Mesenchymal Stem cells (MSCs) in lumbar spine surgery: 
a single institution experience about red bone marrow vs fat tissue 
derived MSCs.  
Clinico-radiological remarks on a consecutive series of 22 patients

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Abstract

**Study Design:** Mesenchymal stem cells (MSCs) are undifferentiated, multipotent cells, which have the ability to self-renew and differentiate into many tissue types. MSCs have shown several therapeutic applications in different medical fields and could represent a successful treatment of degenerative disc disease (DDD).

**Objective:** Several studies have demonstrated, ex vivo or in vivo over animal models, the chondrogenic and osteoblastic MSCs differentiation efficacy in spine surgery for DDD and spine fusion. The authors aim to demonstrate the efficacy of MSCs in humans.

**Summary of Background Data:** 22 consecutive patients, who suffered of spine DDD, were submitted: in 11 cases the MSCs were harvested from red bone marrow, 11 from fat tissue.

**Methods:** The red bone marrow withdrawal was performed from the vertebral bodies, and processed by a fully automated, mobile, closed capability system. The fat tissue withdrawal was acted from the subcutaneous adipose tissue, at skin incision, and processed through a microfluidic fractioning procedure, without enzymatic digestion. MSCs were implanted in the central part of the nucleus pulposus of the DDD or added to bone chips to accelerate posterolateral arthrodesis.

**Results:** All the 14 posterolateral fusions and MSCs implantations showed at three months a complete bone bridge at the CT scans, stable at follow-up. The one intersomatic implantation gained a complete interbody fusion after 1 month; while 80% black discs treated with MSCs presented a new T2-W hyperintensity at postoperative MRI. The mean VAS pain score improved from 70±20 to 10±5 at 12 months, as the ODI score from 70±5% to 20±10.

**Conclusions:** There are several questions that need to be answered but MCSs look promising in lumbar spine surgery, both to block the aging of the disc both to accelerate the fusion processes in arthrodesis.
**Introduction**

MSCs are undifferentiated, multipotent cells, who have the ability to self-renew and differentiate into many tissue types as mesoderm, endoderm and ectoderm tissue (1). Bone marrow (1), periosteum (2), synovial membrane (3), and adipose tissue (4) represent the most important MSCs sources in adults. MSCs have peculiar membrane surface markers such as (CD29, CD44, CD105, CD73, CD90) (5) and can be easily cultured, showing an high ex vivo expansive potential, with robust and persistent engraftment (6, 7). Due to their characteristics, MSCs have shown several therapeutic applications in different fields of the medicine, including regenerating infracted myocardium (8, 9), improving functional recovery from ischemic stroke (10), and rescuing liver failure (11).

The main targets of MSCs use in spine surgery could be represented both by the treatment of degenerative disc disease (DDD) both by the aid and enhancement of fusion processes, which represent two of the greatest challenges of each spine surgeon.

From a biomechanical point of view, disc degeneration can be described as a decrease in water content associated with proteoglycans diminution of the nucleus pulposus and inner annulus. This results in flattening of the disc with loss of proper stability - mobility and eventually destruction of the annular structure (12, 13). From a radiological point of view, the aging and degeneration of the disc is visible at T2 weighted images of MRI, with the so called “black disc”, due to hypointensity because of the loss of capacity of binding water (14).

The DDD’s clinical part of the coin is mainly represented by low back pain (LBP), which is highly widespread and debilitating pathology. Over a lifetime, 70% to 80% of people will at some time experience back pain (15), which is second only to headache as frequent source of pain (16), and represents the second most common reason for sick leave, behind only the common cold (17-19). During each year, 8% of the entire working population will be disabled by LBP (20), resulting also in significant economic losses (21, 22). LBP is considered the leading cause of time lost from work in industrialized countries (16).
Dealing with spine fusion, there are more than 185,000 spinal arthrodesis procedures performed in the USA each year (23) and fusion rates are variable (24,25), because of factors inherent to the spine, contributing to failure of the fusion process including tensile forces, low parent bone surface, and interference by surrounding musculature (26). Concomitant patient conditions such as advanced age and osteoporosis as well as corticosteroid, tobacco, and nonsteroidal anti-inflammatory drug use also interfere with successful spinal fusion (27). Therefore, methods to facilitate the process of spinal fusion will significantly improve patient outcome despite potential complicating factors.

