Introduction: Tissue engineered intervertebral disc could offer an alternative treatment for intervertebral disc degeneration. Different methods have been reported: nucleus pulposus tissue engineering using cell transplantation or hydrogels scaffolds [1,2,3]. An entire composite intervertebral disc was created by seeding annulus fibrosus and nucleus pulposus cells on a hybrid scaffold [4]. It is important from a clinical prospective to analyze the integrative process of newly engineered disc with the vertebral column and in particular with vertebral body endplate as a recipient area for disc transplantation. The goal of this study was to characterize the integration of a composite tissue engineered intervertebral disc to native endplate.

Methods: All procedures were conducted in compliance with Institutional regulations for the use of laboratory animals. Spinal columns of freshly sacrificed sheep were used for cell source. The annulus fibrosus (AF), nucleus pulposus (NP) and vertebral body endplate (VBEP) were harvested separately. The VBEP were shaped in a form of a smalls discs and cultured in Media 199 with antibiotic antimotic. The AF and NP were digested in 0.3% w/v collagenase type II for approximately 4 hours for NP and 7 hours for AF until no visible fragments remain. The cell-collagenase solution were filtered and washed with phosphate buffered saline. Isolated cells were cultured in F-12 media with antibiotic,10% fetal bovine serum and Vitamin C for 30 days and passaged twice in order to obtain sufficient number of cells for transplantation.

Composite intervertebral disc constructs were assembled as described previously [4]. Briefly, AF cells were seeded onto polyglycolic/polyactic acid scaffolds, which were then filled with a 23% solution of Pluronic F127 seeded with NP 50x10^6 cells/ml. A total of 15 samples were implanted subcutaneously in nude mice for a period of 4, 8 and 12 weeks at every time point discs were harvested and analyzed as described below. For histological analysis samples were stained with H&E and Safranin-O. To determine the strength of adhesion, samples were fixed between plexiglass rods using cyanoacrylate glue, with the composite construct fixed to one rod and the VBEP fixed to another. Samples were pulled to failure in tension as indicated by measured load <0.05 N. From the stress-strain curve, the ultimate tensile strength, failure strain, failure energy and dynamic tensile modulus were calculated. For biochemical analysis specimens were lyophilized for 24 hours then digested in papain and analyzed in duplicate for DNA content by fluorimetric assay using Picogreen dye and hydroxyproline using dimethylbenzaldehyde (pDAB) and chloramines T. One way ANOVA statistical analysis was performed with Bonferroni, post-hoc test using Sigma Stat 2.0 software.

Essential Results: On gross morphology examination newly generated composite intervertebral disc resemble a native disc. (Fig. 1).

Figure 1. Histological examination of native (Fig 2A&B) and of engineered tissue (Fig 2C) confirmed that both have similar morphology. No fiber orientation was observed in newly generated annulus fibrosus (Fig 2C) unlike native tissue (Fig 2A&D). Safranin-O of native (Fig 2E) and engineered tissue (Fig 2F) showed similar proteoglycan content.

Figure 2

The composition of the composite intervertebral disc constructs changed with time in vivo. Specifically, water content increased by 10% from 4 to 12 weeks (p<0.05, Fig 3A), while hydroxyproline content increased by a factor of 3 from 4 to 12 weeks (p<0.05, Fig. 3B). There was no change in DNA content with time, suggesting that there was no cell proliferation up to 12 weeks (Fig. 3C).

Figure 3.

Concomitant with changes in the composition of the tissue were changes in the mechanical properties of adhesion. Ultimate tensile strength (Fig 4A), failure energy (Fig 4B), and tensile modulus (Fig 4C) all increased with time, while strain at failure (Fig 4D) did not change.

Figure 4.

Discussion: Generation of tissue engineered intervertebral disc remain a challenge. One of the important steps in bringing this technology to practice is studying adhesion properties and interaction with endplate. This study demonstrated that newly generated composite intervertebral disc could integrate with native VBEP, and that this adhesion became stronger with time. The mechanism by which this integration takes place is not clear although increases in adhesive strength are likely correlated with increased collagen accumulation as indicated by hydroxyproline content (Fig 3B). Future work will also attempt to determine if cells from human tissue have similar reparative capacity as described here.

References:
1) Oegema TR et al., Spine 25: 2742, 2000
2) Yung LJ et al. Spine. 26:2316, 2001