SUPPLEMENTAL DIGITAL CONTENT

Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial

by

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SDC, Table S1. Results from the SF-12 life quality questionnaire.

	Baseline		8 days		3 months		6 months		1 year	
Arm	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**	**PCS**	**MCS**
Control (n=12)	40±3	52±3	43±3	50±2	43±3	46±3	39±3	48±3	42±3	50±3
+MSC (n=12)	39±2	46±3	39±2	47±3	47±3	50±2	46±3	52±2	45±3	48±3

PCS, Physical Component Summary. MCS, Mental Component Summary. Values are given as mean±SE

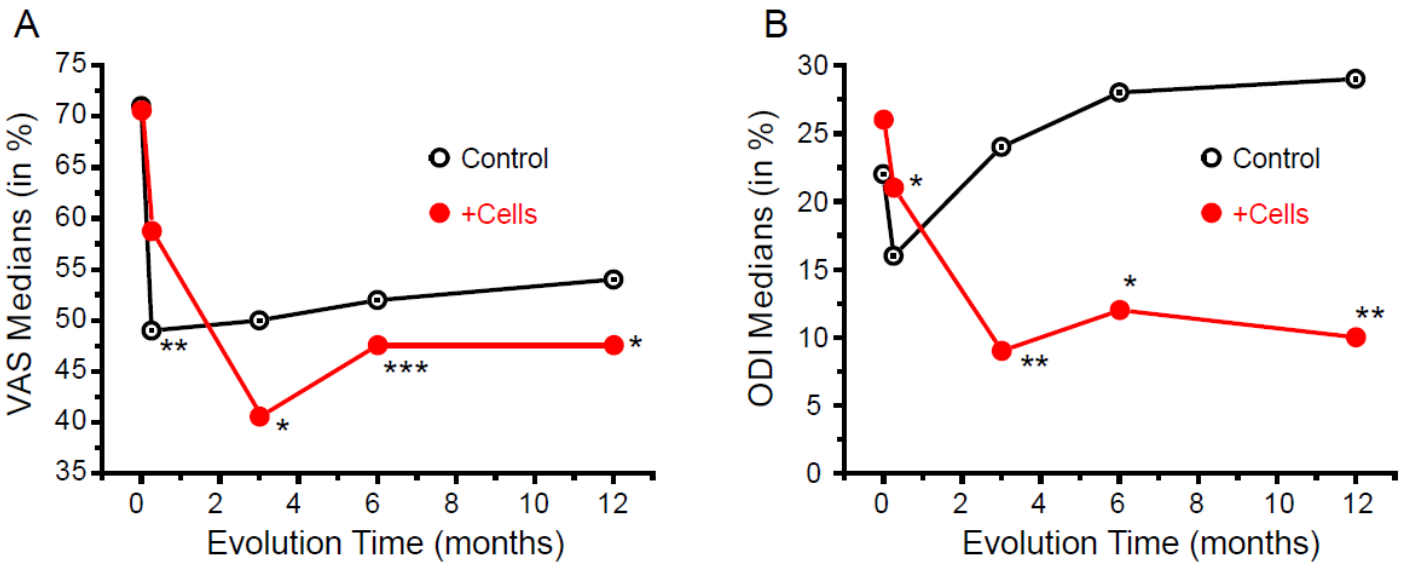
SDC, Table S2. Fluid contents of the discs along the clinical trial period.

	Values are given as mean ± SE (n)			
	Density[a] before Transplantation	**Density[a] 6 months afterwards**	**Density[a] 12 months afterwards**	**Ratio[b] 12 months/6months**
Control	0.48 ± 0.05 (20)	0.51 ± 0.05 (20)	0.49 ± 0.05 (20)	1.06 ± 0.08 (20)
+MSC	0.46 ± 0.05 (18)	0.42 ± 0.05 (18)	0.52 ± 0.06 (18)	1.22 ± 0.11 (18)

[a]Image densities were measured in the T2-weighted MRI images and are normalized to 1 with regard to the healthy discs. The values of density at 6 and 12 months did not significantly differ from baseline ANOVA, Bonferroni test).

[b]Ratio was performed for each individual disc and then the ratio values used for calculations of mean and SE. The ratio values did not differ significantly from 1.