Several studies have demonstrated how MSCs can be very helpful both in the DDD treatment both accelerating the fusion processes after spine surgery, having a chondrogenic and osteoblastic differentiation. (28-36). The chondrogenic differentiation leads to disc cells as well as chondrocytes, which are able to produce collagen type II and proteoglycans, which slow the degeneration of the disc, regenerating the matrix (37-50). Otherwise, the osteoblastic differentiation gives rise to osteoblast cells, who have the important role in bone matrix formation (51-53). The greatest part of these studies have been performed ex vivo or in vivo over animal models.

The authors report their experience about DDD treatment and acceleration of spine fusion with the use MSCs, over a series of 22 consecutive patients, of whom 11 were intraoperatively prelevated from red bone marrow, 11 from fat tissue.

**Patients and Methods**

22 patients underwent spine surgery, with intraoperative MSCs withdrawn and implantation, at the Policlinico Umberto I, Departement of Neurological Sciences – Neurosurgery, University of Rome “Sapienza”, from January to November 2013.

All patients were in detail informed about the purpose of the MSCs utilization and preoperatively signed a detailed informed consent.
In each patient MSCs were intraoperatively withdrawn, from red bone marrow (11 cases) or from fat tissue (11 cases), and implanted in the same patient, during the same surgical procedure (TABLE 1).

The red bone marrow withdrawal was performed from the vertebral bodies, through all the holes of the pedicle’s screws, with a specific syringe and needle 13 G, till the achievement of about 30 cc. Subsequently the blood was processed by a fully automated, mobile, closed capability system, with single use kits, in a completely sterile way, which allowed separation of cellular products (Sepax 2®- Omniaed – Biosafe SA, Eysins, CH), through centrifugation process (FIGURE).

The fat tissue withdrawal was acted from the adipose tissue in the subcutaneous space, at the level of the skin incision, by the use of a specific liposuction cannula (type microharvesting 15 G multihole connected to the vacuum 60 mL syringe, included in the package of the device MyStem® (MyStem LLC, Wilmington, DE, US) till the achievement of 20 cc. Thereafter the lipoaspirate syringe was connected to the top connector of the single use sterile device, and its content injected (FIGURE).

The MyStem device allows a non-enzymatic perivascular regenerative fractioning of cells: the filtration-based cell collection is achieved by direct culture of mechanically dissociated fragments of adipose tissue, obtained by lipoaspiration, avoiding centrifugation of the sample to separate the different components.

The device is able to separate from the adipose tissue stromal vascular fraction of adipose tissue liquid fraction, without the need for enzymatic digestion, through a microfluidic fractioning process. Then an empty 10 ml syringe was connected to the bottom connector and sucked the liquid fraction contained in the bag until the filling of the syringe. It is mandatory that the liquid fraction may end prior to the filling of the syringe. Finally, the syringe was disconnected with the stromal fraction ready for use.

Gained MSCs were utilized in two different ways: in the first one they were implanted in the central part of the nucleus pulposus of the lumbar intervertebral disc, about 1cc, though a sterile needle for
lumbar puncture, in order to prevent the aging of the disc. This happened for those patients who had surgery for herniectomy or segmental spinal stenosis, with evidence of a black disc at MRI examination.

In the second one they were added to bone chips, both autogenous, derived from decompressive laminectomy, both heterologous cancellous collagenated bone (Tecnoss® Chips, Tecnoss s.r.l., Torino, Italy) for the posterolateral arthrodesis after pedicle screws and rods positioning, in order to accelerate spine fusion.

Preoperatively, all patients were subjected to careful medical and neuroclinical case history review, objective neurological examination and underwent X-rays, CT and MRI scan. VAS (Visual Analogue Scale) (54) pain values and ODI (Oswestry Disability Index) questionnaire were also assessed for an accurate neurological examination (55,56) both preoperatively and postoperatively during the follow-up Postoperatively, in those where was necessary to check the evolution of the DDD, MRI examinations were performed at 3,6,12 months, while those patients, in whom the target was to value the evolution of the fusion, underwent X-rays at 1 month and CT scan early postoperatively, at 1 month, 3 and 6 months. All patients underwent clinical follow-up at 1 month, 3,6, 12 months.

In the postoperative assessment of the DDD, it was paid attention to the surgically treated disc potential rehydratation, at the MRI examination, with hyperintensity signal at T2 weighted images. In the postoperative evaluation of the posterolateral fusion, bone bridges were carefully evaluated at CT scans.

**Results**

There were 12 females and 10 patients (F/M ratio 1.2/1). The mean age was 57.4 years, ranging from 37 to 66 years.

