Treatment of Knee Osteoarthritis with Autologous Expanded Bone Marrow Mesenchymal Stem Cells: 50 Cases Clinical and MRI Results at One Year Follow-Up

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Abstract
Knee osteoarthritis is one of the most prevalent joint diseases, causing pain, function loss and disability, leading to a progressive cartilage degeneration induced by biochemical changes in its composition. Current available treatments focus on addressing symptoms and joint replacement is the last treatment option.

Advanced therapies with mesenchymal stem cells build new expectations to improve the results of OA treatments. MSC applied in animal models, show encouraging results in modulating inflammation and joint cartilage repair. Several studies applied autologous mesenchymal stem cells to treat knee osteoarthritis in humans by means of an intra-articular injection.

Our team previously conducted a pilot study applying $4\times10^6$ autologous bone marrow expanded mesenchymal cells in 12 patients affected with knee osteoarthritis through intra-articular infusion. After 2 years we obtained excellent clinical and quantitative MRI outcome measures, no adverse events reported.

Keywords: Knee osteoarthritis; Autologous expanded mesenchymal cells; Stem cells; Regenerative therapy

Introduction
Osteoarthritis (OA) is the most prevalent chronic joint disease and a frequent cause of joint pain, loss of function, and disability [1]. Knee is one of most affected joints. The pathogenesis of knee OA has been linked to biomechanical and biochemical changes in joint cartilage, e.g. inability to withstand normal mechanical stresses, limited nutrients and oxygen supply, inadequate synthesis of extracellular matrix components, increased synthesis of proteinases and overall apoptosis of chondrocytes [2-5]. Synovial inflammation is a response of synovial macrophages to cartilage debris and catabolic mediators entering the synovial cavity and limits knee cartilage repair [6].

Current treatments for knee OA achieve poor clinical results and fail to modify cartilage. Joint replacement is the last treatment option, bearing enormous effort and expenses [7,8]. Its high prevalence and social impact have promoted the development of many therapeutic options to try to stop or slow down its progression. Diagnosis of OA is typically made late in the disease process and there are no disease modifying osteoarthritis drug (DMOADs) currently available for treatment. Mesenchymal Stromal Cells (MSC) opened new therapeutic perspectives provided their regenerative potential and ability to modulate inflammation [9-12].

MSC were first identified by Friedenstein, and have intrinsic characteristics defined by the International Society for Stem cell Therapy: (a) remain plastic-adherent under standard culture conditions; (b) express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR; (c) differentiate into osteoblasts, adipocytes, and chondrocytes in vitro [13]. These cells remain in lethargic status G0, and may be obtained from bone marrow, periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle and deciduous teeth [14,15]. Regardless of their origin they have the capacity to differentiate in vitro into different cell types of connective tissue lineages as bone, fat, muscle and cartilage. Many studies show co-cultured MSC to induce chondrocyte proliferation and extracellular matrix protein synthesis, including aggrecan and type II collagen [16,17]. The capacity to differentiate into cells of the chondrogenic lineage and produce extracellular matrix together with their proven anti-inflammatory potential brought to focus MSC as a potential treatment for osteoarthritis [18].

MSC effects in chondrogenic repair have been documented in mice, rabbits, pigs, sheep, and horses [19-21]. These studies report a dose-dependent effect, which requires a lingering time to be apparent. We published a feasibility and safety study in horse and ovine model, with intra-articular infusion of $4\times10^6$ autologous expanded bone marrow MSC (BM-MSC) with no local or systemic pathologic alterations seen in necropsy after 6 months, and showing clear regenerative findings [22].

These encouraging results in animal model allowed us to attempt translating the procedure to human therapy, with added difficulties when it comes to objectively assess the effect in the whole joint surface. Whilst biopsy is possible, it bears an invasive surgical intervention and limits the analysis to a restricted area of the cartilage.

These limitations of the available treatment tools triggered the search of specific biomarkers for assessing changes in articular

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cartilage. The FDA (Federal Drug Administration) together with the
OARSI (Osteoarthritis Research Society International) established
the OARSI–FDA Biomarkers Working Group. They published a
comprehensive report encompassing the role of biomarkers in the
course of clinical trials that charts the course for future research of
new therapies and disease modifying drugs for OA [23,24]. Research
focuses in the structural components of extracellular matrix, especially
type II collagen degradation markers [25].

