed. Telephone questionnaires from 100 joints, the tumor formation at the implant sites was not reported. Intra-articular injection of autologous adipose-derived SVF in conjunction with PRP, HA and $CaCl_2$ into an osteoarthritic joint are a safe method with no evidence of neoplastic formation in any implant sites. In addition to this, it is therapeutically beneficial by reducing pain at long-term.
Bui et al. 2014 (113)	Knee OA, KL II-III	IA injection of autologous adipose-derived SVF (from abdominal adipose tissue) in combination with activated PRP	None	21 patients	8.5 months	Analysis of MRI revealed an increase of thickness of the cartilage layer and partial regeneration of the injured cartilage. Significant improvement in joint function, VAS pain and Lysholm scores. Intra-articular injection of autologous adipose-derived SVF in conjunction with activated PRP into an osteoarthritic knee are a safe method with no adverse events. In addition to this, it is therapeutically beneficial by improving the patient's quality of life.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Garza et al. 2015 (3) **NCT02276833**	Knee OA, KL II-III	IA injection of 27.3-70.5x10^6 adipose-derived SVF (from the abdomen)	None	6 patients (10 knees)	3 months	No adverse events were reported. Intra-articular injection of autologous adipose-derived SVF into osteoarthritic knee is a safe method with no adverse events. In addition to this, it is therapeutically beneficial by reducing pain and increasing mobility
Kim et al. 2015 (64)	Knee OA	<u>Group A:</u> IA injection of 3.19-4.65x10^6 autologous adipose-derived SVF (from buttocks) in conjunction with PRP after debridement <u>Group B:</u> Underwent to 3.33-4.47x10^6 autologous adipose-derived SVF (from buttocks) implantation on a fibrin glue scaffold after debridement	20 patients	20 patients	28.6 months	Significant improvement of IKDC and Tegner activity scores in both groups at the time of second-look arthroscopy (12 months after the therapy). However, only the surgery group further improved their clinical results at the final follow-up. Only in the injective group, the number of cells administrated were correlated with the clinical outcomes.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Koh et al. 2015 (100)	Knee OA, KL II, III	Arthroscopic lavage before IA injection of $4.0x10^6$ autologous adipose-derived SVF (from buttock subcutaneous fat tissue) in combination with PRP	None	30 patients	24-26 months	Only minor adverse effects were reported. Only 16 patients who received MSC therapy underwent second-look arthroscopy. 10 out of 16 patients demonstrated cartilage formation and 4 of the 16 patients maintained cartilage healing status. Intra-articular injection of MSCs was more effective in "younger" patients and in patients with earlier stage of OA. Improvement of Lysholm, KOOS and VAS scores in almost all patients at the final follow-up. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee of elderly patients (≥65) are a safe method with no serious adverse events. In addition to this, it is therapeutically beneficial by reducing pain, promoting cartilage healing and improving the function of knee OA in elderly patients being treated.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Michalek et al. 2015 (45)	OA various joints (mainly knee and hip joints), KL II-IV	IA injection of adipose-derived SVF	None	1128 patients (1856 joints)	12-54 months	Fourteen patients were lost during follow-up. Only minor adverse effects were reported and the most reported were pain and swelling. These complications were transient or treated with common analgesics. Analysis of MRI demonstrated a slight increase in cartilage thickness, smoothed surface irregularities and defects, sealed chondral fissures and reduced subchondral bone edema. Clinical improvement was faster/ better within 3-6 months after the cell therapy in patients with earlier stages of OA and in non-obese patients. Although the clinical improvement was slower for this same period of time in patients with higher BMI and in patients with higher degree of OA, at the end of 12 months there was no difference in clinical outcomes between these groups. Only 4 patients, whose grade were IV, of 503 TJA candidates required TJA during the follow-up. Intra-articular injection of autologous adipose-derived SVF into osteoarthritic joints is a safe method with a low rate of adverse events and which none of them were serious adverse events. In addition to this, it is therapeutically beneficial by improving the quality of life.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Tantuway et al. 2015 (61)	Joint OA, KL II-IV	IA injection of adipose-derived SVF	None	31 patients (62 joints)	6 months	Only minor adverse effects were reported such as pain and swelling. At the injection site, fever and mild headache. These complications were transient or treated with common analgesics Improvement in all KOOS scores. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee is a safe method with no serious adverse events and it is therapeutically beneficial by reducing pain and improving quality of life.
Correa et al. 2016 (117)	Advanced Knee OA	IA injection of autologous adipose-derived SVF (from subcutaneous fat)	None	1 patient	20 months	Significant improvement in KOOS scores at the end of follow-up. Ultrasound imaging of the knee revealed a progressive increase in both the joint space and cartilage thickness. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee is therapeutically beneficial by reducing clinical symptoms and improving cartilage regeneration.

Table 6: Continued

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Fodor et al. 2016 (123) **NCT02357485** **Phase I**	Knee OA, KL I-III	IA injection of autologous adipose-derived SVF	None	6 patients (8 knees)	12 months	No adverse events were reported related to the injection of adipose-derived SVF in the knee. Improvement in range of motion, WOMAC, VAS pain and TUG. At the end of follow-up, only one knee had a modest improvement in the WOMAC score but had an improvement in the VAS score. The patient who had better results (no pain on the WOMAC and VAS at both 3 months and 1 year) was the patient who received the highest SVF yield. A significant improvement in WOMAC and VAS were achieved 3 months after MSC injection and were maintained for 1 year. Intra-articular injection of autologous adipose-derived SVF into an osteoarthritic knee is a safe method with no adverse effects and it is therapeutically beneficial by reducing pain and improving range of motion.

Abbreviations: BMI, body mass index; $CaCl_2$, calcium chloride; FRI, functional rating index; HA, hyaluronic acid; HTO, high tibial osteotomy; *IA,* intra-articular; ICRS, international cartilage repair society; IKDC, international cartilage repair society; KL, kellgren lawrence; KOOS, knee injury and osteoarthritis outcome score; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; OA, osteoarthritis;; PRP, platelet-rich plasm; SVF, stromal vascular fraction; TJA, total joint arthrosplasty; TKA, total knee replacement; TUG, timed up-and go; VAS, visual analogue scale; WOMAC, western ontario and McMaster Universities Osteoarthritis Index; WORMS whole-organ magnetic resonance imaging.

The injective intra-articular delivery of SVF has emerged as the trend in clinical trials, contrarily to the tendency found in the preclinical studies (58, 104, 105). SVF is a heterogeneous fraction that contains a varied population of cells: AD-MSCs, pre-adipocytes, smooth muscle cells, fibroblasts, endothelial cells, leukocytes (lymphocytes, monocytes and macrophages), erythrocytes and pericytes (11, 55, 58, 61, 71, 81, 100, 117, 125). The main advantage of using SVF, as BMAC, is the elimination of the period of time between harvest and implantation, providing the opportunity to perform the therapy in the same day (55).

In the clinical studies presented in Table 3, intra-articular injection of autologous adipose-derived SVF was applied in several joints: knee, hip, ankle, foot, shoulder, hand, wrist and elbow. These cells were used alone (5 studies), combined with HA and activated PRP (2 studies), activated PRP (2 studies), or PRP following surgery methods (4 studies). In general, the intra-articular injection of adipose-derived SVF promoted regeneration of cartilage and reduction of pain as well as improvement in joint mobility and quality of life.

