Annular Repair using High Density Collagen Gel. An in vivo Study.

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Introduction:
Unrepaired annular defects potentially increase the re herniation rate after lumbar discectomies (1) and have shown to accelerate degenerative changes after discographies (2). This is the first study to test an injectable biological substance to repair annular defects in vivo. We used high-density collagen gel in a needle-punctured rat-tail model. Needle puncturing leads to extrusion of NP tissue with subsequent degenerative changes. Restoring annular integrity will help to retain the NP material and therefore inhibit these changes. In addition the repair of annular defects is expected to restore biomechanical function to injured discs as shown in preliminary in vitro tests by our group (3).

Methods: We punctured the S3/S4 intervertebral disc (IVD) of 58 athymic rats using an 18-gauge needle. Subsequently high-density collagen (HDC) gel was injected to seal the defect. Riboflavin (RF) was added to increase the stiffness of the collagen gel by inducing cross-link formation. The cross-linking process was initiated in situ after exposing the collagen gel to blue light. The animals were subdivided into four groups. The first group was injected with un cross-linked HDC gel, the second with cross-linked collagen using 0.5mM RF and the third with using a higher concentration of 0.75mM RF. The fourth group served as control and was left untreated after needle puncture. The animals were followed up at week 1, 2, 5, 12 and 18 with X-ray measurements to assess the disc heights and MR imaging to evaluate degenerative changes according to a modified Pfirrmann grading system. We developed an algorithm based on T2-relaxation time measurements to assess the size of the nucleus by the number of NP voxels that compose it.

The animals were sacrificed at 5 (n=32) or 18 weeks (n=26). Tails were collected either for histological analysis to assess disc degeneration and NP size or for mechanical tests carried out on a load frame to assess the functionality of the punctured discs. We performed stress relaxation tests to measure the stiffness of the discs and the ability to pressurize. Damping quality was measured by imposing sinusoidal strains on the explanted segments at variable frequencies ranging from 0.01Hz to 0.3Hz at amplitudes of ±10% strain.

Results: Over 18 weeks the RF cross-linked groups retained significantly more NP tissue in the disc space than the uncross linked and the control group according to the NP voxel count and histological NP cross section measurements (Fig 1). However there was no significant difference in NP size between 0.5mM and 0.75mM over 18 weeks. Both groups retained about 70% of NP size when compared to healthy discs and maintained a disc height of over 80% compared to the pre-puncture state. Both cross-linked groups showed no significant histological degenerative changes. The NP was reduced in size with a regular cellularity and matrix morphology. There was a clear border to the AF visible. The AF maintained its organization and lamellar structure. The annular defect remained visible until the 18-week time point. However, after 5 weeks the cross-linked collagen treated discs showed the formation of a fibrous cap at he outer part of the annulus which bridged the defect and thereby partially repaired it (Fig. 2). This fibrous cap appeared to be formed from host fibroblasts, which infiltrated the injected collagen and subsequently remodeled it. After 18 weeks this fibrous cap formed into a fibrous sheet, which connected both endplates covering the defect. After 18 weeks, both cross-linked groups showed similar damping qualities according to frequency sweep tests as well as a similar stiffness and ability to pressurize when compared to healthy adjacent discs.

At the 5-week time point the uncross-linked collagen and the control group showed no residual NP tissue in the disc space with terminal degenerative changes on histological sections and MRIs. The NP was replaced by connective tissue. Extruded NP tissue was visible in the paravertebral space. There was no repair tissue visible at the outer part of the AF. The IVDs of both groups were stiffer then healthy discs and showed less damping qualities.

Discussion: HDC is capable of repairing annular defects induced by needle puncture in a rat-tail model and thereby inhibit subsequent degenerative changes and improve mechanical properties of injured discs. Cross-linking of HDC positively influences the repair mechanism. Cross-linking of collagen gel has been shown to increase its stiffness (4), which might result in a more robust barrier sealing the defect. In addition cross-linking of polymer gels have shown to increase adherence to AF tissue (5) and enhance host cell infiltration with subsequent tissue reorganization (6).

Significance: The repair of annular defects could have a significant impact on the treatment of annular defects during discsectomies and could prevent degenerative changes of IVDs after discographies. To our knowledge this is the first study testing an injectable biomaterial for this purpose. Injectable substances could potentially be used through minimally invasive or percutaneous approaches. Both components collagen gel as well as riboflavin are already in clinical use individually but have never been combined to treat annular defects.

Experiments in larger animals will be necessary to further evaluate the clinical applicability of the presented biomaterial.
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Fig. 1: Five-week outcome after needle puncture. The 0.5mM RF group showed only a slightly reduced NP size according to the NP voxel count and histological cross-section measurements compared to healthy discs. On T2 weighted images (second row from the top), the NP appears oval shaped and homogeneous hyper intense resulting in a Pfirrmann grade of I. The uncrosslinked group showed a decrease of NP size. The NP appeared more heterogeneous. The disc height was decreased compared to healthy discs. The untreated disc shows terminal degenerative changes with a black disc sign combined with collapsed disc space seen on the X-Ray image. The NP tissue has been completely replaced by connective tissue.

Fig. 2: Punctured IVD, 0.5mM RF group after 5 weeks. A, Low magnification. The NP displays a standard size and a clear border between the AF and the endplate bone. B. The box marks the needle puncture defect. B, Higher magnification displays the needle puncture defect piercing through all AF layers (red arrow). Marked by the box, there is a fibrous cap visible, bridging the annular defect at the outer portion of the AF. C, D Higher magnifications of the fibrous cap.