The most validated MRI image biomarker is T2 mapping. It
correlates with the collagen structure and the water interaction with the
extracellular matrix. It has been suggested that 60% of T2 variation is
due to collagen fibres orientation and organization and the rest could be
secondary to water content and other macromolecules [26,27].
Although T2 mapping relation to collagen structure is well known,
recent studies suggest that it is also sensible to PG content, since the
GAG negative changes affect the water protons interaction [28,29].

Small changes in collagen hydration and organization lead to cartilage
degradation and OA early findings may be evident through T2 mapping
before any standard procedure such as radiologic tests [30-32].

Both animal studies and human clinical trials report increased T2
values in OA patients, thus validating T2 values as hyaline cartilage
structure marker for reparative procedures.

Quantitative MRI has meant an important step in OA research
and became an essential tool in epidemiologic investigation and
development of therapies attempting to modify cartilage structure,
allowing us to noninvasively assess cartilage and rendering consistent
outcome measures, preventing the need to perform an arthroscopy or
a biopsy [33-35].

Previous studies show that patients with OA have a mean cartilage
volume decrease of 4% - 6% per year [33]. Studies from the OA1
(Osteoarthritis Initiative) stated that T2 values increased at 3 year
follow-up in patients with OA risk factors and established OA [36]. Our
findings suggest that cell therapy may revert this tendency.

Clinical application of MSC in humans is limited. The European
Union (EU) Regulation on advanced therapies considered expanded
MSCa medicinal product [37-39]. Therefore, prior to clinical application
it is mandatory to conduct a clinical trial in order to prove the potential
benefits and the absence of side effects of the investigational new drug.

We reported satisfactory clinical results in a preliminary study
applying expanded MSC in degenerative disc disease, with no adverse
side effects [40]. Afterwards we published the outcomes of a pilot study
for knee OA treated with autologous expanded MSC (EudraCT 2009-
017405-11 and NCT01183728). Twelve patients were treated by means of
intra-articular infusion of 40 × 10^6 autologous expanded MSC. Statistically
significant changes were observed in cartilage quality, assessed by means of MRI T2 mapping. We also reported excellent
results according to pain (VAS) algofunctional and disability tests
(Lequesne and WOMAC). No adverse side effects were described
[22,41]. Similar results were obtained in a multicentre clinical trial
applying allogenic MSC with HA as control [42].

The European Regulation (EC)No1394/2007 and (EC)No 668/2009,
as well as the Spanish Regulation RD 477/2014 which set the regulatory
framework for advanced therapy, allows the research team, once proven
the viability, security and efficacy, to carry on with the treatment under
the supervision of the Spanish Medicines Agency (AEMPS).

We present the results at 12 months of the first 50 patients treated
after the previous clinical trial, following the same procedure described in
the pilot study [22].

Materials and Methods

The therapeutic procedure started in Centro Médico Teknon in
Barcelona, where under sedation and local anaesthetics 100 mL
of bone marrow were collected from the iliac crest. The product
obtained was shipped to "Instituto de Biología y Genética Molecular"
(IBGM), University of Valladolid, for selection and culture under GMP
regulation (The distance between Barcelona and Valladolid is 800 Km).

The number of mononuclear cells obtained was 1,13 ± 0,21×10^6;
expansion time 22 ± 1 days; number of MSCs 40 ± 1×10^6 suspended in
Ringer-lactate and Albumina at 5×10^6 cells/mL; cells viability 91 ± 6%.

After 7 to 10 days in culture, cells became relatively homogeneous
and demonstrated a fibroblastic appearance when approaching
confluence. In this gap cells went out from G0 status, and came to
replication period. This morphology remained unchanged until use
[22].

The product is presented in 8 mL suspension and transported by
plane to Barcelona, where it is applied by intra-articular infusion in the
operating theatre. The time interval between cell release and infusion
is less than six hours. The average cell viability in a Neubauer camera
measured after application was consistently above 85%.

Clinical evaluation was assessed through physical examination and
the validated VAS, Lequesne and WOMAC indices, performed prior to
the treatment and at 6 and 12 months.

Cartilage was assessed by T2 mapping average values (ms). 88
regions of interest (ROIs) were well defined, including patellar cartilage
(24 ROIs), femoral condyles (32 ROIs) and tibial plateau (32 ROIs).