It should be emphasized that only 3 out of 13 studies had a control group. In the first study, performed by Koh and Choi, the authors evaluated the effects of injecting autologous SVF following knee surgery in patients with knee OA. A total of fifty patients, who underwent arthroscopic debridement, were divided in two groups: study and control. The study group received an injection of autologous adipose SVF (a mean of 1.89×10^{6} stem cells) with activated PRP, whereas the control group received intra-articular injection of activated PRP. At the final follow-up, patients in both groups experienced a similar clinical improvement. However, the degree of improvement was greater in the study group once preoperative clinical scores and ICRS grade in this group were significantly worse. Another interesting point presented in this study is that the cell therapy was more effective in younger patients and in patients with earlier OA stage. In conclusion, intra-articular injections of adipose SVF derived from infrapatellar fat pad is safe and effective by reducing pain and improving function in patients with knee OA (37). In the second study, performed by Koh et al, the authors compared the clinical and second-look arthroscopic outcomes of patients undergoing HTO and intra-articular injection of SVF in conjunction with PRP to intra-articular injection of PRP alone. A total of forty-four patients were included in the study, and randomized into treatment and control groups. After following the patients for 24 months, the treatment group showed greater clinical improvement and better cartilage regeneration compared to the control group (125). The last study, performed by Kim et al., was

the first matched-pair study that compared the effects of intra-articular SVF with surgical implantation of SVF by comparing their clinical and second-look arthroscopic outcomes. For that purpose, 40 patients were chosen from a pool of 115 patients after a matching process, where the authors pair-matched patients treated with the injective therapy to patients treated with surgical implantation. This pair matched was based on gender, age and lesion size. Of note, the injective group included 20 patients treated with an injection of SVF in combination with PRP, whereas the surgery group included 20 patients who underwent SVF implantation on a fibrin glue scaffold. At the time of second-look arthroscopic surgery, the authors found comparable clinical outcomes in both groups but the surgical group had better ICRS grades. At the final of follow-up, only the surgery group further improved their clinical results. The researchers pointed out that a single simple injection of SVF is insufficient for the cartilage repair and the optimal dose of SVF remains to be determined. Another interesting finding was that only in the injective group the number of cells administered was correlated with the clinical outcomes (64).

Another meaningful study about SVF was performed by Michalek et al. (45), who administrated single dose SVF injections to the largest available group of patients with OA. During the follow up, the researchers observed no adverse events related with the therapy. In addition, they observed that patients with higher BMI or patients with higher degree of OA had a slower clinical improvement. The authors concluded that intra-articular injection of autologous SVF into osteoarthritic joints is safe and effective by improving the quality of life. Further, this study shows the effectiveness of SVF in regenerating cartilage because no adjuvants was used (PRP or HA).

In summary, the injective treatment with autologous adipose-derived SVF can be considered a feasible, safe (no serious adverse events) and effective treatment for OA. Yet, it should be taken into account that the study performed by Kim demonstrated better results in the surgical groups and future studies are needed to determine the optimal numbers of SVF cells and injections for the cartilage repair. Besides, the intra-articular injection of AD-MSCs in the form of SVF was more effective in younger patients, in patients with earlier stage of OA and in patients who received a larger number of cells (37, 45, 64, 100, 112, 123).

Table 7: Clinical studies conducted using injective PBMSCs for cartilage repair in patients with OA

Reference/ Clinical Trial Identifier	Indication	Intervention (n° of injection)	Control group	Study group	Follow-up	Outcomes/Results
Turajane et al. 2013 (35)	Knee OA, KL II ICRS III-IV	IA injection of autologous PBMSCs in combination with GFAP and HA following arthroscopic microdrilling	None	5 patients	6 months	No adverse events were reported. Significant improvement in WOMAC and KOOS at the end of follow-up in all patients. Electron microscopy scanning shown cell attachment and proliferation. Histological analysis revealed an increase in proteoglycan and glycosaminoglycan content. Intra-articular injection of autologous PBMSCs in conjunction with GFAP and HA into OA knees are therapeutically beneficial by improving the quality of life and promoting regeneration of articular cartilage in early knee OA.

Abbreviations: GFAP, growth factor addition/preservation; HA, hyaluronic acid; IA, intra-articular; ICRS, international cartilage repair society; KL, kellgren lawrence; KOOS, knee injury and osteoarthritis outcome score; OA, osteoarthritis; PBMSCs, peripheral blood mesenchymal stem cells; WOMAC, western Ontario and McMaster Universities Osteoarthritis Index.

Only one study used PBMSCs as source of MSCs for the treatment of OA as shown Table 7.

In this study, performed by Turajane et al. (35), intra-articular injection of autologous activated PBMSCs in combination with growth factor addition/preservation (GFAP) and HA was combined with arthroscopic microdrilling for endogenous mesenchymal cell stimulation in 5 patients with knee OA. At the end of the follow-up of 6 months, the authors observed an improvement in the quality of life of patients and regeneration of articular cartilage in earlier OA stage. They suggested that this injective treatment with PMSCs is safe because no adverse events were reported and effective in early stage of OA. On the other hand, they admitted that more controlled studies with larger groups are vital not only to confirm the results but also to elucidate the mechanism of hyaline cartilage regeneration. Nonetheless, the follow-up period is too short for definite conclusions both on efficacy and safety to be drawn.

These cells have also been used in clinical trials for cartilage repair of chondral defects. In clinical trials performed by Saw et al. in 2011 and in 2013, it was demonstrated that these cells contribute to cartilage repair of chondral defects in patients with cartilage damage grade III-IV in the International Cartilage Repair Society (ICRS) grading system (140, 141). These results may be more trustworthy than the results presented by Turajane et al. (35), as it has a longer follow-up. Also, in the study performed by Saw et al. (141) in 2013 not only the number of patients evaluated was larger, but also the intervention was compared to a group control.

In conclusion, despite the results obtained by Turajane et al. (35) are similar to the results obtained by Saw et al. (140, 141), showing the capacity of these cells to improve joint damage, it is also mandatory not only have more controlled studies with lager groups but also to demonstrate the effectiveness of PBMSCs alone, i.e., without the combination of surgical methods or adjuvants.

Table 8: Completed clinical trials of injective MSCs registered at clinicaltrials.gov for the treatment of OA with no results disclosed

Clinical Trial Identifier/ Title	Sponsor/ Phase	Indication	Intervention (n° of injection)	Number of patients	Follow-up	Outcomes/Results
NCT01504464 **(142)** (The Effects of Intra-articular Injection of Mesenchymal Stem Cells in Knee Joint Osteoarthritis)	Royan Institute Phase II	Severe Knee OA	<u>Group A:</u> (3) IA injection of autologous BM-MSCs <u>Group B:</u> (2) IA placebo injection and (1) IA injection of autologous BM-MSCs	40 patients (control and MSC group)	6 months	Endpoint: Efficacy Parameters evaluated: Adverse events, WOMAC, VAS and MRI
NCT01453738 **(143)** (Allogeneic Mesenchymal Stem Cells in Osteoarthritis)	Stempeutics Research Pvt Ltd Phase II	Knee OA, KL II-III	<u>Group A:</u> IA injection of (different doses of) expanded allogeneic MSCs in 2-4 ml Plasmalyte-A followed by 2ml hyaluronan <u>Group B:</u> IA injection of 2ml plasmalyte-A	60 patients (control and MSC groups)	24 months	Endpoint: safety and efficacy Parameters evaluated: Adverse advents, WOMAC, ICOAP, VAS, x-ray, WORMS
NCT01227694 **(144)** (Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis)	Banc de Sang I Teixits Phase I/II	Knee OA, KL II-III	IA injection of $4x10^7$ autologous expanded BM-MSCs	15 patients	12 months	Endpoint: safety and efficacy Parameters evaluated: Adverse events, VAS, HAQ, SF-36 and MRI quantitative T2

Table 8: Continued

Clinical Trial Identifier/ Title	Sponsor/ Phase	Indication	Intervention (n° of injection)	Number of patients	Follow-up	Outcomes/Results
NCT 01300598 **(145)** (Autologous Adipose Tissue Derived Mesenchymal Stem Cells Transplantation in Patients with Degenerative Arthritis)	Biostar Phase I/II	Knee OA	Group A: IA injection of $1x10^7$ autologous AD-MSCs; Group B: IA injection of $5x10^7$ autologous AD-MSCs; Group C: IA injection of $1x10^8$ autologous AD-MSCs cells	18 patients	6 months	Endpoint: safety and efficacy of three different doses of autologous MSCs in patients with OA. Parameters evaluated: WOMAC, MRI, x-ray, KSCRS, VAS, histological evaluation, Arthroscopy and adverse advents
NCT02118519 **(146)** (Mesenchymal Stem Cells in Knee Cartilage Injuries)	University of Jordan Phase II	Knee OA, Articular Cartilage Disorder of Knee	Group A: IA injection of autologous MSCs Group B: IA injection of autologous MSCs with platelet lysate	13 patients	12 months	Endpoint: Safety and efficacy Parameters evaluated: Clinical assessment and MRI
NCT02142842 **(147)** (Autologous Adipose Stem Cells and Platelet Rich Plasma Therapy for Patients With Knee Osteoarthritis)	University of Science Ho Chi Minh City Phase I/II	Knee OA, KL II-III	Group A: IA injection of autologous AD-MSCs in combination with PRP	30 patients (control group and MSC group)	18 months	Endpoint: Safety and efficacy The clinical efficiency is evaluated by VAS, Lysholm score and MRI. The safety is evaluated by adverse events. Parameters evaluated: Adverse events, VAS, Lysholm score, MRI

