Morphologic and quantitative mapping of biological disc contructs in a rat tail model

S. Pownder[1], M. F. Koff[1], A. James[2], H. H. Gebhard[2], R. Hartl[2], R. D. Bowles[3], L. J. Bonassar[3], and H. G. Potter[1]
[1]Department of Radiolgy and Imaging - MRI, Hospital for Special Surgery, New York, NY, United States, [2]Department of Neurological Surgery, Weill Cornell Brain and Spine Center, [3]Department of Biomedical Engineering, Cornell University

Introduction. The rat is commonly used as a pre-clinical model to evaluate intervertebral disc disease in humans, as previous studies have validated the use of the rat intervertebral disc as a model of the human disc based on mechanical and geometric parameters [1,2]. A difficulty of this animal model are the tail dimensions which limit non-invasive evaluation of experimental sites [3]. High field magnetic resonance imaging (MRI) is often used on rodent models as the specimens are small and SNR is improved with increasing field strength. Few research studies have been performed at clinical magnetic field strengths [4] and limited quantitative MRI of the rat intervertebral disc has been performed. The purpose of this study was to determine the feasibility of using a clinical magnet (3T) to assess morphologic features of a rat tail biologic disc model and the quantitative MR techniques of T2 and T1ρ mapping to evaluate collagen orientation and proteoglycan (PG) content, respectively.
Materials and Methods. Eight athymic rats were used in this trial. A tissue engineered disc was used and surgically implanted at a caudal vertebral disc site in each rat. The rats were allowed free cage activity following surgery. The rats were euthanized 10 months post-operatively and the tails were immediately prepared for imaging. Image Acquisition: MR imaging was performed on a 3.0 Tesla clinical imaging system (14.0 HDx, GE Healthcare, Milwaukee WI), using a prototype birdcage coil (∅=2cm, length=7cm). Morphologic axial and sagittal PD FSE images were acquired with the parameters: TE: 24.4-30.6 ms, TR: 5867-7417 ms, ETL: 12, BW: ±50.0 kHz, Matrix: 512x(416-512), FOV: 5-6 cm, slice thickness: 1.0 mm, slice spacing: 0 mm. Quantitative T2 mapping of articular cartilage was performed using a multi-slice, multi-echo acquisition [5] using parameters: TE: 9.6, 19.3, 28.9, 38.6, 48.2, 57.8, 67.5, and 77.1 ms, TR: 1000 ms, BW: ±50.0 kHz, Matrix: 384x256, FOV: 6 cm, slice thickness: 3.0 mm, slice spacing: 0 mm. Quantitative T1ρ imaging (GE Healthcare, Milwaukee, WI) was performed with the parameters: spin lock frequency: 500 Hz, TSLs: 0, 20, 40 and 60 ms, FOV: 8 cm, acquisition matrix: 256 x 160, slice thickness: 3 mm, NEX: 0.65. Image analysis: Morphologic images were evaluated at the surgical level and at a normal disc. Qualitative features evaluated included: new bone formation, sclerosis, osteolysis, endplate irregularity, herniation pits. Linear measurements were also taken of the disc height, disc width, and annulus thickness. Quantitative T2 and T1ρ values were calculated on a pixel-by-pixel basis by fitting the echo time or spin lock time to the corresponding signal intensity data (Functool 3.1, GE Healthcare, Milwaukee WI) using a mono-exponential decay equation: $SI(TE) \propto \exp(-TE/T2)$ and $SE(TSL) \propto \exp(-TSL/T1\rho)$, respectively. Local T2 and T1ρ values were evaluated by placing regions of interest (ROIs) on the nucleus pulposus, annulus and endplate of the normal and tissue engineered disc. Statistical analysis: Frequency tables were generated for qualitative morphologic scoring variables. A paired t-test was performed for morphologic measurements and T2 and T1ρ values to detect differences between the normal disc and tissue engineered disc. Statistical significance was set at $p<0.05$.