T2 relaxation times were averaged for each area, those greater than
50 ms were considered to calculate the Poor Cartilage Index (PCI),
expressed as the percentage of all values greater than 50 ms obtained
from the 88 ROIs. Values above 99 ms were dismissed for the statistical
analysis.

Image

T2 measurements are not absolute and values are MR System
dependent, thus the characteristics of the device should be specified
when analysing the results. Interpretation of T2 values is challenging
due to the range of acquisition parameters and analysis methods used.
It is thus important to understand the variables, including MR system
components that may influence T2 values.

This technique requires a multichannel coil with a min. 1.5 T
scan able to perform a T2 mapping. Sequences for global cartilage
assessment and qualitative measures of the cartilage morphology have
been validated both in vitro and in vivo and are available for clinical use
through 1.5T and 3T scanners [32,43-48].

For this study, images were acquired on 1.5T MR systems (General
Electric) using a 4 channel knee coil with 40 mT/m gradients. We
followed the standard knee protocol including sagittal T1, axial T2,
sagittal eco gradient and coronal T2 density sequences. We added a
sagittal multi-slice, multi-echo spin echo (MSME-SE) acquisition for
T2 relaxation time measurement in the axial plane for the study of the
femoro-patelar joint and in the sagittal plane for the study of the
femoro-tibial joint.
Evolution of pain and disability

Table 1 summarizes the distribution of knee pain and disability indexes throughout the observation period. The starting point was quite homogeneous in the cohort, with mean values of 57 for the Visual Analogue Scale (VAS) and 10.9 for the Lequesne Index. The Lequesne score is one of the most used outcome measures for knee OA. It was created to assess pain and function in patients with knee and hip OA, in order to aid the orthopaedic surgeon when indicating prophylaxis [49]. It comprises 10 questions addressing pain, stiffness and function. Scores above 10 support prophylaxis indication. We used the Lequesne score before treatment and after cell infusion (6 months, 12 months). At 12 months the score decreased far below prophylaxis indication, because bad and very bad values decreased to moderate values (Figure 1).

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a measurement system for knee OA assessing pain associated to daily activities; VAS-SP, Visual Analogue Scale for pain associated to sports activities; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

<table>
<thead>
<tr>
<th>Test</th>
<th>time</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
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</thead>
<tbody>
<tr>
<td>Knee pain VAS -DA (0-100)</td>
<td>0</td>
<td>50</td>
<td>57</td>
<td>22</td>
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<tr>
<td></td>
<td>12 months</td>
<td>50</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Knee pain VAS -Sp (0-100)</td>
<td>0</td>
<td>32</td>
<td>74</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>32</td>
<td>28</td>
<td>22</td>
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<tr>
<td>WOMAC (0-100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pain subscale</td>
<td></td>
<td>50</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>50</td>
<td>3</td>
<td>2.5</td>
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<tr>
<td>Rigidty subscale</td>
<td></td>
<td>50</td>
<td>2.2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>50</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Function loss subscale</td>
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<td>50</td>
<td>18</td>
<td>8.8</td>
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<tr>
<td></td>
<td>12 months</td>
<td>50</td>
<td>10</td>
<td>7.8</td>
</tr>
<tr>
<td>Total WOMAC scale</td>
<td></td>
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<td>11.9</td>
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<td></td>
<td>12 months</td>
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<tr>
<td>Lequesne (0-100)</td>
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<td>10.9</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>50</td>
<td>5.2</td>
<td>4.1</td>
</tr>
</tbody>
</table>

In all cases, the scale was from 0 to 100%. Measurements were performed before cell transplantation (0) and 12 months afterwards.; VAS-DA, Visual Analogue Scale for pain associated to daily activities; VAS-SP, Visual Analogue Scale for pain associated to sports activities; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Table 1: Total score sum of VAS, WOMAC, and Lequesne severity indices.