Table 8: Continued

Clinical Trial Identifier/ Title	Sponsor/ Phase	Indication	Intervention (n° of injection)	Number of patients	Follow-up	Outcomes/Results
NCT01931007 **(148)** (Use of Autologous Bone Marrow Aspirate Concentrate in Painful Knee Osteoarthritis (BMAC))	Mayo Clinic Phase I	Bilateral knee OA, KL I-III	Group A: IA injection of autologous BMAC Group B: The contralateral knee was injected with sterile placebo.	25 patients (control and MSC groups)	12 months	Endpoint: Safety and efficacy Parameters evaluated: Adverse events, x-ray, MRI
NCT01873625 **(149)** (Transplantation of Bone Marrow Derived Mesenchymal Stem Cells in Affected Knee Osteoarthritis by Rheumatoid Arthritis)	Royan Institute Phase II/III	Knee OA	Group A: IA injection of placebo Group B: IA injection of BM-MSC	60 patients (control and MSC groups)	12 months	Endpoint: Safety and efficacy Parameters evaluated: WOMAC, VAS, DAS28 scoring, x-ray, MRI, biochemical analysis
NCT01809769 **(150)** (Autologous Adipose Tissue Derived Mesenchymal Stem Cells Therapy for Patients With Knee Osteoarthritis)	Cellular Biomedicine Group Ltd Phase I/II	Knee OA	Group A: (2) IA injection of $1x10^7$ autologous AD-MSCs.; Group B: (2) IA injection of $2x10^7$ autologous AD-MSCs.; Group C: (2) IA injection of $5x10^7$ autologous AD-MSCs.	18 patients	24 months	Endpoint: safety and efficacy of three different doses of autologous MSCs in patients with OA. Parameters evaluated: Adverse events, WOMAC, SF-36, NRS-11, the volume of articular cartilage, KSCRS score

Table 8: Continued

Clinical Trial Identifier/ Title	Sponsor/ Phase	Indication	Intervention (n° of injection)	Number of patients	Follow-up	Outcomes/Results
NCT01448434 **(151)** (Allogeneic Mesenchymal Stem Cells for Osteoarthritis)	Stempeutics Research Pvt Ltd Phase II	Knee OA, KL II-III	<u>Group A:</u> IA injection of expanded allogeneic MSCs in combination with Plasmalyte-A followed by hyaluronan <u>Group B:</u> IA injection of expanded allogeneic MSCs in combination with Plasmalyte-A followed by hyaluronan <u>Group C:</u> IA injection of 2 ml Plasmalyte-A followed by hyaluronan	72 patients (control and MSC groups)	12 months	Endpoint: safety and efficacy of two different doses of allogeneic MSCs in patients with OA. Parameters evaluated: Adverse events, WOMAC, ICOAP, x-ray, WORMS score, VAS, reduction of the intake of analgesic
NCT01601951 **(152)** (Outcomes Data of Bone Marrow Stem Cells to Treat Hip and Knee Osteoarthritis)	Regenerative Pain Center	Hip and Knee OA	IA injection of autologous BMAC	12 patients	12 months	Parameters evaluated: VAS, Harris Hip score or KSS, x-ray

Table 8: Continued

Clinical Trial Identifier/ Title	Sponsor/ Phase	Indication	Intervention (n° of injection)	Number of patients	Follow-up	Outcomes/Results
NCT01485198 **(153)** (Autologous Stem Cells in Osteoarthritis)	Hospital Universitario Dr. Jose E. Gonzalez Phase I	Knee OA, KL II-III	Group A: IA injection of autologous hematopoietic stem cells (from bone marrow) (BMASC) Group B: Acetaminophen 750mg orally every 8 hours	61 patients (control and MSC groups)	6 months	Endpoint: safety and efficacy Parameters evaluated: Adverse events, WOMAC, KSS, SF-36, VAS

Abbreviations: AD-MSCs, adipose-derived mesenchymal stem cells; BMAC, bone marrow aspirate concentrate; BM-MSCs, bone marrow-derived mesenchymal stem cells; HAQ, health assessment questionnaire; HHS, harris hip score; IA, intra-articular; ICOAP, ; KL, kellgren Lawrence; KSCRS, ;KSS, knee society score; MRI, magnetic resonance imaging; MSC, mesenchymal stem cells; NRS-11, ; OA, osteoarthritis; PRP, platelet-rich plasm; SF-36, short form health survey ;VAS, visual analogue scale; WOMAC, western ontario and McMaster Universities Osteoarthritis Index; WORMS, whole-organ magnetic resonance imaging.

Only completed studies registered at clinicaltrials.gov were introduced in the Table 8. The majority of these studies presented in Table 8 involves the use of autologous MSCs from bone marrow or adipose tissue. Only two clinical trials used allogeneic expanded cells. These studies not only evaluate the safety and efficacy of different doses of MSCs but also they compared their outcomes to the control group. Interestingly, more than half (7) of the 12 studies in the Table 8 had a control group, whereas in the other tables the majority of studies did not have a control group. Another interesting point present in two studies of this table is that patients with knee OA received multiple intra-articular injections of MSCs. Therefore, these two studies may answer to the question of the number of injection needed for cartilage repair. In studies from Table 8, the duration of the follow-up was in the range of 6-24 months, thus the evaluation of safety and efficacy of injective cell therapy in this period of time will not added more information. Unfortunately, none of these studies has yet released its results.

4. CONCLUSIONS

MSCs cell therapy has demonstrated promising results for the treatment of many diseases thanks to its ideal characteristics for regenerative medicine, such as homing potential, differentiation capacity, trophic and immunomodulatory properties. The injective MSC treatment of OA in preclinical and clinical studies have also demonstrated encouraging results. According to the clinical studies presented above, this injective therapy shows promise as a safe, effective and feasible method independently of the sources or doses of MSCs.

Despite these promising results, too many questions still remain open. Firstly, as discussed above, MSCs have been used either expanded or concentrated. The main advantages of using expanded stem cells are to allow a more reproducible treatment, having higher number of stem cells and knowing the exact number used in each patient (11). On the other hand, the cell culture presents high costs and some regulatory issues, since these products are considered as pharmacological treatments by regulatory agencies (11, 58, 105). Besides, the danger of bacterial contamination, xenogenic risk or cellular transformation represent additional hurdles (6, 11, 45, 83). In contrast, the utilization of concentrated cell products, such as BMAC and SVF, reduces costs, minimizes exposure to risks, increases patient compliance and decreases logistic difficulties by reducing the turnaround time from cell harvest to treatment (45, 55, 105). However, they offer a lower number of stem cells (105) and potential consequences of the presence of other cell types are still unknown. It should be noted that some sources of MSC, such as PBMSCs and S-MSCs, require expansion to be exploited due to their low yield in stem cells (105). Up to now, there is no evidence of which cell manipulation produces superior outcomes, as analysis of the studies in the tables presented shows and is widely acknowledged by the scientific community (82, 105).

Secondly, the donor source of MSCs can be autologous or allogeneic. As previously mentioned, allogenic MSCs are advantageous over autologous MSCs. The main advantages of using allogeneic MSCs in OA are their immediate availability, higher quality, higher homogeneity and lower cost (136). However, despite these cells being non-immunogenic and possessing immunomodulatory and immunosuppressive proprieties, their clinical use has been very limited (11). In addition, the efficacy of allogeneic MSCs to treat OA seems to be inferior. However, this claim needs to be confirmed in studies in which autologous and allogeneic cells are directly compared (120). Thus, to date, the majority of studies have been using autologous MSCs because the safety and predictability of these cells is established and they have are subjected to less strict regulatory requisites (57).

