Riboflavin-Crosslinked Collagen for Annulus Fibrosus Repair In Vivo

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Disclosures:

Introduction: A weak or damaged annulus fibrosus (AF) often manifests itself in the form of a bulging and herniated intervertebral disc (IVD). This damage is the initial stage in a debilitating cascade, eventually leading to full disc degeneration. Current treatments to impede the progression of early disc degeneration have exhibited limited success [1], with full disc degeneration often prevailing after delivery of a therapeutic. Our group has developed a high density collagen gel formulation that can be delivered to the defect site, sealing the damaged AF. Although our formulation showed some success both in vitro and in vivo, the gels still exhibited inferior mechanical properties to the native annulus. Crosslinking with riboflavin has been shown to improve the mechanical properties of collagen gels while not compromising cell viability in culture [2]. The goal of this study is to apply riboflavin crosslinking to our collagen gel formulation to enhance AF repair in vivo.

Methods: Type I collagen was harvested from rat tail tendon as previously described [3]. Harvested collagen was stored in 0.1% acetic acid at 4°C until use. Working solutions containing saline, NaOH and riboflavin at concentrations of 0.00, 0.25 or 0.50mM were prepared and stored at 4°C. According to approved IACUC protocol, athymic rats were anesthetized and prepared for surgery. The Cd3-Cd4 IVD was located via x-ray and marked prior to the first incision. After surgical exposure of the IVD, an 18ga needle was used to puncture the AF. Working solutions were mixed with the stock collagen to initiate the polymerization process immediately before injection. Defects were either filled with one of the three described collagen gel formulations, or left without gel treatment. Tails were exposed to 40s blue light to initiate crosslinking [2], and the tails closed. Each tail was x-rayed immediately post-operation to qualitatively assess disc height. Both x-ray and MRI were done on each tail at time points of 1, 2 and 5 weeks. At 5 weeks, animals were euthanized and the tails harvested and placed in formalin in preparation for histology. Disc height index (DHI) was calculated from x-ray films using a modified version of previously described methods [4]. Quantitative T2 MRI was performed to assess NP tissue hydration using a novel method for analysis. Briefly, each MRI was processed against a threshold set using an individualized relaxation time denoting hydrated NP tissue. The number of voxels at or above this threshold in the disc space was counted for each disc of interest as well as the adjacent healthy control. Histological slides were prepared and stained with either picrosirius red, Alcian blue or Safranin-o to look at disc content and tissue organization. A subset of motion segments from each group was subjected to mechanical testing in the form of frequency sweeps from 0.01 to 0.3 Hz at amplitudes of ±10% strain around 20% strain.

Results: Samples treated with collagen gel maintained higher DHI and NP voxel counts than untreated controls throughout the duration of the study. Samples treated with gels containing 0.50mM riboflavin maintained DHIs of over 80% of the adjacent healthy disc compared to samples treated with plain collagen (70%) and untreated samples (less than 65%). Quantitative T2 showed similar results; treated samples maintained higher number of voxels representing hydrated NP throughout the entire study. Samples treated with gels containing 0.50mM riboflavin exhibited values of at least 60% of healthy discs while samples treated with plain collagen or untreated exhibited less than 40% of healthy disc values. Histological slides confirmed the quantitative results. All treated samples showed intense staining for glycosaminoglycans in the NP region and preserved endplate structure. Samples treated with crosslinked collagen gels maintained AF organization and lamellar structure. Furthermore, samples treated with collagen containing 0.50mM riboflavin had a distinct AF/NP border similar to that seen in the
native, healthy IVD. Dynamic stiffness of discs treated with 0.50mM riboflavin exhibited similar behavior to tested healthy discs, which the puncture only segments were stiffer than both treated and healthy segments.

**Discussion:** This study reports the use of a novel high density collagen gel formulation for annulus fibrosus repair. Our formulation contains the nontoxic crosslinker riboflavin, and can be crosslinked in situ after delivery to an AF defect upon exposure to blue light. Our crosslinked collagen gels showed enhanced repair ability over uncrosslinked collagen gels. Furthermore, treatment with collagen containing a higher amount of riboflavin exhibited superior repair ability, with DHI and T2 values close to healthy discs. Histological slides showed samples treated with collagen containing 0.50mM riboflavin were similar in organization and tissue content to healthy discs. These crosslinked collagen gel formulations have inhibited the progression of degenerative symptoms such as loss of disc height and NP hydration in a rat tail model of disc degeneration.

**Significance:** This study demonstrated the development of a crosslinked collagen gel that prevents IVD degeneration after puncture injury in vivo. Crosslinking greatly enhanced the ability of the gel to prevent damage, with the highest concentration of crosslinker maintaining IVD morphology, disc height, and NP hydration up to 5 weeks after puncture. To our knowledge, this is the first injectable biomaterial shown to prevent IVD degeneration after puncture in vivo.

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