![Figure 1](image.png)

**Figure 1:** (A) Graph showing evolution of knee pain, as measured by VAS (VAS-DA), over time. Mean ± standard error (SE) values of 50 patients treated with MSC. **p<0.01 (0-6 months); **p>0.001 (0-12 months) (ANOVA; Bonferroni test for paired values) (B) Graph showing evolution of knee pain associated to sports activity, as measured by VAS SPORT over time. Mean ± standard error (SE) values of 32 patients treated with MSC. Data from 18 patients were not included because they did not practice sports. p<0.001 (ANOVA; Bonferroni test for paired values).
painless (5 questions, 0-20 points), stiffness (2 questions, 0-8 points) and joint functionality (17 questions, 0-68 points) [50]. The initial mean value was 27.5, with pain dominating over rigidity and function loss. Compared with the basal pain level, improvement was statistically significant at 12 months (Table 1).

The pattern of 1-year improvement was parallel for VAS, WOMAC, and Lequesne indices and resulted in the displacement of the whole distribution toward smaller values, with a strong decrease of median values. Pain relief during sports performance, followed systematically in 32 patients, was even greater (74 to 28 - 63%) (Table 1).

All patients were satisfied with the treatment, and 43 out of 50 (86%) patients reported lasting pain relief greater than 45% throughout 1-year observation period. The median pain reduction was 60% for daily activities and 63% for sport activities. Figure 1 shows knee pain relief at the 1-year follow up assessed by VAS, as a function of the initial pain score. A good positive correlation was observed between the amount of improvement and the initial score ($r=0.55$), indicating that MSC treatment had a clear pain-relieving effect ($P<0.001$). The slope of the line was 0.52 (Figures 2 and 3).

**Cartilage MRI**

Magnetic resonance imaging (MRI) quantitative T2 mapping was used to assess cartilage quality. T2 relaxation time is sensitive to both changes in cartilage hydration and collagen fibril orientation [51,52].

T2 relaxation time is longer in inflammatory tissue versus hyaline cartilage [53] and increases in osteoarthritis [51]. Consistent with previous results in healthy knee [51,52], the mean TSD T2 value was 37.0 ± 6.8 ms. Because 95% of values should be smaller than (mean ± SD), 50 was chosen as the threshold above which T2 values were considered inordinately high. To quantify T2 mapping, a Poor Cartilage Index (PCI) was estimated as the percentage of T2 values larger than 50 ms. A PCI of 100 is the worst possible value, and a value near 5 is considered healthy. The mean PCI significantly decreased from 25 to 5 at 12 months after injection (Figure 4). The PCI decreased in 37 of 50 patients (74%), 10 remained the same (20%) and 3 worsened between 7%-10% (6%). Additionally, when PCI improvement was plotted against the initial PCI, a positive correlation ($r=0.38$; $P=0.044$) was noted (Figure 5).

**Discussion**

We believe that the outcomes obtained support the research path and suggest expanded MSC at a given dose may play an important role in the therapeutic approach of OA.

Cell expansion is a key factor. Cell quality and dose are critical for the healing potential of cellular therapy. The average MSC content of healthy young human BM is about 100 MSC/million MNC, yielding a few thousands (1500-3000) MSC/ml of bone marrow. Furthermore, research on stem-cell transplantation suggests that the results largely
situation has never been reported in any cell therapy study: neoplastic
produced tumors when injected into immunodeficient mice. This
although MSC lack tumoral epigenome, so they cannot lead to
achieve the best outcomes. Regarding the cell source we know that,
autologous MSC improved function and pain as well as cartilage defects
patients in 3 groups, administrating 10×10^6 and 100×10^6 MSC. The outcomes showed intra-articular knee injection of 100×10^6
received hyaluronic acid 3 weeks after surgery, whereas the control group
articular injection of cultured MSC (from bone marrow) with
OA knees and genu varum: the cell-recipient group received intra-
yield of several millions MSC in a few weeks [54].

Other authors have published studies applying cultured MSC, but using different doses and cell sources.

Wong et al. treated randomly 56 patients with unicompartimental OA knees and genu varum: the cell-recipient group received intra-articular injection of cultured MSC (from bone marrow) with hyaluronic acid 3 weeks after surgery; whereas the control group received hyaluronic acid alone. They described clinical and MRI improvement in the cell-recipient group [55].

Jo et al. treated 18 patients with intra-articular injection of autologous adipose tissue derived MSC for knee OA. They divided patients in 3 groups, administrating 10×10^6, 50×10^6 and 100×10^6 MSC. The outcomes showed intra-articular knee injection of 100×10^6 autologous MSC improved function and pain as well as cartilage defects by regeneration of hyaline-like articular cartilage [56].