Thirdly, the optimal source of MSCs remains to be determined (20, 83). Despite MSCs can be harvested from many anatomical locations (135), the most common source of MSCs used in clinical studies is bone marrow followed by adipose tissue. Nonetheless, it should be noted that all cellular sources above mentioned present advantages and drawbacks that will be determinant for their choice.

Fourthly, several points of this injective therapy remain undetermined: the optimal dose of MSCs, the number and the frequency of injections as well as the use of adjuvants. The current clinical studies have been using different doses of MSC, ranging from $0.3x10^6$ to $1.5x10^8$ per injection. Despite it is expected that higher concentrations of MSCs have higher rates of improvement (64, 106, 112), in the studies performed by Pers et al. and Vangsness et al., the best outcomes were achieved by the group which received the lowest dosage of MSCs (22, 115). Thus, the dose-response relationship of MSCs injected is unclear and other factors may be involved, namely the donor age and presence of comorbidities (11, 22, 83). In addition to the foregoing, according to some studies, it also remains to be determined whether a single injection is sufficient for efficient cartilage repair or repeated injections are needed (37, 64, 112, 122, 125). In these circumstances, determining the frequency of administration will be another relevant issue to be resolved. Also, several studies used PRP or HA to improve the effect of MSCs. However, up to now, no studies have proven that adding these adjuvants provide an increased benefit in comparison with the administration of MSCs alone. In fact, the combination of MSCs with adjuvants makes the distinction of the effect of MSCs from those of PRP or HA more difficult (11, 37, 105, 112). Therefore, it is vital to design studies that will demonstrate if the addition of these adjuvants is essential and if they improve cartilage repair with regard to the administration MSCs alone (105).

Fifthly, the determination of the best method to deliver MSCs also needs to be established. These cells can be delivered by intra-articular injection or surgically implanted. Using injective delivery is advantageous as it is minimally invasive, simple and cost-effective (11, 16, 65, 104). In addition, this approach has a better patient compliance because not only it allows the reduction of the time of recovery but also the rates of morbidity and adverse events are lower (11, 55, 65, 104). It should be emphasized that this approach allows MSCs to target not only the articular cartilage but also the whole joint environment (11, 104). As already discussed, OA is a complex condition that affects the whole joint and, thus this procedure may be advantageous over surgical implants, but more studies are mandatory to determine which delivery method is more effective. (16, 55, 65)

Finally, it is important to define the profile of patient that will benefit the most from this kind of therapy. Several studies suggest that the injective therapy is more effective in younger patients, in patients with earlier OA stages and in patients with lower BMI.

In summary, more quality studies appropriately controlled are required both to determine the efficacy and safety of injective cell therapy for OA and to identify the best conditions for success. In particular, such studies are expected to determine the duration of the benefit of injective MSCs therapy, since there is no guarantee that MSC therapy can lead to a definitive cure for OA, but also to optimize this approach by identifying the best dose of MSCs, the most effective frequency of injections and the best source of MSCs (41, 45). Furthermore, it may also become apparent that not a single MSC source can fit every situation. Indeed, as consequence of the increasing recognition of different OA phenotypes may be that such phenotypes may require distinct therapeutic approaches. In what concerns MSC therapies, the possibility that different stem cell sources may be more effective to treat a specific phenotype than the other ones cannot be excluded and will have to be investigated. In this regard, existing evidence that the BMI influences the outcome of injective autologous MSC therapy suggests that metabolic OA, a recently acknowledged OA phenotype, may be one the OA phenotypes in which MSC source may have the largest influence in the outcome (45, 60).

5. REFERENCES

1. KIM, M. J. *et al.* - Generation of human induced pluripotent stem cells from osteoarthritis patient-derived synovial cells. **Arthritis Rheum**. ISSN 0004-3591. 63:10 (2011) 3010–3021. doi: 10.1002/art.30488.
2. KANG, M. L.; IM, G. I. - Drug delivery systems for intra-articular treatment of osteoarthritis. **Expert Opin Drug Deliv**. ISSN 1742-5247. 11:2 (2014) 269–282. doi: 10.1517/17425247.2014.867325.
3. GARZA, J. R. *et al* - Use of Autologous Adipose- Derived Stromal Vascular Fraction to Treat Osteoarthritis of the Knee: A Feasibility and Safety Study. **Journal of Regenerative Medicine**. ISSN 2325-9620. (2015) doi: 10.4172/2325-9620.1000119.
4. TOGHRAIE, F. *et al.* - Scaffold-free adipose-derived stem cells (ASCs) improve experimentally induced osteoarthritis in rabbits. **Arch Iran Med**. ISSN 1029-2977. 15:8 (2012) 495–499. doi: 012158/aim.0011.
5. BARRY, F.; MURPHY, M. - Mesenchymal stem cells in joint disease and repair. **Nat Rev Rheumatol**. ISSN 1759-4790. 9:10 (2013) 584–594. doi: 10.1038/nrrheum.2013.109.
6. KRISTJANSSON, B.; HONSAWEK, S. - Current perspectives in mesenchymal stem cell therapies for osteoarthritis. **Stem Cells Int**. ISSN 1687-966X. (2014) 194318. doi: 10.1155/2014/194318.
7. MICHAEL, J. W.; SCHLUTER-BRUST, K. U.; EYSEL, P. - The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. **Dtsch Arztebl Int**. ISSN 1866-0452. 107:9 (2010) 152–162. doi: 10.3238/arztebl.2010.0152.
8. JANSSEN, M. *et al.* - Drugs and Polymers for Delivery Systems in OA Joints: Clinical Needs and Opportunities. **Polymers**. 6:3 (2014) 799–819. doi: 10.3390/polym6030799.
9. SINGH, J. A. - Stem cells and other innovative intra-articular therapies for osteoarthritis: what does the future hold? **BMC Med**. ISSN 1741-7015. 10 (2012) 44. doi: 10.1186/1741-7015-10-44.
10. THYSEN, S.; LUYTEN, F. P.; LORIES, R. J. U. - Targets, models and challenges in osteoarthritis research. **Dis Model Mech**. 8:1 (2015) 17-30. doi: 10.1242/dmm.016881.
11. GIROLAMO, L. DE *et al.* - Regenerative approaches for the treatment of early OA. **Knee Surg Sports Traumatol Arthrosc**. ISSN 0942-2056. 24:6 (2016) 1826–1835. doi: 10.1007/s00167-016-4125-y.

12. FAQEH, H. AL *et al.* - The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. **Exp Gerontol**. ISSN 0531-5565. 47:6 (2012) 458–464. doi: 10.1016/j.exger.2012.03.018.
13. EMADEDIN, M. *et al.* - Long-Term Follow-up of Intra-articular Injection of Autologous Mesenchymal Stem Cells in Patients with Knee, Ankle, or Hip Osteoarthritis. **Arch Iran Med**. ISSN 1029-2977. 18:6 (2015) 336–344. doi: 015186/aim.003.
14. YU, S. P.; HUNTER, D. J. - Emerging drugs for the treatment of knee osteoarthritis. **Expert Opin Emerg Drugs**. ISSN 1472-8214. (2015). doi: 10.1517/14728214.2015.1037275.
15. TURKIEWICZ, A. *et al.* - Current and future impact of osteoarthritis on health care: a population-based study with projections to year 2032. **Osteoarthritis Cartilage**. ISSN 1063-4584. 22:11 (2014) 1826–1832. doi: 10.1016/j.joca.2014.07.015.
16. NOTH, U.; STEINERT, A. F.; TUAN, R. S. - Technology insight: adult mesenchymal stem cells for osteoarthritis therapy. **Nat Clin Pract Rheumatol**. ISSN 1745-8382. 4:7 (2008) 371–380. doi: 10.1038/ncprheum0816.
17. HUNTER, D. J.; SCHOFIELD, D.; CALLANDER, E. - The individual and socioeconomic impact of osteoarthritis. **Nat Rev Rheumatol**. ISSN 1759-4790. 10:7 (2014) 437–441. doi: 10.1038/nrrheum.2014.44.
18. SOLER, R. *et al.* - Final results of a phase I-II trial using ex vivo expanded autologous Mesenchymal Stromal Cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. **Knee**. ISSN 0968-0160. (2016). doi: 10.1016/j.knee.2015.08.013.
19. OROZCO, L. *et al.* - Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. **Transplantation**. ISSN 0041-1337. 95: 12 (2013) 1535–1541. doi: 10.1097/TP.0b013e318291a2da.
20. WOLFSTADT, J. I. *et al.* - Current concepts: the role of mesenchymal stem cells in the management of knee osteoarthritis. **Sports Health**. ISSN 1941-7381. 7:1 (2015) 38–44. doi: 10.1177/1941738114529727.
21. MEHRABANI, D. *et al.* - The Healing Effect of Bone Marrow-Derived Stem Cells in Knee Osteoarthritis: A Case Report. **World Journal of Plastic Surgery**. (2016).
22. PERS, Y. M. *et al.* - Adipose Mesenchymal Stromal Cell-Based Therapy for Severe Osteoarthritis of the Knee: A Phase I Dose-Escalation Trial. **Stem Cells Transl Med**. ISSN 2157-6564. 5:7 (2016) 847–856. doi: 10.5966/sctm.2015-0245.

