INTRODUCTION: Rabbit models with degenerated intervertebral discs (IVD) have been used to study the therapeutic intervention of IVD repair. Nucleus augmentation, using injectable biomaterials such as hydrogels, is a promising technique that can provide both immediate weight bearing and long-term biological treatment. To evaluate disc repair, MRI and radiography have been used to assess disc morphology and joint space height. The objective of this study was to evaluate effects of an injectable HA-based hydrogel with and without supplementation of TGF-beta3 (TGFβ3) growth factor on repair of degenerated rabbit IVD, using MRI, plain radiography and micro CT (µCT).

METHODS: Samples - With IACUC approval, New Zealand White rabbits (n = 24, ~3 kg and 9 mo old) were obtained. Using an 18G needle device, the annulus fibrosus (AF) was punctured 5 mm deep in the ventral aspect into the nucleus pulposus (NP) at the L2/3 and L4/5 levels. Unpunctured L3/4 served as a control. After 4 wks, 20 µl of the following solution were injected into the center of the NP at the punctured levels: sterile saline (n=8), a Hyaluronic Acid-based Hydrogel, TGFβ3 (10 ng/ml) combined with Hydrogel (n=8) were injected into the center of the NP at the same level. Animals were sacrificed at wk 16. PLAIN RADIOGRAPHY & DISC HEIGHT INDEX (DHI) – Animals were x-rayed laterally under anesthesia before the initial surgery (wk 0) and every two wks afterwards until sacrifice. On digital radiographs, vertebral body height and disc height were measured using a custom Matlab program, which then calculated IVD height (Fig.1) as an index where DHI = IVD height / adjacent vertebral body height. MRI & T2 PROPERTIES – After sacrifice at wk 16, lumbar spine segments were harvested and imaged on the same day, using a 3T GE Signa HDx with a 3rd birdcage coil. T2-weighted images (Fig.2A) in the mid-sagittal plane were obtained using a multi-echo spin echo sequence: TR = 2000 ms; TE = 10–70 ms; matrix = 384x384; FOV = 80 mm; and slice = 2.5 mm. T2 values in the AF and NP were determined in selected regions of interest (ROI; ~2 mm² area); after median filtering, signal intensities in each ROI were averaged and then fit to a mono-exponential curve using a least-squares method. For the AF, T2 values in two ROI were averaged to obtain a single value. This method was highly repeatable (ICC=0.97) between observers in a pilot study. µCT & 3D DISC HEIGHT – The spine segments were also imaged using µCT (Skyscan 1076) at 35 µm isotropic resolution. Using Mimics, images were segmented semi-automatically (after sampling signal intensity of bone and air) and 3D surfaces were reconstructed (Fig.2B top). Using a custom software, the minimum distance between apposing endplates was determined (Fig.2B bottom) as well as an average disc height for the entire disc.

Statistics – All measurements were normalized internally to L3/4 values from the same animal. For DHI, L2/3 and L4/5 values were averaged, and effects of treatment and time were determined using repeated measures ANOVA and posthoc comparison at each time point. For MRI and µCT measures, effects of treatment and level were determined using repeated measures ANOVA. Data are represented as mean±SEM, n=8. The measures were compared to each other using linear regression.

RESULTS: DHI (Fig.1) varied with time (p<0.05) and with treatment at certain time points (* p<0.05 compared to saline group). Normalized T2 MR values of the NP (Fig.3A) were generally higher in the gel group, while those of the AF (Fig.3B) were higher at L4/5 than L2/3 (p<0.01) and in the gel group relative to saline (p<0.01). Normalized disc height from µCT data (Fig.4) was higher at L2/3 than L4/5 (p<0.001), and was higher in the gel group than the saline group (p<0.05). T2 values of the NP and disc height from µCT correlated strongly (Fig.5; R²=0.45, p<0.001).

DISCUSSION: These results suggest sensitivity of the imaging measures to our model of disc degeneration as well as disc repair using hydrogel. Hydrogel-treated samples had higher (i.e., closer to normalized value of 1) DHI (Fig.1), T2 (Fig.3) and disc height (Fig.4) compared to the untreated control, suggesting the effectiveness of the treatment to restore structural and MR properties of the disc closer to a normal level. Addition of TGFβ3 to the hydrogel had a significant effect on DHI (Fig.1) but its effect on other measures was subdued. Additionally, T2 values of the NP were found to be a strong predictor of disc height (Fig.5); the reduced disc height may be related to dehydration of the NP, which reduces T2 values. The mechanism of the repair remains to be elucidated: structural support along with hydration property of the hydrogel are likely to provide immediate increase in disc height and T2 values, and additional studies are needed to understand degradation characteristics. Nonetheless, the present results provide a positive outlook the use of injectable hydrogel materials for augmentation of degenerated IVD in patients.


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