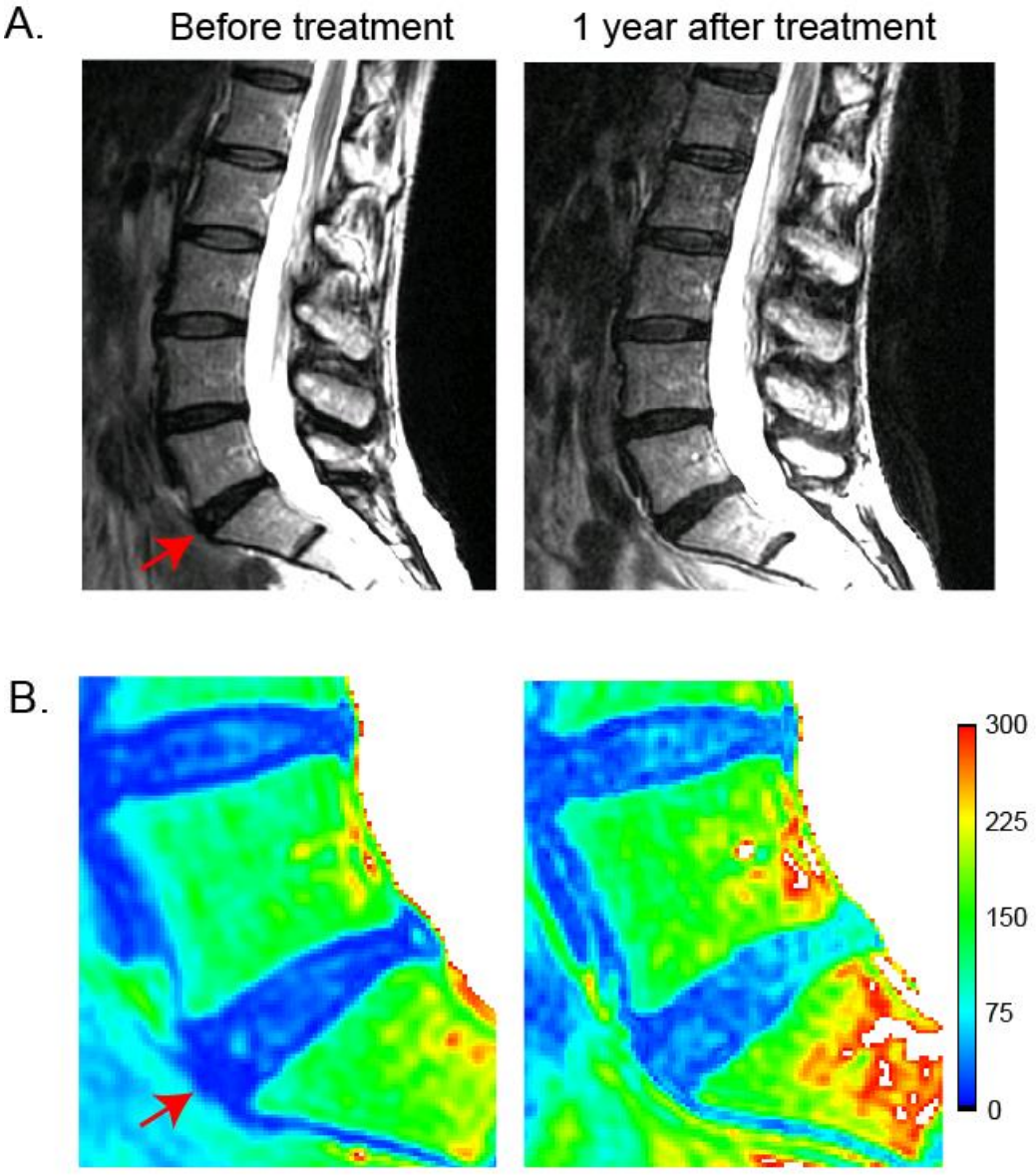
SDC, Figure S1.



SDC, Figure S1. Evolution of medians of lumbar pain (A) and disability (B) in the control (black empty circles) and the MSC-treated cohorts (red filled cycles) one year after the intervention.

The values are the medians of the two 12-patient cohorts. Statistical significance of the differences with the baseline values (t=0) was assessed by the Wilcoxon matched-pairs signed-ranks test; *p<0.05; **p<0.01; ***p<0.005.

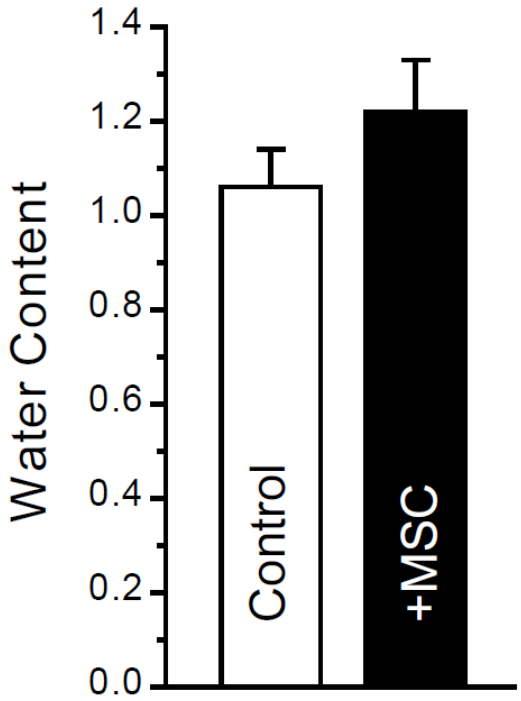
SDC, Figure S2.



SDC, Figure S2. Representative MRI changes after treatment with MSC.