The 11 patients, in whom MSCs were taken from red bone marrow, underwent surgery for lumbar degenerative or spondylolytic spondylolisthesis and lumbar canal stenosis, treated by decompressive laminectomy and posterolateral fusion with pedicle screws and rods.
Among the 11 patients, with MSCs taken from fat tissue, 3 with lumbar spondylolisthesis were operated for gaining posterolateral fusion with pedicle screws and rods. In the remnant 8 MSCs were used to achieve the blocking of the DDD and therefore were implanted in the disc: 5 of them, affected by lumbar canal segmental stenosis, underwent dynamic stabilization with UniWallis® (Zimmer Spine, Zimmer Inc., Warsaw, IN) dynamic interspinous blocking device and bilateral interhemilaminectomy with undercutting technique, 3 herniectomy through microdiscectomy.

In 3 patients who underwent surgery for lumbar spondylolisthesis with pedicle screws and rods, MSCs were implanted in the intersomatic space. 2 had the MSCs implanted in a black disc, in order to see if it was possible to block the aging of the disc, one of them, who had an advanced spinal degeneration, developed till a quite proximal bony contact (FIGURE), in the intersomatic space, with the purpose to point out if it was possible to accelerate the intersomatic fusion process.

The postoperative hospitalization had an average value of three days, ranging from 2 to 4 days. No intraoperative or postoperative complications were detected.

All the 14 patients treated with posterolateral fusion showed at three months a complete bone bridge at the CT scans, stable at 1 year of follow-up (FIGURE).

The intersomatic implantation gained a complete interbody fusion after 1 month (FIGURE), while among the remaining 10 cases in whom MSCs were implanted in the black disc, 8 (80%) presented a new hyperintensity at postoperative MRI in the T2 weighted images (FIGURE), stable at 1 year.

Preoperatively most patients (80%) made frequent use of analgesics, the mean VAS pain score was 70±20, the ODI was 70±5%. In the follow-up neuroclinical examinations an improvement was found in both the VAS (10±5 at 12 months), ODI (20±10), as well as the use of analgesics.

The mean time in order to obtain MSCs withdrawal from red bone marrow was of 20 ±5 minutes, due to the times needed for centrifugation, while, from adipose tissue, the procedure was shorter, lasting 10±5 minutes.
Discussion

In 1982, Kirkaldy-Willis and Farfan proposed three biomechanical stages underpinning spinal degeneration: Dysfunction, Instability and Stabilisation (57). The term Degenerative Spinal Pathology includes prolapsed disc degeneration and disc bulging, osteoarthritis of the facet joints and degeneration of the ligamentum flavum, with consequent hypertrophy of the above structures, and muscular deterioration.

Regenerative medicine aims for the replacement, regeneration and remodeling of tissue or the functional enhancement of impaired tissues.

The intervertebral disc is the most important component in determining the segmental stability of the spine, as it bears the heaviest load. Its degeneration (DDD) is characterised both by an alteration in the glycoproteins and glycosaminoglycans with accumulation of lactates, resulting in constant dehydration of the disc and evolution towards a black disc, and a true mechanical distortion, generally morphologically reflected by a large, bulging protrusion, the disappearance of a distinct limit between the nucleus and the annulus and cracking of the disc (58-60). In fact, in the discs of healthy individuals, we observe a constant balance between catabolic and anabolic activity, maintaining an excellent extra-cellular matrix structure. Alteration of this balance leads to the beginning of a degenerative cascade that includes apoptotic mechanisms supported by inflammation with macrophage infiltration, the presence of a network of inflammatory cytokines Il-1a, Il-1b, Il-6, TNF-alfa (58-60) and the production of catabolic enzymes such as matrix metalloproteinases (MMPs) (61,62).

From the clinical point of view, DDD is strongly associated with LBP (63,64), which is highly widespread and costly, representing the fifth most common reason for physician visits, constituting approximately 2.3% of all appointments (65-66).

Current treatment options for DDD comprise either conservative approach with physiotherapy and pain management or invasive surgical procedures like vertebral interbody fusion or spinal arthroplasty (67). Nevertheless, the expanding comprehension of processes involved in DDD and
disc repair, however, presents the possibility of developing strategies for restoring disc tissues, the so called regenerative medicine. One of the cornerstones of regenerative medicine is based on cell therapy, such as implantation of mesenchymal stem cells into the intervertebral disc.

