# New MRI Methods for the Monitoring of the Intervertebral Disc Ablation

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**Introduction:** The intervertebral disc (IVD) is composed of three regions: the nucleus pulpous (NP) – a gelatinous core containing proteoglycan (PG) and type-II collagen, the annulus fibrosus (AF) – the outer ring composed of type-I collagen lamellae, and the cartilaginous end plates. Disc degeneration, a major cause for back pain, is characterized by the loss of PG from the NP, which results in reduced water content, reduction of disc width and disorganization of the annular ring. In cases of severe pain caused by bulging of the NP and pressure application on surrounding nerves, disectomy or nucleus ablation are used. Tissue engineering provides an approach that could potentially regenerate a damaged disc by the introduction of repair cells, like Mesenchymal Stem Cells (MSCs). One of the major goals in tissue regeneration research is the establishment of valid imaging modalities that will provide a solid quantitative analytical tool for the evaluation of the repair processes. We have previously shown that Double Quantum Filtered (DQF) MRI can be used for the analysis of tendon repair using MSCs, based on the organization of collagen fibers (1).
In this study we used quantitative MRI parameters such as $T_2$ , magnetization transfer ratio (MTR) and dipolar echo refocusing techniques as well as $^2$H double quantum filtering (DQF) for monitoring early changes after nucleus ablation in order to provide a baseline for future work of disc repair.

**Materials and Methods:** Using Fluoroscan guidance and Micromanipulator two needles were inserted into the NP through the right and left postero-lateral aspect of WISTAR rats coccyges spine (C3 or C4). NP was removed by wash with PBS till the leakage of NP substance through the opposite needle was completed. Animals were sacrificed on day 5, tails fragments containing C2-C3 or C3-C4 segments were dissected and samples were immersed in fluorinated oil for the MRI measurements. $T_2$ relaxation times, and $T_2$ maps, were acquired using the multi slice multi echo pulse sequence (128x128, TR/TE=3000/3 ms, 128 echoes, 8.45T). The contribution of the residual dipolar interaction to $T_2$ was refocused by dipolar echo refocusing: $90^o - [\tau_{cp} - 90^o - \tau_{cp}]_n$ – Imaging (2, 3), where $\tau_{cp}$ is short (50 μs) and n = 20-5000. The relaxation time obtained by this method is denoted $T_{DE}$. MT images were acquired using a saturation pulse with a magnitude of 6 μT at different off resonance frequencies. The MTR results are expressed by: $(1-Mz/M_0)x100$. $^2$H double quantum filtered (DQF) images were acquired with the basic DQF pulse sequence: $90^o - \tau/2 - 180^o - \tau/2 - 90^o - t_{DQ} - 90^o$ – Imaging (4), where $t_{DQ}$ is the double quantum evolution time and τ is the creation time of the 2nd rank tensors. For $^2$H measurements the tails fragments were equilibrated for a few hours in deuterated saline

**Table 1.**

| | | $T_2$, ms | $T_{DE}$, ms | $1/T_{dipol}$, $sec^{-1}$ | MTR, % |
|---|---|---|---|---|---|
| Intact IVD (n=5) | NP | 83.1 ± 7.5 | 162.6 ± 10.5 | 5.9 ± 1.0 | 8.8 ± 1.9 |
| | AF | 19.1 ± 3.7 | 36.4 ± 3.8 | 26.2 ± 8.6 | 61.4 ± 4.3 |
| Ablated IVD (n=2) | all the disc | 15.0 ± 1.4 | 32.6 ± 5.6 | 35.7 ± 11.9 | 45.4 ± 1.4 |

