INTRODUCTION: The rate-limiting factor in the development of novel tissue engineering based treatments of intervertebral disc degeneration (IDD) has been lack of a reproducible animal model of disc degeneration. This report describes a slowly progressive and reproducible rabbit model of disc degeneration based on anterior annular “stab” by hypodermic needle, validated by longitudinal MRI, X-ray, and histology.

METHODS: 18 skeletally mature female NZW rabbits were used with IACUC approval. With the rabbits under general anesthesia, the anterior aspects of the L2-3, L3-4, and L4-5 discs were surgically exposed using a retro-peritoneal approach. Anterolateral stab was performed by advancing a 16 ga. hypodermic needle 5 mm into the anterolateral annulus fibrosus of the L2-3, L3-4, and L4-5 discs. MRI scans (1.5 Tesla clinical magnet) were taken at regular intervals up to 24 weeks post-stab. 3-mm-thick mid-sagittal views (12cm field of view, 256 x 224 matrix size) of the lumbar spine were analyzed. A T2-weighted imaging sequence was applied (TR 3500ms, TE 100ms) to highlight the signal from the nucleus pulposus (NP). Nucleus pulposus (NP) signal intensity and NP area were measured using RS 13 IMPAX medical software. An “MRI index” (product of NP area and average signal intensity) was defined to evaluate the degree of disc degeneration. Complementary X-rays were also taken. At sacrifice, histological analysis was performed on the discs of 3 NZW rabbits each at different 3, 6, 12, and 24 weeks post-stab. Samples were fixed in 10% neural buffered formalin for 1 wk., decalcified in EDTA, and sections stained with H&E.

RESULTS: MRI analysis revealed progressive decrease in NP signal intensity, NP area, and MRI index in the stabbed discs over the course of 24 weeks (Fig.1). By the week 6 post-stab, the MRI index of the stabbed L2-3, L3-4, and L4-5 discs had significantly decreased to 49.0 ± 10.6%, 54.6 ± 11.2%, and 51.1 ± 17.6% of their pre-operative values (Fig. 2). Further decreases were noted at weeks 12 and 24. Intra- and inter-observer studies demonstrated excellent reproducibility of the measurements. X-ray revealed disc space narrowing, disc wedging, and osteophyte formation in stabbed discs as compared with healthy discs. Histological examination demonstrated decrease in the number of notochordal cells in the NP, which was replaced by disorganized
fibrocortilaginous tissue. Infolding of the annulus and the clefts and fissures was also evident, suggestive of widespread degenerative changes throughout the disc.

DISCUSSION: Numerous animal models of intervertebral disc degeneration (IDD) have been described in the literature. The classic work by Lipson et al. (1981) described a stab model of disc degeneration wherein a scalpel blade was introduced into the anterolateral wall of the rabbit annulus to produce a full thickness defect. Histological and biochemical analyses were performed that documented a rapid degenerative process.

In the present study, we achieved a more gradual progression of degeneration by stabbing the anterolateral annulus of rabbit lumbar discs by a 16 ga. hypodermic needle, and documented the degenerative changes via longitudinal MRI—which have great relevance in determining whether the animal model mimics those changes seen in human disc degeneration—as well as by X-Ray and histology. The observed MRI, radiographic, and histological findings in this study are consistent with observations of disc degeneration in humans.

With the successful development of a slowly progressive and reproducible animal model of IDD, it becomes possible to rigorously test the efficacy of tissue engineering strategies (including intradiscal gene transfer and stem cell therapy) in the treatment of disc degeneration.