23. DIEKMAN, B. O.; GUILAK, F. - Stem cell-based therapies for osteoarthritis: challenges and opportunities. **Curr Opin Rheumatol**. ISSN 1040-8711. 25:1 (2013) 119–126. doi: 10.1097/BOR.0b013e32835aa28d.
24. LOESER, R. F. *et al.* - Osteoarthritis: a disease of the joint as an organ. **Arthritis Rheum**. ISSN 0004-3591. 64:6 (2012) 1697–1707. doi: 10.1002/art.34453.
25. CHEVALIER, X.; EYMARD, F.; RICHETTE, P. - Biologic agents in osteoarthritis: hopes and disappointments. **Nat Rev Rheumatol**. ISSN 1759-4790. 9:7 (2013) 400–410. doi: 10.1038/nrrheum.2013.44.
26. DESANDO, G. *et al.* - Intra-articular delivery of adipose derived stromal cells attenuates osteoarthritis progression in an experimental rabbit model. **Arthritis Res Ther**. ISSN 1478-6354. 15:1 (2013) R22. doi: 10.1186/ar4156.
27. MADRY, H.; CUCCHIARINI, M. - Advances and challenges in gene-based approaches for osteoarthritis. **J Gene Med**. ISSN 1099-498x. 15:10 (2013) 343–355. doi: 10.1002/jgm.2741.
28. MURPHY, J. M. *et al.* - Stem cell therapy in a caprine model of osteoarthritis. **Arthritis Rheum**. ISSN 0004-3591. 48:12 (2003) 3464–3474. doi: 10.1002/art.11365.
29. SATO, M. *et al.* - Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. **Arthritis Res Ther**. ISSN 1478-6354. 14:1 (2012) R31. doi: 10.1186/ar3735.
30. JIANG, Y.; TUAN, R. S. - Origin and function of cartilage stem/progenitor cells in osteoarthritis. **Nat Rev Rheumatol**. ISSN 1759-4790. 11:4 (2015) 206–212. doi: 10.1038/nrrheum.2014.200.
31. UTH, K.; TRIFONOV, D. - Stem cell application for osteoarthritis in the knee joint: A minireview. **World J Stem Cells**. ISSN 1948-0210. 6:5 (2014) 629–636. doi: 10.4252/wjsc.v6.i5.629.
32. BAUGE, C.; BOUMEDIENE, K. - Use of Adult Stem Cells for Cartilage Tissue Engineering: Current Status and Future Developments. **Stem Cells Int**. ISSN 1687-966X (2015) 438026. doi: 10.1155/2015/438026.
33. ESLAMINEJAD, B. M.; POOR, M. E. - Mesenchymal stem cells as a potent cell source for articular cartilage regeneration. **World J Stem Cells**. ISSN 1948-0210. 6:3 (2014) 344–354. doi: 10.4252/wjsc.v6.i3.344.
34. SACCHETTI, C. *et al.* - Polyethylene-glycol-modified single-walled carbon nanotubes for intra-articular delivery to chondrocytes. **ACS Nano**. ISSN 1936-0851. 8:12 (2014) 12280–12291. doi: 10.1021/nn504537b.

35. TURAJANE, T. *et al.* - Combination of intra-articular autologous activated peripheral blood stem cells with growth factor addition/ preservation and hyaluronic acid in conjunction with arthroscopic microdrilling mesenchymal cell stimulation Improves quality of life and regenerates articular cartilage in early osteoarthritic knee disease. **J Med Assoc Thai**. ISSN 0125-2208. 96:5 (2013) 580–588.
36. BUZHOR, E. *et al.* - Cell-based therapy approaches: the hope for incurable diseases. **Regen Med**. ISSN 1746-0751. 9:5 (2014) 649–672. doi: 10.2217/rme.14.35.
37. KOH, Y. G.; CHOI, Y. J. - Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis. **Knee**. ISSN 0968-0160. 19:6 (2012) 902–907. doi: 10.1016/j.knee.2012.04.001.
38. FRISCH, J. *et al.* - Current progress in stem cell-based gene therapy for articular cartilage repair. **Curr Stem Cell Res Ther**. ISSN 1574-888X. 10:2 (2015) 121–131.
39. REDMAN, S. N.; OLDFIELD, S. F.; ARCHER, C. W. - Current strategies for articular cartilage repair. **Eur Cell Mater**. ISSN 1473-2262. 9 (2005) 23–32.
40. LEE, K. B. *et al.* - Injectable mesenchymal stem cell therapy for large cartilage defects--a porcine model. **Stem Cells**. ISSN 1066-5099. 25:11 (2007) 2964–2971. doi: 10.1634/stemcells.2006-0311.
41. RICH S. *et al.* - Treatment of Knee Osteoarthritis with Autologous Expanded Bone Marrow Mesenchymal Stem Cells: 50 Cases Clinical and MRI Results at One Year Follow-Up. **Journal of Stem Cell Research & Therapy**. ISSN 2157-7633. (2015). doi: 10.4172/2157-7633.1000285.
42. DAVATCHI, F. *et al.* - Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. **Int J Rheum Dis**. ISSN 1756-1841. 14:2 (2011) 211–215. doi: 10.1111/j.1756-185X.2011.01599.x.
43. SHEN, W. *et al.* - Intra-articular injection of human meniscus stem/progenitor cells promotes meniscus regeneration and ameliorates osteoarthritis through stromal cell-derived factor-1/CXCR4-mediated homing. **Stem Cells Transl Med**. ISSN 2157-6564. 3:3 (2014) 387–394. doi: 10.5966/sctm.2012-0170.
44. MATTHEWS, G. L.; HUNTER, D. J. - Emerging drugs for osteoarthritis. **Expert Opin Emerg Drugs**. ISSN 1472-8214. 16:3 (2011) 479–491. doi: 10.1517/14728214.2011.576670.
45. MICHALEK, J. *et al.* - Autologous adipose tissue-derived stromal vascular fraction cells application in patients with osteoarthritis. **Cell Transplant**. ISSN 0963-6897. (2015). doi: 10.3727/096368915x686760.