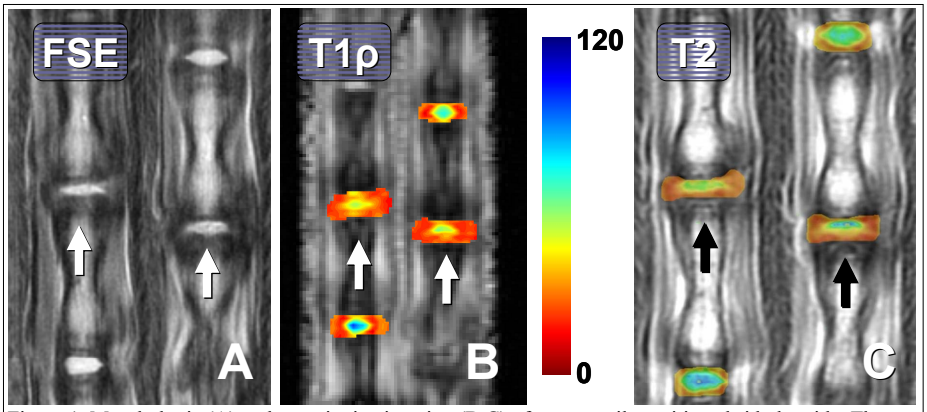

Figure 1. Morphologic (A) and quantitative imaging (B,C) of two rat tails positioned side-by-side. The tissue engineered intervertebral discs are indicated with arrows.

Results: Morphologic: None of the tissue engineered disc sites demonstrated reactive osteolysis. One construct demonstrated new bone formation and two construct displayed sclerosis. A majority of sites showed some degree of herniation of endplate into the disc space (6/8, 75%). Most sites (5/8, 63%) had an annulus that was isointense to normal annulus; however, these tissue engineered disc sites were hypertrophied compared to normal disc. The tissue engineered disc nucleus was significantly wider, p=0.049, and shorter, p=0.0001, than the normal disc, and the annulus was significantly thicker, p=0.0001. Quantitative: The tissue engineered discs had significantly shorter T2 values at the nucleus (p = 0.0003), the annulus (p=0.0004), and the endplate (p=0.0006) than the native discs. Only the nucleus of the tissue engineered discs had significantly lower T1ρ values than normal discs, p=0.0007.

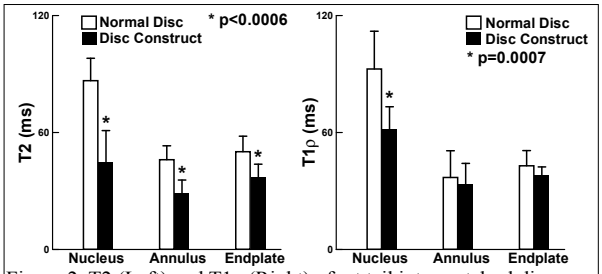

Figure 2. T2 (Left) and T1ρ (Right) of rat tail intervertebral disc. The disc construct had significantly shorter T2 values at all ROIs evaluated, and tended to have shorter T1ρ values.

Discussion. This study evaluated a novel tissue engineered intervertebral disc construct in a rat model using a clinical MR scanner and prototype solenoid coil. The morphologic images provided good image quality data to evaluate the tissue engineered disc and to display anatomic features of the intervertebral disc to assess sclerosis, herniation, and osteolysis of the tissue engineered construct. The differences seen in the T2 values reflected the orientation of collagen in the annulus and hydration of the disc and are similar to those reported in the human literature with normal discs demonstrating lower T2 values in regions of higher collagen fiber content (peripheral annulus and intranuclear cleft) [6]. T1rho values were only significantly different in the nucleus, reflecting the presence of proteoglycan and reduced water content [7]. Prior studies have demonstrated a negative correlation between relaxation time of T2 and T1ρ and disc degenerative grade. In two sites, the tissue engineered disc showed subtle differences on gray scale images; however, the corresponding T2 and T1ρ maps highlighted biochemical differences at the construct sites. This may reflect a difference in the construct to the native disc or represent a stage of immature biologic incorporation. The current data suggest MRI at clinical field strengths can be used to assess rat tail intervertebral disc structure, and quantitative MRI can show differences between the tissue engineered discs and the native tissue. The preliminary data suggest that at the 10 month interval, minimal differences were noted in the relative PG content in the annulus of engineered versus native discs, and in the nucleus, the engineered discs demonstrated approximately 2/3 PG content relative to the native disc. Quantitative MRI may be helpful in assessment of the healing of intervertebral disc constructs. Histopathology is pending to correlate qMRI findings.

References. 1.O'Connell GD, et al. *Spine* 32(3), 2007. 2.Elliott DM, et al. *Spine* 29(7), 2004. 3.Lai A, et al. *Med Eng Phys* 29(7), 2007. 4.Saldanha KJ, et al. *Eur Cell Mater* 16 2008. 5.Maier CF, et al. *J Magn Reson Imaging* 17(3), 2003. 6.Perry J, et al. *AJNR Am J Neuroradiol* 27(2), 2006. 7.Blumenkrantz G, et al. *Magn Reson Med* 63(5), 2010. **Acknowledgements.** Institutional research support was provided by General Electric Healthcare.