Reproducible Disc Degeneration Scale In A Large Animal Model

Gianluca Vadalà, MD, PhD1, Fabrizio Russo, MD, PhD1, Francesca De Strobel, DVM2, Marco Bernardini, DVM3, Devid Eglin, PhD3, Luca Denaro, MD, PD2, Mauro Alini, PhD3, Roberto Busetto, DVM2, Domenico D’Avella, MD2, Vincenzo Denaro, MD1.
1Campus Bio-Medico University, Rome, Italy, 2University of Padua, Padua, Italy, 3AO Research Institute, Davos, Switzerland.


Introduction: Numerous animal models of intervertebral disc degeneration (IDD) have been proposed, each with attendant advantages and disadvantages in order to study pathogenesis and pathophysiology of IDD and test novel therapies. In order to study regenerative strategies of the nucleus pulposus (NP), it is paramount to maintain the annulus fibrosus (AF) intact. Indeed, a recent establishment in intervertebral disc (IVD) research is that even a small AF lesion may biomechanically alter the disc making it unable to support the physiological intradiscal pressure [1-2] and avoid NP and cell/material leakage [3]. To study the efficacy of novel regenerative strategies it is necessary to develop a new model that does not implement AF damage.

We hypothesize that an ideal preclinical model to study novel biological therapies for NP regeneration could be achieved by approaching the NP via the endplate (EP) route through a minimal invasive transpedicular approach [4]. The aim of the study is to characterize a preclinical ovine model that resembles different degrees of IDD, triggering EP damage and repair with or without mechanical nucleotomy, whilst keeping the AF intact.

Methods: Female Biellese sheep (n = 18), approximately 3 years of age, were used. In each sheep, five lumbar IVDs (L1-2, L2-3, L3-4, L4-5, L5-S6) were used for the experiment. Throughout a posterior surgical access to the lumbar spine, a 2 mm tunnel was drilled, under fluoroscopy, using a k-wire via the transpedicular approach to access the NP, as already described [4]. Nucleotomy was performed using a 2 mm shaver resector under aspiration. The tunnel was sealed/repaired using a press-fit porous polyurethane (PU) cylinder [5-6] (2.2 mm diameter and 10 mm length) that was placed at the EP edge using a 2 mm cannula inserted through the tunnel. The five lumbar discs were randomly assigned to different groups of treatment: EP tunnel (A); EP tunnel + nucleotomy (B); EP tunnel + repair with the PU scaffold (C); EP tunnel + nucleotomy + repair with the PU scaffold (D); no treatment (E - control). The effect of different treatments on IVDs was evaluated through imaging follow-up. Sheep lumbar IVDs were studied through X-ray and MRI at time 0, 1, 3 and 6 months after surgery. Disc height and MRI indexes were calculated at each time point. Disc macro- and micro-morphology were analyzed after euthanasia at each time point. Extracellular matrix content was also evaluated. MRI images and gross anatomy photographs were graded using both Pfirrmann [7] and Thompson [8] grading criteria.

Results: The MRI analysis showed a progressive decrease of MRI signal intensity of NP in T2-wheigthed midsagittal images with different degrees of degeneration according to the group of treatment. According to Pfirrmann degenerative grade of the human lumbar spine, the C group showed a grade II at all time point, group A appeared as grade III, group D looked as grade IV and group B appeared as grade V (Figure). Morphologically, all stages of the degenerative process from Thompson grade I to grade V were also observed with the same association of the Pfirrmann degenerative grade (Figure). Disc height index on radiographs showed a trend of decrease overtime in groups D and B. MRI index decreased progressively starting at 1-month time point to 6 months according with the degenerative grade.

Histological analysis revealed progressive disc narrowing, fragmentation of the NP matrix in D and B group. The scaffold in the tunnel of C and D groups appeared colonized by cells without sign of bone formation at all time point. NP tissue was in the tunnel with infiltration of inflammatory cells in A and B groups.

Discussion: This stepwise model could be suitable for studying pathogenesis and pathophysiology of IDD evaluated at the different stages of degeneration. Moreover, the model could be used to test safety and efficacy of novel treatments for IDD. The sealing of the tunnel prevents the NP to leak allowing the restoration of intradiscal pressure. Therefore, growth factors and drugs could be tested in grade II model (EP tunnel and repair), while biomaterials and cells as well as tissue engineering constructs might be tested in grade IV model (EP tunnel, nucleotomy and EP repair). For this purpose, grade IV model allows the injection in the NP of high viscose materials using a 14 g needle trough the 2mm transpedicular tunnel. Moreover the intact AF would prevent leakage trough the AF allowing also reliable biomechanical tests.

Significance: A new preclinical model to study tissue-engineering strategies for NP regeneration has been developed and characterized by approaching the NP via the EP route through a minimal invasive transpedicular approach [4]. Keeping the AF intact, the different degrees of IDD have been observed according to Pfirrmann [7] and Thompson [8] grading system. This will be a significant contribution towards the translation to the therapeutic arsenal of new regenerative strategies for biological restoration of early and mild degenerative changes in the IVD.

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References:

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