46. CHAGANTI, R. K.; LANE, N. E. - Risk factors for incident osteoarthritis of the hip and knee. **Curr Rev Musculoskelet Med**. 4:3 (2011) 99–104. doi: 10.1007/s12178-011-9088-5.
47. TOGHRAIE, F. S. *et al.* - Treatment of osteoarthritis with infrapatellar fat pad derived mesenchymal stem cells in Rabbit. **Knee**. ISSN 0968-0160. 18:2 (2011) 71–75. doi: 10.1016/j.knee.2010.03.001.
48. NEOGI, T.; ZHANG, Y. - Epidemiology of osteoarthritis. **Rheum Dis Clin North Am**. ISSN 0889-857x. 39:1 (2013) 1–19. doi: 10.1016/j.rdc.2012.10.004.
49. HAWKER, G. A.; STANAITIS, I. - Osteoarthritis year in review 2014: clinical. **Osteoarthritis Cartilage**. ISSN 1063-4584. 22:12 (2014) 1953–1957. doi: 10.1016/j.joca.2014.06.018.
50. VALDES, A. M. *et al.* - Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. **Arthritis Rheum**. ISSN 0004-3591. 62:8 (2010) 2347–2352. doi: 10.1002/art.27530.
51. DONG, J. *et al.* - Intra-articular delivery of liposomal celecoxib-hyaluronate combination for the treatment of osteoarthritis in rabbit model. **Int J Pharm**. ISSN 0378-5173. (2013). doi: 10.1016/j.ijpharm.2012.11.031.
52. GOLDRING, M. B.; BERENBAUM, F. - Emerging targets in osteoarthritis therapy. **Curr Opin Pharmacol**. ISSN 1471-4892. 22 (2015) 51–63. doi: 10.1016/j.coph.2015.03.004.
53. DEMANGE, M. K.; SISTO, M.; RODEO, S. - Future trends for unicompartmental arthritis of the knee: injectables & stem cells. **Clin Sports Med**. ISSN 0278-5919. 33:1 (2014) 161–174. doi: 10.1016/j.csm.2013.06.006.
54. EVANS, C. H.; KRAUS, V. B.; SETTON, L. A. - Progress in intra-articular therapy. **Nat Rev Rheumatol**. ISSN 1759-4790. 10:1 (2014) 11–22. doi: 10.1038/nrrheum.2013.159.
55. COUNSEL, P. D. *et al.* - Cell therapy in joint disorders. **Sports Health**. ISSN 1941-7381. 7:1 (2015) 27–37. doi: 10.1177/1941738114523387.
56. OLIVER, K. S. *et al.* - Clinical Outcome of Bone Marrow Concentrate in Knee Osteoarthritis. **Journal of Prolotherapy**. (2015).
57. WYLES, C. C. *et al.* - Mesenchymal stem cell therapy for osteoarthritis: current perspectives. **Stem Cells Cloning**. ISSN 1178-6957. 8 (2015) 117–124. doi: 10.2147/sccaa.s68073.
58. PAK, J. *et al.* - Cartilage Regeneration in Human with Adipose Tissue-Derived Stem

Cells: Current Status in Clinical Implications. **Biomed Res Int**. (2016). doi: 10.1155/2016/4702674.

59. CENTENO, C. J. *et al.* - Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. **Curr Stem Cell Res Ther**. ISSN 1574-888X. 6:4 (2011) 368–378.
60. CENTENO, C. *et al.* - Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. **Biomed Res Int**. (2014). doi: 10.1155/2014/370621.
61. TANTUWAY1, V. *et al.* – Use of autologous adipose tissue derived stromal vascular fraction in treatment of knee osteoarthritis and chondral lesions. **Journal of evidence based medicine and healthcare**. 2:41 (2015) 7085–7098. doi: 10.18410/jebmh/2015/964.
62. FREITAG, J. *et al.* - Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy - a review. **BMC Musculoskelet Disord**. ISSN 1471-2474. 17:1 (2016) 230. doi: 10.1186/s12891-016-1085-9.
63. CENTENO, C. J. *et al.* - A dose response analysis of a specific bone marrow concentrate treatment protocol for knee osteoarthritis. **BMC Musculoskelet Disord**. ISSN 1471-2474. 16 (2015) 258. doi: 10.1186/s12891-015-0714-z.
64. KIM, Y. S. *et al.* - Comparative Matched-Pair Analysis of the Injection Versus Implantation of Mesenchymal Stem Cells for Knee Osteoarthritis. **Am J Sports Med**. ISSN 0363-5465. 43:11 (2015) 2738–2746. doi: 10.1177/0363546515599632.
65. ORTH, P. *et al.* - Current perspectives in stem cell research for knee cartilage repair. **Stem Cells Cloning**. ISSN 1178-6957. 7 (2014) 1–17. doi: 10.2147/sccaa.s42880.
66. CUCCHIARINI, M.; MADRY, H. - The potential of gene transfer for the treatment of osteoarthritis. **Regen Med**. ISSN 1746-0751. 9:1 (2014) 5–8. doi: 10.2217/rme.13.70.
67. GUPTA, P. K. *et al.* - Mesenchymal stem cells for cartilage repair in osteoarthritis. **Stem Cell Res Ther**. ISSN 1757-6512. 3:4 (2012) 25. doi: 10.1186/scrt116.
68. DIEDERICHS, S.; SHINE, K. M.; TUAN, R. S. - The promise and challenges of stem cell-based therapies for skeletal diseases: stem cell applications in skeletal medicine: potential, cell sources and characteristics, and challenges of clinical translation. **Bioessays**. ISSN 0265-9247. 35:3 (2013) 220–230. doi: 10.1002/bies.201200068.
69. OSCH, G. J. VAN - Osteoarthritis year in review 2014: highlighting innovations in basic research and clinical applications in regenerative medicine. **Osteoarthritis Cartilage**. ISSN 1063-4584. 22:12 (2014) 2013–2016. doi: 10.1016/j.joca.2014.07.022.

70. PEETERS, C. M. *et al.* - Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review. **Osteoarthritis Cartilage**. ISSN 1063-4584. 21:10 (2013) 1465–1473. doi: 10.1016/j.joca.2013.06.025.
71. MIZUNO, H.; TOBITA, M.; UYSAL, A. C. - Concise review: Adipose-derived stem cells as a novel tool for future regenerative medicine. **Stem Cells**. ISSN 1066-5099. 30:5 (2012) 804–810. doi: 10.1002/stem.1076.
72. BRAGANÇA, J.; TAVARES, A.; BELO, J.A. – Células estaminais e medicina regenerativa – um admirável mundo novo. **Canal BQ**. 7 (2010) 4-17
73. KANG, L. *et al.* - Induced pluripotent stem cells (iPSCs)--a new era of reprogramming. **J Genet Genomics**. ISSN 1673-8527. 37:7 (2010) 415–421. doi: 10.1016/s1673-8527(09)60060-6.
74. ILLICH, D. J. *et al.* - Concise review: induced pluripotent stem cells and lineage reprogramming: prospects for bone regeneration. **Stem Cells**. ISSN 1066-5099. 29:4 (2011) 555–563. doi: 10.1002/stem.611.
75. TAKAHASHI, K.; YAMANAKA, S. - Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. **Cell**. ISSN 0092-8674. 126:4 (2006) 663–676. doi: 10.1016/j.cell.2006.07.024.
76. TAKAHASHI, K. *et al.* - Induction of pluripotent stem cells from adult human fibroblasts by defined factors. **Cell**. ISSN 0092-8674. 131:5 (2007) 861–872. doi: 10.1016/j.cell.2007.11.019.
77. DIEKMAN, B. O. *et al.* - Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. **Proc Natl Acad Sci U S A**. ISSN 0027-8424. 109:47 (2012) 19172–19177. doi: 10.1073/pnas.1210422109.
78. LEE, J. *et al.* - Generation of disease-specific induced pluripotent stem cells from patients with rheumatoid arthritis and osteoarthritis. **Arthritis Res Ther**. ISSN 1478-6354. 16:1 (2014) R41. doi: 10.1186/ar4470.
79. ROY, S. S. *et al.* - A hypothetical relationship between the nuclear reprogramming factors for induced pluripotent stem (iPS) cells generation--bioinformatic and algorithmic approach. **Med Hypotheses**. ISSN 0306-9877. 76:4 (2011) 507–511. doi: 10.1016/j.mehy.2010.12.003.
80. FENG, B. *et al.* - Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. **Cell Stem Cell**. 4:4 (2009) 301–312. doi: 10.1016/j.stem.2009.03.005.
81. MARMOTTI, A. *et al.* - Bone marrow derived stem cells in joint and bone diseases: a

concise review. **Int Orthop**. ISSN 0341-2695. 38:9 (2014) 1787–1801. doi: 10.1007/s00264-014-2445-4.