(A) Grey images with red arrow pointing to the affected disc, L5S1. Note that after treatment the disc is denser. **(B)** Close-up of the affected disc and his upper neighbour. Density coded in pseudocolor with calibration scale at right. The density of L5S1 was increased by about 50 %, but the healthy discs were little affected. Data from patient 5. On average, the density of the affected discs increased by 22 % after MSC treatment (Table S2).

SDC, Figure S3.



SDC, Figure S3. Evolution of relative fluid content of the affected discs.

Changes in density of T2-weigthed images in the nucleus pulposus area 1 year after the intervention were measured. Density was normalized with regard to the baseline level (1.00). Values are mean ± SE for the control (open bar) and for the MSC-treated patients (filled bar). Differences with 1.00 are not significant.

SDC, SUPPLEMENTARY METHODS.

Bone marrow harvesting, purification and culture

Puncture and bone marrow aspiration were performed in an ambulatory surgery session. The patient, in prone position, underwent light sedation. The surgical field was brushed with alcoholic povidone-iodine solution (chlorhexidine if a history of allergy to iodine exists) and delimited with sterile fields, leaving free both posterior iliac crests. After local anesthesia (20 ml of 1% lidocaine without epinephrine diluted v/v with saline), two members of the extractor team, placed on both sides of the operating table, performed several punctures with a 11-G trocar under the iliac spine, aiming towards the posterior sacroiliac joint (this is the iliac area with higher trabecular density). The technique involves sudden cortical perforation and repeated aspiration of small bone marrow volumes (2-4 ml) to minimize contamination with the peripheral blood. The aspirate was injected into a heparinized bag for transport. Two successive aspirations were performed by rotating 90 degrees clockwise the beveled trocar. The same puncture hole allows a further 1-2 mm deepening twice, repeating the same methodology with 2-4 ml suction, syringe change, 90º bezel rotation and new aspiration. Then the trocar can be removed by sliding it slightly, and a few millimeters above the cortical pelvic, puncture can be repeated, continuing on both sides of the pelvis until about 80 ml are collected. Bone marrow (sterile bag heparinized with a volume of about 80 ml of aspirate) was refrigerated to 4ºC, conditioned and shipped to the Cell Therapy Unit. Further processing should was done within 24 h.

Donor serum samples were also obtained at this time in order to carry out the required screenings for excluding VIH and hepatitis A and B contamination (Annex VIII and Directive 2004/23/EC of the European Parliament and of the Council). Tests must be performed by Nucleic Acid Amplification Testing (NAAT) to circumvent quarantine, which would otherwise be necessary to avoid the window effect [1]. An aliquot of the serum sample must be stored to allow further analysis if required in future.

Bone marrow samples were transported to the Cell Production Unit at 4-12°C within 24 hours of harvesting. The mononuclear cell fraction was isolated by density-gradient centrifugation, resuspended, and cultured in MSC expansion culture medium [2] in 175-cm^2 tissue culture flasks, with periodic washing to remove non-adherent cells. When cells reached 80% confluence, they were trypsinized and re-plated, and the process was repeated for two more passages. At the end of this period (21-24 days), cells were harvested, resuspended in Ringer's lactate solution containing 0.5% human albumin (CSL Behring GmbH, Marburg, Germany) and 5 mM glucose, and transported at 4-20°C to the hospital application. In addition to quality-control tests, viability and flow cytometric immunophenotypic profiles [2,3] were determined at this stage.

Details on cell injection.

This intervention was performed under slight sedation and radiological control. Cells were injected by disc puncture avoiding neurovascular elements [4]. After double brushing of the region with aqueous povidone-iodine (chlorhexidine in patients allergic to iodine), the field was delimitated with sterile sheets and local anesthesia (Mepivacaine-1%) was applied to skin, subcutaneous tissue and muscle close to the puncture. With fluoroscopy in anteroposterior position, a vertical line corresponding to the projection of the spinous processes was marked on the skin with a sterile dermographic pencil. Then a perpendicular line corresponding to the projected image of a Kirschner needle aligned with the intervertebral space to be treated was drawn. At a point of located 8 to 9 cm (depending on the patient morphotype) from the midline, a 20G spinal needle was inserted with an inclination of 25 to 35 degrees towards the midline. Fluoroscopy was then changed to lateral position. This view ensures that the penetration of the needle follows the right direction until the nucleus pulposus is reached. After the correct position of the needle into the nucleus pulposus was verified in both, the anteroposterior and lateral fluoroscopic views, the suspension of MSC was slowly injected. No incidents have been recorded in none of the 10 patients using this procedure.

Following the cell infusion, the patient was generally discharged after a 2 hour-observation period. Lumbostat corset was not prescribed and moderate walking was permitted. Labour activity was suspended for one week. Exercises for tonifying paravertebral and abdominal muscles were started 1 month after intervention. Analgesic medication was adapted to the needs of each patient and anti-inflammatory drugs were not used.

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