Stem cells are defined as unspecialized cells capable of long-term self-renewal and differentiation into more specialized cells. Cells capable of producing mesenchymal tissues are referred to as mesenchymal stem cells (MSCs) and are capable to differentiate to adipocytic, osteoblastic and chondrocytic lineages under appropriate conditions (1). MSCs have been isolated from almost every organ in adulthood (68), but actually, the two main sources are represented by the red bone marrow and adipose tissue (1, 69-72). Chronologically the first source of MSCs is represented by red bone marrow, in which 1 per $10^5$ nucleated cells is a MSC (73). MSCs derived from adipose tissue were firstly identified by Zuk et al. in 2001 (71,72) as a source of adult MSCs and the incidence in adipose tissue is estimated to be about 1 per $10^3$ nucleated cells (74), which is two magnitudes higher than the number of MSCs in bone marrow.

Initially we started our surgical experience with MSCs derived from the red bone marrow. At the beginning, it was recommended to act the withdrawal from the iliac crest, but when we proposed this procedure to the patients, although they were very interested to the MSCs use, they refused. Iliac crest biopsy had no appeal for a patient who was hospitalized for undergoing lumbar spine surgery because of donor site pain, surgical unaesthetism and also for the fear of another surgical procedure. Obviously also the vertebral body has red bone marrow, therefore we had the idea to easily withdraw the red bone marrow from the vertebrae during spine surgery, when we make the holes for pedicle screws placement. In this way, the withdrawal was performed in the same time of the spine surgery, with no other surgical fields. The only recommendation, according to our experience, is to take the red bone marrow from all the holes and not from only one, just to avoid the shortage of MSCs. Nevertheless, not all spine surgery is based on screws positioning, so we had the challenge to find the way to have MSCs for patients, in whom there was not surgery, which allowed direct approach to vertebral body.
Therefore, we started to use MSCs taken from adipose tissue. The most important features of adipose tissue as a MSC source are the relative expendability and easy accessibility. Adipose tissue can be obtained in substantial quantities with minimal risk, as liposuction is a common procedure to obtain adipose tissue with zero reported deaths on 66,570 procedures and a serious adverse event rate of 0.68 per 1000 cases (75). Adipose tissue is also accessible at most sites used for a surgical procedure, neutralizing the need for a separate harvest site and its concomitant morbidity. Moreover, it is 100 times richer of MSCs than red bone marrow (1 per 10^3 nucleated cells, 1 per 10^5 nucleated cells respectively). Thus, MSCs are the most important source of stem cells for tissue engineering, in one-step procedure for tissue regeneration. The particularly innovative aspect of MyStem® device is the filtration-based cell collection, which avoids centrifugation of the sample to separate the different components. The filtration is unanimously recognized as safe and efficient method for the separation of cells from a liquid suspension. Among the methods of bio-separation, in fact, filtration has several advantages: it ensures the treatment of the biological sample within a closed system, limiting the risks of contamination and greatly reducing the machining time; it allows careful selection of the cellular component based on dimensions, thanks to the presence of meshes of a size comparable to the cell diameter, obtaining a sample with cellularity homogeneous and free of subcellular fractions and unwanted debris; the sample is separated and concentrated within the same system, allowing to obtain a final product enriched in autologous multipotent somatic perivascular progenitor cells ready for use; and it considerable reduces processing times. Therefore, to date, we prefer to use fat tissue as MSCs origin.

The high rate of disc rehydratation (80%) stable at 1 year of follow up shows how MSCs implant is effective and easily feasible in a short time with no complications.

Dealing with the spine, the focus is not only on disc repair, but also to aid arthrodesis and accelerate times of ossification which nowadays are 3 months for a partial bone bridging in the posterolateral ossification, and 1 year for a complete ossification (76). Infact mechanisms to
Accelerate the fusion process will decrease patient morbidity and contribute to improved outcomes. MSCs have been successfully applied in animal models of long bone fracture and calvarial defect healing (77-81), and the enhancing properties on bone fusion has been detected also in posterior lumbar arthrodesis in rat model (82), where MSCs on a biocompatible scaffold resulted in superior callus maturation and remodeling 8 weeks after implantation compared to scaffold alone or no treatment in a rat model of lumbar fusion. According to our experience we had a good posterolateral arthrodesis in 3 months, which represents a shorter time than that reported in international literature. Although MSCs show multiple-lineage differentiation potentials (i.e. adipogenic, myogenic, chondrogenic, osteogenic, endothelial, cardiomyogenic and potentially neurogenic) (70-72) the microenvironment regulates stem cell commitment toward specific lineages through intrinsic and extrinsic factors (13, 83-88). This represents the reason why, we saw two different MSCs’ way of behavior after the implant in the intersomatic space in patients who had lumbar posterolateral arthrodesis. Infact in the 2 patients with a preoperative black disc, we observed a rehydration of the same, probably because in the DDD cascade it was not passed the point of not return (Pfirman grade II-III) (14). It is plausible that implanted MSCs found the growth factors, the interleukine and all the other network of molecules (13, 83-88), who had showed to have anabolic effect on the disc and which influenced their chondrogenic differentiation, glycoproteins and glycosaminoglycans production, which are able to attract and bind water (58-60), providing rehydration and resilience to compression. Otherwise in the case where we had a very advanced DDD, with an important loss of height of the disc (Pfirman grade V) (14), MSCs found a local network of molecules that may lead them to an osteogenic differentiation, explaining why we had a complete intersomatic fusion after only one month.