82. VONK, L. A. *et al.* - Autologous, allogeneic, induced pluripotent stem cell or a combination stem cell therapy? Where are we headed in cartilage repair and why: a concise review. **Stem Cell Res Ther**. ISSN 1757-6512. 6 (2015) 94. doi: 10.1186/s13287-015-0086-1.
83. FILARDO, G. *et al.* - Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics. **Knee Surg Sports Traumatol Arthrosc**. ISSN 0942-2056. 21:8 (2013) 1717–1729. doi: 10.1007/s00167-012-2329-3.
84. COLEMAN, C. M. *et al.* - Mesenchymal stem cells and osteoarthritis: remedy or accomplice? **Hum Gene Ther**. ISSN 1043-0342. 21:10 (2010) 1239–1250. doi: 10.1089/hum.2010.138.
85. DOMINICI, M. *et al.* - Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. **Cytotherapy**. ISSN 1465-3249. 8:4 (2006) 315–317. doi: 10.1080/14653240600855905.
86. CHEN, F. H.; TUAN, R. S. - Mesenchymal stem cells in arthritic diseases. **Arthritis Res Ther**. ISSN 1478-6354. 10:5 (2008) 223. doi: 10.1186/ar2514.
87. CAPLAN, A. I. - Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. **J Cell Physiol**. ISSN 0021-9541. 213:2 (2007) 341–347. doi: 10.1002/jcp.21200.
88. STOLTZ, J. F. *et al.* - Stem Cells and Regenerative Medicine: Myth or Reality of the 21th Century. **Stem Cells Int**. ISSN 1687-966X. (2015). doi: 10.1155/2015/734731.
89. CAPLAN, A. I.; DENNIS, J. E. - Mesenchymal stem cells as trophic mediators. **J Cell Biochem**. ISSN 0730-2312. 98:5 (2006) 1076–1084. doi: 10.1002/jcb.20886.
90. MUSCHLER, G. F. *et al.* - Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. **J Orthop Res**. ISSN 0736-0266. 19:1 (2001) 117–125. doi: 10.1016/s0736-0266(00)00010-3.
91. MURPHY, J. M. *et al.* - Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. **Arthritis Rheum**. ISSN 0004-3591. 46:3 (2002) 704–713. doi: 10.1002/art.10118.
92. SAKAGUCHI, Y. *et al.* - Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. **Arthritis Rheum**. ISSN

0004-3591. 52:8 (2005) 2521–2529. doi: 10.1002/art.21212.

93. SHI, Y. *et al.* - Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. **Cell Res**. ISSN 1001-0602. 20:5 (2010) 510–518. doi: 10.1038/cr.2010.44.
94. PENG, L. *et al.* - Comparative analysis of mesenchymal stem cells from bone marrow, cartilage, and adipose tissue. **Stem Cells Dev**. ISSN 1547-3287. 17:4 (2008) 761–773. doi: 10.1089/scd.2007.0217.
95. SHEN, W. *et al.* - Osteoarthritis prevention through meniscal regeneration induced by intra-articular injection of meniscus stem cells. **Stem Cells Dev**. ISSN 1547-3287. 22:14 (2013) 2071–2082. doi: 10.1089/scd.2012.0563.
96. UCCELLI, A.; PISTOIA, V.; MORETTA, L. - Mesenchymal stem cells: a new strategy for immunosuppression? **Trends Immunol**. ISSN 1471-4906. 28:5 (2007) 219–226. doi: 10.1016/j.it.2007.03.001.
97. BLANC, K. LE; RINGDEN, O. - Immunomodulation by mesenchymal stem cells and clinical experience. **J Intern Med**. ISSN 0954-6820. 262:5 (2007) 509–525. doi: 10.1111/j.1365-2796.2007.01844.x.
98. WANG, W.; CAO, W. - Treatment of osteoarthritis with mesenchymal stem cells. **Sci China Life Sci**. ISSN 1674-7305. 57:6 (2014) 586–595. doi: 10.1007/s11427-014-4673-7.
99. HUURNE, M. TER *et al.* - Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. **Arthritis Rheum**. ISSN 0004-3591. 64:11 (2012) 3604–3613. doi: 10.1002/art.34626.
100. KOH, Y. G. *et al.* - Clinical results and second-look arthroscopic findings after treatment with adipose-derived stem cells for knee osteoarthritis. **Knee Surg Sports Traumatol Arthrosc**. ISSN 0942-2056. 23:5 (2015) 1308–1316. doi: 10.1007/s00167-013-2807-2.
101. QI, Y.; FENG, G.; YAN, W. - Mesenchymal stem cell-based treatment for cartilage defects in osteoarthritis. **Mol Biol Rep**. ISSN 0301-4851. 39:5 (2012) 5683–5689. doi: 10.1007/s11033-011-1376-z.
102. BUUL, G. M. VAN *et al.* - Mesenchymal stem cells secrete factors that inhibit inflammatory processes in short-term osteoarthritic synovium and cartilage explant culture. **Osteoarthritis Cartilage**. ISSN 1063-4584. 20:10 (2012) 1186–1196. doi: 10.1016/j.joca.2012.06.003.
103. CHAHLA, J. *et al.* - Concentrated Bone Marrow Aspirate for the Treatment of

Chondral Injuries and Osteoarthritis of the Knee: A Systematic Review of Outcomes. **Orthop J Sports Med**. ISSN 2325-9671. 4:1 (2016) 2325967115625481. doi: 10.1177/2325967115625481.

104. PERDISA, F. *et al.* - Adipose-Derived Mesenchymal Stem Cells for the Treatment of Articular Cartilage: A Systematic Review on Preclinical and Clinical Evidence. **Stem Cells Int**. ISSN 1687-966X. (2015). doi: 10.1155/2015/597652.

105. FILARDO, G. *et al.* - Stem cells in articular cartilage regeneration. **J Orthop Surg Res**. ISSN 1749-799x. 11 (2016) 42. doi: 10.1186/s13018-016-0378-x.

106. JO, C. H. *et al.* - Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. **Stem Cells**. ISSN 1066-5099. 32:5 (2014) 1254–1266. doi: 10.1002/stem.1634.

107. CENTENO, C. J. *et al.* - Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. **Pain Physician**. ISSN 1533-3159. 11:3 (2008) 343–353.

108. MOKBEL, A. N. *et al.* - Homing and reparative effect of intra-articular injection of autologus mesenchymal stem cells in osteoarthritic animal model. **BMC Musculoskelet Disord**. ISSN 1471-2474. 12 (2011) 259. doi: 10.1186/1471-2474-12-259.

109. DIEKMAN, B. O. *et al.* - Intra-articular delivery of purified mesenchymal stem cells from C57BL/6 or MRL/MpJ superhealer mice prevents posttraumatic arthritis. **Cell Transplant**. ISSN 0963-6897. 22:8 (2013) 1395–1408. doi: 10.3727/096368912x653264.

110. HORIE, M. *et al.* - Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. **Osteoarthritis Cartilage**. ISSN 1063-4584. 20:10 (2012) 1197–1207. doi: 10.1016/j.joca.2012.06.002.

111. PAK, J. *et al.* - Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. **BMC Musculoskelet Disord**. ISSN 1471-2474. 14 (2013) 337. doi: 10.1186/1471-2474-14-337.

112. KOH, Y. G. *et al.* - Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. **Arthroscopy**. ISSN 0749-8063. 29:4 (2013) 748–755. doi: 10.1016/j.arthro.2012.11.017.

113. BUI, K. H.T. *et al* - Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study. **Biomedical**

Research and Therapy. ISSN 2198-4093. 1:1 (2014). doi: 10.7603/s40730-014-0002-9.

114. PAK, J. - Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. **J Med Case Rep**. ISSN 1752-1947. 5 (2011) 296. doi: 10.1186/1752-1947-5-296.

115. VANGSNESS C. T., Jr. *et al.* - Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study. **J Bone Joint Surg Am**. 96:2 (2014) 90–98. doi: 10.2106/jbjs.m.00058.

116. BRAUN, H. J.; GOLD, G. E. - Diagnosis of osteoarthritis: imaging. **Bone**. ISSN 1873-2763. 51:2 (2012) 278–288. doi: 10.1016/j.bone.2011.11.019.

117. CORREA, D. *et al.* - 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 - CellR4. **CellR4 2016**. ISSN 2329-7042 (2016).

118. MARLOVITS, S. *et al.* - Magnetic resonance observation of cartilage repair tissue (MOCART) for the evaluation of autologous chondrocyte transplantation: determination of interobserver variability and correlation to clinical outcome after 2 years. **Eur J Radiol**. ISSN 0720-048X. 57:1 (2006) 16–23. doi: 10.1016/j.ejrad.2005.08.007.

119. CENTENO, C. J. *et al.* - Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. **Curr Stem Cell Res Ther**. ISSN 1574-888X. 5:1 (2010) 81–93.

