Intervertebral Disc Regeneration Using Mesenchymal Stem/Stromal Cells Transplanted Via The End-Plate Route in a Large Animal Model

Gianluca Vadalà, MD, PhD1, Fabrizio Russo, MD1, Maria Musumeci, PhD1, Francesca De Strobel, DVM2, Marco Bernardini, DVM2, Giulia De Benedictis, DVM3, Luca Denaro, MD, PhD3, Domenico D'Avella, MD3, Rosaria Giordano, MD3, Vincenzo Denaro, MD1.  
1Campus Bio-Medico University of Rome, Rome, Italy, 2University of Padua, Padua, Italy, 3Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy.


Introduction: Recent evidences showed that intradiscal injection of bone marrow Mesenchymal Stem/Stromal Cells (MSC) effectively alter the course of Intervertebral disc degeneration (IDD) in vivo and is clinically safe [1]. However, many unanswered questions remain in the translation to the clinical phase, such as the most reliable transplantation method including the surgical approach to the disc. Indeed, drugs, cells or biomaterials are commonly delivered into the NP by injection through the AF. However, evidences showed that small needle disc puncture for discography resulted in accelerated disc degeneration with progression of disc degeneration grade and new disc herniation on the side of the disc injection compared to matched controls discs [2-3]. Moreover, it has been recently shown that injecting MSC into the NP from the AF of a model of disc degeneration may lead to cell leakage and osteophyte formation [4]. All these considerations suggest that intradiscal injection through the AF route itself is not completely innocuous and may disable the treatments to therapeutic agents delivered.

The Purpose of the study was to test MSCs/hydrogel transplantation for IVD regeneration in a grade III/IV preclinical model of IDD on large size animals [5] via the novel transpedicular approach [6] to the disc with cell dose escalation using a clinical relevant hydrogel.

Methods: Female adult sheep (n=18) were used. Sheep underwent bone marrow aspiration two month before surgery. Cells were isolated, characterized, expanded and frozen. Four lumbar IVDs (L1-2, L2-3, L3-4, L4-5) were used for the experiment (L5-L6 was considered a normal control). Before surgery peripheral blood was withdrawal for autologous Platelet Reach Plasma (PRP) preparation. Then, autologous MSC were thawed, washed and suspended in PRP and brought to the OR along with Hyaluronic Acid 1,6% + Batroxobin (HA/BTX) compound. Throughout a posterior surgical access to the lumbar spine, a 2 mm tunnel was drilled via the transpedicular approach to access the NP under fluoroscopy [6]. Nucleotomy was performed using a shaver resector under aspiration. The discs were randomly assigned to different treatments and received an injection using 14G needle of a 100ul volume of 1) hydrogel (PRP/HA/BTX), 2) Low doses of MSC (5x10^6 cell/ml in PRP/HA/BTX), 3) High doses of MSC (1x10^7 cell/ml in PRP/HA/BTX), 4) no injection (CTRL). The tunnel was sealed using a press-fit porous polyurethane (PU) scaffold shaped as a cylinder placed at the endplate edge using a biopsy cannula. The animals underwent X-ray and MRI at baseline and at 1, 3,
6 and 12 months following operation. Disc height and MRI indexes were calculated at each time point. Disc macro- and micro-morphology were analyzed after euthanasia at each time point.

**Results:** The MRI index showed a significant decrease in the untreated group, the disc injected with hydrogel and those injected with low MSC dose compared to healthy discs in all time points. The discs treated with high dose of MSC showed maintenance of the MRI index compared to the healthy disc. MRI signal intensity of NP in T2-weighted midsagittal images evaluated according to Pfirrmann degenerative grade [7] of the human lumbar spine showed that the high MSC dose group looked as a grade II at all time point, the low MSC dose group appeared as grade III, the hydrogel group looked as grade IV and the untreated group appeared as grade V. Morphologically, the grade of degeneration evaluated using the Thompson grade system [8] were in agreement with the grades observed at the MRI.

The High MSC dose treated discs demonstrated abundant cartilage formation at 3 months, and to a lesser extent at 6 and 12 months. For the carrier and low MSC dose treated groups, however, there was less proteoglycan matrix.

**Discussion:** An effective dose of autologous MSC (1x10^7 cell/ml) delivered with a clinical relevant carrier (PRP/HA/BTX) via the alternative transpedicular approach regenerates the NP in a preclinical model of grade III/IV IDD. These data highlight as the disc regeneration can be achieved maintaining the AF intact via the end-plate route, overcoming the unsolved problem of reliable transplantation method including the surgical approach to the disc necessary for an effective clinical translation of a stem cell therapy for the treatment of IDD.

This preclinical study has high translational value as the carrier that was used is designed using clinically available drags and materials for the treatment of musculoskeletal disorders. The 2mm transpedicular tunnel allows the injection of viscose materials using a 14 G needle. The sealing of the tunnel prevents the leakage of the transplanted tissue engineering construct.

**Significance:** The results of this study showed that an effective MSC dose delivered with a clinical relevant hydrogel trough the end-plate route effective alters the course of IDD in a transpedicular nucleotomy model in large size animal. This study bring a significant contribution towards the translation of regenerative therapies for the biological restoration of degenerative changes in the IVD, which is crucial to improve present clinical treatment and life quality of several patients.

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