It is clear that there are several questions that need to be answered but, according to our experience followed the initial phase of experimenting MSCs over patients, we certainly assert that they are really useful in lumbar spine surgery, both to block the aging of the disc both to aid and accelerate the fusion processes in arthrodesis.
Due to complex interactions between stem cells and their environments, characterization of their behavior for specific applications is vital to optimizing their full therapeutic potential. In the near future, in spine surgery, MSCs could be easily harvested in a day surgery from the patient. This procedure could be followed by in vitro induction of autologous progenitor cells differentiation into differentiated cells (i.e. chondrogenic, osteogenic differentiation), in vitro proliferation and, after a short period, their implantation in the same patient.
Bibliography:


Figures:

Figure 1: Female, 47 yrs. Low Back Pain and bilateral sciatalgia with neurogenic claudication. The patient underwent fixation with pedicle screws L4-S1 and decompressive bilateral laminectomy. MSCs were both added to bone chips for posterolateral fusion and implanted in L5-S1 disc.

1A: Preoperative CT scan pointing out bilateral isthmic lysis and spondololysthesis with Pfirrmann grade V.
**1B:** MSCs withdrawal from vertebral body, harvested through each pedicle hole for screw positioning (Biosafe processing) plus autologous and heterologous cancellous bone.

**1C:** Early postoperative XRay showing L4-S1 pedicle screws and rods fixation.

**1D, E and F:** Respectively Early postoperative, 1 month and 3 months follow-up CT scans, sagittal reconstruction, pointing out the bone chips with air (D); the starting bone bridging (E) and the complete fusion (F).
1G: Early postoperative CT scan showing the Pfirrmann grade V at L5-S1.
1H: 1 month postoperative CT scan showing the complete intersomatic fusion.
Figure 2:
Female 66 yrs. Low Back Pain resistant to medical therapy, due to degenerative L2-L3-L4 lysthesis. The patient underwent L2-L3-L4 miniinvasive fixation with pedicle screws and rods. MSCs were harvested from subcutaneous fat tissue.

2A: Preoperative T2 Weighted MRI showing L2-L3-L4 lysthesis with black discs.
2B: MSCs harvested from subcutaneous fat tissue and processed by MyStem® device.
2C: Intraoperative XRay showing the mini-invasive insertion of pedicle screws and the percutaneous needle positioned in L3-L4 disc.

2D: Postoperative 1 month T2 W image pointing out a clear hyperintensity in L3-L4 space with the mark of the needle.
The instrumentation procedure allowed the reduction of vertebrae malalignment.
2E: Postoperative 3 months T2Weighted image with complete hyperintensity in the L3-L4 space.
2F: Postoperative 6 months T2Weighted image with complete hyperintensity in the L3-L4 space.
| Table 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Patient** | 22 |
| **Mean Age** | 57.4 (range 37-66 yy) |
| **Sex** | 12F : 10M (Ratio 1.2:1) |
| **Spine Disease** | 11 * Spondylolisthesis + Stenosis | 3 † Segmental Spondylolisthesis | 5 † Segmental Stenosis | 3 † Disc Herniation |
| **Level** | 2 L4-L5 9 L5-S1 | 3 L5-S1 | 2 L3-L4 3 L4-L5 | 1 L3-L4 1 L4-L5 1 L5-S1 |
| **Surgical Treatment** | Decompressive Laminectomy + Posterolateral Fusion | Posterolateral Fusion | Bilateral Interhemilaminectomy + Undercutting + Uniwallis® | Interhemilaminectomy + Microdiscectomy |

*Red Bone Marrow Withdrawal
†Fat Tissue Withdrawal