120. VEGA, A. *et al.* - Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells: A Randomized Controlled Trial. **Transplantation**. ISSN 0041-1337. 99:8 (2015) 1681–1690. doi: 10.1097/tp.0000000000000678.

121. MARLOVITS, S. *et al.* - Definition of pertinent parameters for the evaluation of articular cartilage repair tissue with high-resolution magnetic resonance imaging. **Eur J Radiol**. ISSN 0720-048X. 52:3 (2004) 310–319. doi: 10.1016/j.ejrad.2004.03.014.

122. EMADEDIN, M. *et al.* - Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. **Arch Iran Med**. ISSN 1029-2977. 15:7 (2012) 422–428. doi: 012157/aim.0010.

123. FODOR, P. B.; PAULSETH, S. G. - Adipose Derived Stromal Cell (ADSC) Injections for Pain Management of Osteoarthritis in the Human Knee Joint. **Aesthet Surg J**. ISSN 1090-820x. 36:2 (2016) 229–236. doi: 10.1093/asj/sjv135.

124. CENTENO, C. J. *et al.* - Efficacy and Safety of Bone Marrow Concentrate for Osteoarthritis of the Hip; Treatment Registry Results for 196 Patients. **Journal of Stem Cell Research & Therapy**. ISSN 2157-7633. (2014). doi: 10.4172/2157-7633.1000242.

125. KOH, Y. G. *et al.* - Comparative outcomes of open-wedge high tibial osteotomy with platelet-rich plasma alone or in combination with mesenchymal stem cell treatment: a prospective study. **Arthroscopy**. ISSN 0749-8063. 30:11 (2014) 1453–1460. doi: 10.1016/j.arthro.2014.05.036.

126. FEISE, R. J.; MENKE, M. J. - Functional rating index: a new valid and reliable instrument to measure the magnitude of clinical change in spinal conditions. **Spine (Phila Pa 1976)**. ISSN 0362-2436. 26:1 (2001) 78–86.

127. BRIGGS, K. K. *et al.* - Reliability, validity, and responsiveness of the Lysholm knee score and Tegner activity scale for patients with meniscal injury of the knee. **J Bone Joint Surg Am**. ISSN 0021-9355. 88:4 (2006) 698–705. doi: 10.2106/jbjs.e.00339.

128. BRIGGS, K. K. *et al.* - The reliability, validity, and responsiveness of the Lysholm score and Tegner activity scale for anterior cruciate ligament injuries of the knee: 25 years later. **Am J Sports Med**. ISSN 0363-5465. 37:5 (2009) 890–897. doi: 10.1177/0363546508330143.

129. WHITE, D. K.; WILSON, J. C.; KEYSOR, J. J. - Measures of adult general functional status: SF-36 Physical Functioning Subscale (PF-10), Health Assessment Questionnaire (HAQ), Modified Health Assessment Questionnaire (MHAQ), Katz Index of Independence in activities of daily living, Functional Independence Measure (FIM), and Osteoarthritis-Function-Computer Adaptive Test (OA-Function-CAT). **Arthritis Care Res (Hoboken)**. ISSN 2151-464x. 63 Suppl 11 (2011) S297-307. doi: 10.1002/acr.20638.

130. CENTENO, C. J. *et al.* - Partial regeneration of the human hip via autologous bone marrow nucleated cell transfer: A case study. **Pain Physician**. ISSN 1533-3159. 9:3 (2006) 253–256.

131. CENTENO, C. J. *et al.* - Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. **Med Hypotheses**. ISSN 0306-9877. 71:6 (2008) 900–908. doi: 10.1016/j.mehy.2008.06.042.

132. LEE, K. B. *et al.* - A novel, minimally-invasive technique of cartilage repair in the human knee using arthroscopic microfracture and injections of mesenchymal stem cells and hyaluronic acid--a prospective comparative study on safety and short-term efficacy.

Ann Acad Med Singapore. ISSN 0304-4602. 41:11 (2012) 511–517.

133. WONG, K. L. *et al.* - Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with 2 years' follow-up. **Arthroscopy**. ISSN 0749-8063. 29:12 (2013) 2020–2028. doi: 10.1016/j.arthro.2013.09.074.

134. CENTENO, C. J.; FREEMAN, M. D. - Percutaneous injection of autologous, culture-expanded mesenchymal stem cells into carpometacarpal hand joints: a case series with an untreated comparison group. **Wien Med Wochenschr**. ISSN 0043-5341. 164:5–6 (2014) 83–87. doi: 10.1007/s10354-013-0222-4.

135. CENTENO, C. J. - Clinical challenges and opportunities of mesenchymal stem cells in musculoskeletal medicine. **Pm r**. ISSN 1934-1482. 6:1 (2014) 70–77. doi: 10.1016/j.pmrj.2013.08.612.

136. SWART, J. F.; WULFFRAAT, N. M. - Mesenchymal stromal cells for treatment of arthritis. **Best Pract Res Clin Rheumatol**. ISSN 1521-6942. 28:4 (2014) 589–603. doi: 10.1016/j.berh.2014.10.023.

137. VARMA, H. S.; DADARYA, B.; VIDYARTHI, A. - The new avenues in the management of osteo-arthritis of knee--stem cells. **J Indian Med Assoc**. ISSN 0019-5847. 108:9 (2010) 583–585.

138. HAUSER, R. A.; ORLOFSKY, A. - Regenerative injection therapy with whole bone marrow aspirate for degenerative joint disease: a case series. **Clin Med Insights Arthritis Musculoskelet Disord**. ISSN 1179-5441. 6 (2013) 65–72. doi: 10.4137/cmamd.s10951.

139. KIM, J. D. *et al.* - Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee. **Eur J Orthop Surg Traumatol**. ISSN 1633-8065. 24:8 (2014) 1505–1511. doi: 10.1007/s00590-013-1393-9.

140. SAW, K. Y. *et al.* - Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid after arthroscopic subchondral drilling: a report of 5 cases with histology. **Arthroscopy**. ISSN 0749-8063. 27:4 (2011) 493–506. doi: 10.1016/j.arthro.2010.11.054.

141. SAW, K. Y. *et al.* - Articular cartilage regeneration with autologous peripheral blood stem cells versus hyaluronic acid: a randomized controlled trial. **Arthroscopy**. ISSN 0749-8063. 29:4 (2013) 684–694. doi: 10.1016/j.arthro.2012.12.008.

142. NCT01504464. The Effects of Intra-articular Injection of Mesenchymal Stem Cells in Knee Joint Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01504464?term=NCT01504464&rank=1 >
143. NCT01453738. Allogeneic Mesenchymal Stem Cells in Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01453738?term=NCT01453738&rank=1 >
144. NCT01227694. Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01227694?term=NCT01227694&rank=1 >
145. NCT01300598. Autologous Adipose Tissue Derived Mesenchymal Stem Cells Transplantation in Patient With Degenerative Arthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01300598?term=NCT+01300598&rank=1 >
146. NCT02118519. Mesenchymal Stem Cells in Knee Cartilage Injuries. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT02118519?term=NCT02118519&rank=1 >
147. NCT02142842. Autologous Adipose Stem Cells and Platelet Rich Plasma Therapy for Patients With Knee Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT02142842?term=NCT02142842&rank=1 >
148. NCT01931007. Use of Autologous Bone Marrow Aspirate Concentrate in Painful Knee Osteoarthritis (BMAC). [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01931007?term=NCT01931007&rank=1 >
149. NCT01873625. Transplantation of Bone Marrow Derived Mesenchymal Stem Cells in Affected Knee Osteoarthritis by Rheumatoid Arthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01873625?term=NCT01873625&rank=1 >
150. NCT01809769. Autologous Adipose Tissue Derived Mesenchymal Stem Cells Therapy for Patients With Knee Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01809769?term=NCT01809769&rank=1 >
151. NCT01448434. Allogeneic Mesenchymal Stem Cells for Osteoarthritis.

[Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01448434?term=NCT01448434&rank=1 >

152. NCT01601951. Outcomes Data of Bone Marrow Stem Cells to Treat Hip and Knee Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01601951 >

153. NCT01485198. Autologous Stem Cells in Osteoarthritis. [Online][Accessed May 13, 2016] Available from WWW:<URL: https://clinicaltrials.gov/ct2/show/NCT01485198?term=NCT01485198&rank=1 >