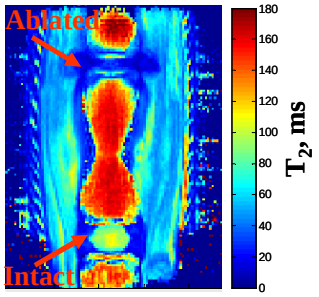


**Fig 1 - $T_2$ map of intact and ablated IVD in rat tail 5 days after NP removal.**

**Results:** The $T_2$ map of intact and ablated IVDs is given in Fig. 1. For the intact disc there is a clear distinction between the nucleus and the annulus, with a relative short $T_2$ is obtained for the AF and a much longer $T_2$ for the NP (see Table 1). After removal of the NP the width of the disc is significantly decreased, the distinction between the AF and the NP is lost and $T_2$ obtained is short in the entire disc. $T_2$ and $T_{DE}$ for intact and ablated discs are listed in Table 1. The results of the difference in relaxivity between these two measurements $1/T_{dipol} = 1/T_2 - 1/T_{DE}$ is a measure of the contribution of the residual dipolar interaction due to anisotropic reorientation of the water molecules to the relaxivity. This contribution is very small in the nucleus pulpous and high in the ablated discs. Magnetization transfer experiments, given in Fig. 2, followed the same trend: high MTR for the intact AF, almost negligible MTR for the intact NP and a relatively high MTR for the ablated disk (Table 1). The intact AF is clearly depicted by $^2$H DQF (Fig. 3). However in the $^2$H DQF image of the ablated disc most of the disc area is highlighted. $^2$H DQF spectra of NP from human IVDs was previously reported (5).

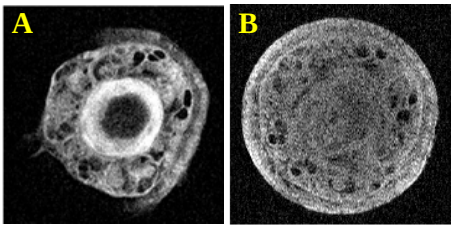


**Fig. 2 – magnetization transfer images of intact (A) and ablated (B) IVDs.**

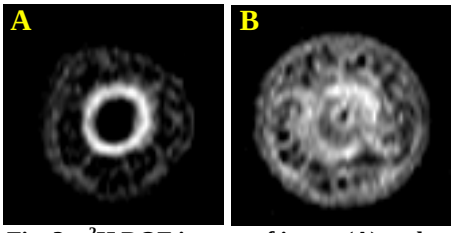


**Fig. 3 – $^2$H DQF images of intact (A) and ablated (B) discs. τ = 1.6 ms.**

**Discussion:** In the intact AF and throughout the ablated disc, $T_2$ and $T_{DE}$ are short and $1/T_{dipol}$, a measure of the contribution of the dipolar interaction to the transverse relaxivity, as well as MTR, are large. These results indicate a large amount of collagen fibers in the intact AF and the ablated disc relative to the intact NP. The increase in the collagen fiber concentration is caused by: a spreading of the collagen fibers of the annulus into the void left after the removal of the NP, and the decrease of the disc width due to the loss of the NP matrix and its ability to withstand pressure. The $T_2$ values and $T_{DE}$ results are in accordance to $T_2$ (6) and $T_{1\rho}$ results (7, 8), respectively, obtained for human intact and degenerate IVDs. The results of the variety of the NMR methods, all indicating the spread of the collagen fibers into the inner part of the ablated disc, are supported by histology (Fig. 4), where the collagen fibers (collagen stained green) are seen in the inner part of the disc as well as $^2$H DQF results.

**Conclusions:** The results presented demonstrate our ability to monitor the early changes in the IVD post-nucleus ablation, serving as a model for disc degeneration. They also provide us with a baseline that will aid future attempts for disc regeneration and with a detection method for evaluating our success.


**Fig. 4 – Histology (Masson Trichrom 5 days after NP removal.**

**References:** 1. Hoffmann et al., J Clin Invest, 116, 940, 2006. 2. Muller K et al., J. Magn. Reson. 90, 19, 1990. 3. Eliav U et al., Proc. ISMRM 6th Mtg. p. 602, 1998. 4. Tsoref L et al., Magn. Reson. Med. 40, 720, 1998. 6. Watanabe A et al., AJR, 189, 936, 2007. 6. Perea W. et al., Magn. Reson. Med. 57, 990, 2007 7. Johannessen W et al., Spine, 31, 1253, 2006. 8. Majumdar S, NMR Biomed, 19, 894, 2006.