

MR Monitoring of Minimally Invasive Delivery of Mesenchymal Stem Cells into the Porcine Intervertebral Disc

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Introduction:

Back pain is one of the most frequent complaints affecting millions of people worldwide. It usually stems from degenerative disc disease. Under physiological conditions, the intervertebral disc (ID) consists of the annulus fibrosus tightly surrounding the nucleus pulposus. The latter is composed of chondrocytes suspended in a highly hydrated mucoprotein gel, which is the key component responsible for flexibility and compressive loads securing structural support for the body. Degenerative disc disease occurs at each age and is one of the frequent causes of long-term absence at work. While surgical treatment and an appropriate rehabilitation plan may offer relief of acute symptoms, degenerative disc changes usually progress further not allowing full recovery and usually leading to lifelong morbidity. Consequently, new approaches aiming at full restoration of ID are being investigated. Mesenchymal stem cells (MSCs) with their potential to differentiate towards multiple types of connective tissue cells including those of the nucleus pulposus (1) represent an excellent candidate for restorative treatment of degenerative disc disease. It has been shown in animal models that transplantation of MSCs into degenerating ID can be beneficial (2). On the other hand, it has been reported that transplanted cells can leak out from the intervertebral space, which can lead to the formation of undesirable bone structures (osteophytes) complicating the restorative process (3). For these reasons, precise cell delivery with verification of their deposit within the nucleus pulposus is of utmost importance. To this end, we have developed a method of real-time MR monitoring of minimally invasive percutaneous delivery of magnetically labeled MSCs into to intervertebral space.

Materials and Methods:

Isolation and labeling of MSCs: Iliac crest bone from porcine donors (n=3, 30 kg) was punctured and bone marrow aspirate was collected under sterile conditions. A phosphate buffered saline (PBS)-diluted cell fraction of heparinized bone marrow was layered over a Ficoll density gradient (1.077 g/mL, GE Healthcare) followed by centrifugation at 400G at room temperature for 35 min. Nucleated cells were collected, diluted with two volumes of PBS, centrifuged twice at 100G for 10 min, and finally resuspended in culture medium. MSCs were maintained in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Forty-eight hours prior to transplantation, MSCs cultured as a monolayer at 70% confluence were labeled with BrdU (10 µM, Sigma) and iron oxide (Molday ION/Rhodamine, Biopal; 25 µgFe/ml). Immediately prior to transplantation, cells were washed with PBS, harvested by trypsinization, and suspended in HyStem hydrogel (Glycosan Biosystems) at a concentration of 1x10⁶/ml.

ID lesion and cell transplantation procedure: Experiments were performed in juvenile female pigs (30 kg, n=3). Degenerative disc disease was induced by injuring the disc with an energy pulse of 1000 J over one minute (Dornier Medilas D MultiBeam, Germany). A laser fiber optics probe was introduced into the intervertebral space using an epidural anaesthesia needle under guidance with C-arm fluoroscopy (**Fig. A**). After this procedure, the fiber optics probe was replaced with a 26G plastic soft catheter. To avoid imaging artifacts, the metal needle was removed and the catheter was secured in place prior to scanning at 3T MRI scanner (Magnetom TRIO, Siemens). Axial T2-w images (TE/TR=100/4500, AV=1, Res=560x560x3,000 µm) were acquired with the same geometry, without repositioning of the animal. Images were taken before, after injecting 150µl/15x10⁵ cells, and again after injecting a second dose of 150µl/15x10⁵ cells.

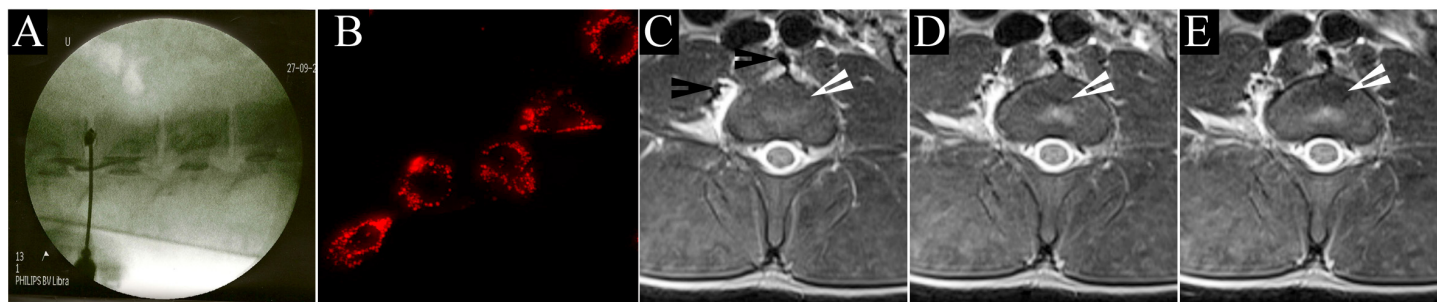
Results:

Magnetic labeling rendered nearly 100% of MSCs positive, as visualized by the red fluorescence of rhodamine (**Fig. B**). MRI prior to cell injection detected several hypointense regions around the vertebrae (**Fig. C, black arrowheads**), likely due to the presence of extravasated blood from the needle puncture. Following the first injection, a new hypointense region appeared within the intervertebral space (**Fig. D, white arrowhead**). Following the second injection, this hypointense region significantly expanded as a result of the new influx of cells (**Fig. E, white arrowhead**).

Conclusions:

Percutaneous injection of stem cells to the intervertebral disc space is feasible. MR monitoring can be used to verify the accurate delivery of cells and provide a detailed picture of initial cell engraftment and biodistribution. As our experimental setup closely resembles a clinical scenario, given the large animal model, use of catheters, C-Arm fluoroscopy and 3T MRI, this approach should be readily translatable.

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