Future studies will have to determine the most accurate dose to achieve the best outcomes. Regarding the cell source we know that, at least in vitro, adipose tissue MSC behaviour is different from Bone Marrow MSC, which generate cartilage lineage cells when cultured in TGF-β enriched medium. This is to be taken into account when attempting to regenerate joint cartilage.

Nevertheless, methods involving cell culture under GMP may be considered safe and show clear regenerative findings [57].

There has been concern about MSC inducing neoplasm formation, although MSC lack tumoral epigenome, so they cannot lead to neoplasm formation or transformation of adjacent cells. These cells produced tumors when injected into immunodeficient mice. This situation has never been reported in any cell therapy study: neoplastic transformation of MSC has the same prevalence as the rest of human cells [58].

It seems appropriate to comment that cell products obtained after aspiration and simple centrifugation, which contain a low and undetermined amount of MSC in G0 phase, are being applied as “stem cells” [59-63]. Selection and culture imply supporting MSC with nutrients in a controlled medium. This situation is quite different from the administration of a low and indeterminate number of MSC mixed with other cells which will be disputing for oxygen and nutrients. Moreover, cell products obtained after direct centrifugation contain maximum 5% of MSC, but we shall not forget the unknown effects of the remaining 95%.

Our team, together with other authors, consider important to adapt the nomenclature to the product features of the so called “one-step procedures” [62]. The product obtained from simple aspiration and centrifugation, without culture or cell characterization should not be considered MSC and should not be compared to procedures such as the treatment we herein present.

The availability of non-invasive diagnostic techniques as T2 mapping MRI allows us to accurately determine the grade of disorganization of the extracellular matrix. There is evidence that cell therapy may lead to cartilage reorganization, until recently thought impossible.

Cartilage T2 relaxation time measurements may be a sensitive biomarker for monitoring cartilage quality in subjects with knee OA, since they offer interesting insights in the condition of cartilage matrix beyond morphologically detectable focal knee lesions.

When assessing the outcomes, it becomes important to differentiate weight-bearing cartilage areas. Knee articular cartilage T2 values can vary with plate and coil, with the lateral femoral condyle having the longest value (52 ms), and the lateral tibial plateau having the shortest value (40.6 ms) [64].

In order to accurately evaluate cartilage, in would be necessary to perform a zonal assessment. Normal hyaline cartilage shows an increase of T2 values from deep to superficial cartilage. Deeper zones with dense collagen mesh and greater PG content have shorter T2 values than the transitional zone with greater water content and thus greater T2 values [18].

We assess T2 values for the whole cartilage, assuming a significant difference is enough to establish the tendency towards cartilage organization or degeneration.

The main goal of this therapeutic approach is the osteoarthritic cartilage, which is a diffuse deterioration of different joint areas, not a focal injury. We are approaching the joint as a complex organ where other components (as the synovial membrane) play an important role in normal function. MSC’s anti-inflamatory effect is thus a key factor [65].

Injuries in other knee structures such as the meniscus might be treated with the same procedure. Vangsness et al. published a randomized, double-blind, controlled study showing meniscus regeneration following partial meniscectomy in 55 patients after intra-articular knee injection of 50×10^6 and 150×10^6 MSC compared with control (intra-articular injection of hyaluronic acid). They concluded that patients treated with MSC achieved a significant reduction in pain and partial meniscus regeneration. Our team conducted a study in sheep model achieving complete meniscus regeneration (meniscal free margin injury) [22].

New studies assessing different cell doses and carriers to enhance cell viability and efficacy are indeed necessary, but in the meantime,
and focusing on the outcomes obtained so far, we believe the term “regeneration” should be accepted since the internal structure of cartilage is being modified.

Conclusions

The intra-articular infusion of a dose of 40×10^6 expanded BM-MSC, suspended in 8 mL of Ringer-lactate and Albumina at 5×10^6 cells/mL, has no local or systemic adverse effects, may significantly improve symptoms derived from joint inflammation in a short period of time and improve OA cartilage organization assessed by means of T2 mapping MRI.

Future studies are necessary to assess this improvement over time and to optimize results by modifying variables such as dose or medium composition.

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References

17. Wu L, Prins HJ, Helder MN (2012) Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. Tissue Eng Part A 18: 1542. [PubMed]


