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A non-destructive method to distinguish the internal constituent architecture of the intervertebral discs using 9.4 Tesla MRI.

Introduction

The intervertebral disc (IVD) plays a crucial role in the biomechanics of the spine, allowing articulation between the vertebrae. Even minor changes in the IVD, whether biological or structural, have the potential to cascade into greater irregularities and significantly affect the biomechanical functions of the spine. Consequently, it is one of the major sites for back pain, which approximately 70% of the population will experience at some point in their lives [1].

Detailed information on the substructure of the IVD is usually obtained from histological slices [2, 3]. This technique has limitations due to being two-dimensional and destructive. Two dimensional micrographs cannot provide accurate height-width-depth as well as spatial orientation details due to sectioning plane alignment and orientation. Hence, there is an element of "educated guesswork" involved in understanding such information, which may affect the reliability and consistency of the interpretations. Lack of coherent information along the 3rd direction is particularly disadvantageous when characterising highly three dimensional structures such as IVDs. For instance, the lamellae in the annulus fibrosus (AF) are curved in their macro state and there exists an alternating fibre pattern in the adjoining lamellae. This fibre orientation is nearly impossible to visualise on a 2D histological section. A three-dimensional method could provide clearer and further information, for example on the morphology of the lamellae within the AF, or the location of annular tears, and could have diagnostic applications if it were non-destructive. Such a technique could also be used pre-clinically during in vitro testing in which different interventions are compared, or for generating accurate computational models of the IVD.

Ultra high-field strength magnetic resonance imaging (MRI) systems (4 - 9.4T) are an emerging technology for clinical applications. Their capabilities, such as improved signal to noise ratio (SNR) and higher spatial resolution, have potential for improved imaging of tissues. The technology has been shown to produce favourable results for imaging of brain tissue, but cardiac and abdominal applications have

posed a greater challenge [4-8]. As yet, the use of ultra-high field MRI for spinal applications has been relatively limited. Therefore there is a need to examine this imaging methodology for spinal tissue structures, to assess the feasibility of obtaining relevant structural and biological information, as well as identifying suitable imaging protocols.

Visualisation of the IVD would benefit from higher resolution due to the multiple structurally distinctive constituents. The hydrated nucleus pulposus (NP) provides a high signal. The AF consists of multiple lamellae and the fibre orientation is completely different in these alternating layers, exhibiting a criss-cross pattern. Consequently the signal intensity will change depending on the fibre direction. The IVD endplate (EP) also gives rise to different signal intensities due to its cartilaginous and bony components. The aim of this study was to investigate the potential of ultra-high field strength MRI to obtain higher quality 3D volumetric MRI data sets of the IVD in order to better distinguish structural details. Two potentially suitable protocols were investigated and comparisons were made with histological imaging of the same IVD.

Materials and Methods

Sample Preparation

Two spines from 5-6 year old sheep (Texel-cross) were obtained from a local abattoir, within 24-hrs of slaughter, with veterinary approval. The sheep weighed approximately 40 kg. Two spinal segments (one from each spine), containing the C7-T1 and T2-T3 IVD and adjoining vertebrae were separately harvested. Each segment, after securing in a bench grip, was trimmed cranially and caudally by sawing through the vertebral bone close to the vertebral endplate. The posterior bony elements were removed close to the posterior margin of the vertebral bodies. The surrounding soft-tissue including the ligamentous structures was also removed. The final trimmed segment was approximately 25 mm in diameter and 20 mm in height. The segments were wrapped in phosphate buffered saline (PBS) soaked paper tissues in order to keep them moist and to avoid drying up during the

handling and freezing. PBS was used due to its isotonicity. Subsequently the segments were stored at -20°C.

Prior to MRI scanning, samples were defrosted overnight at 3°C. The sample dimensions were checked during every intermediate step of the process and no measureable changes were found. The samples were positioned in a MRI-safe polymer container with the anterior-posterior direction through the specimen mid-sagittal plane marked on the exposed vertebral bony surface of the specimen. This marked direction was used to align the specimen during mounting in order to acquire images in the anterior-posterior sagittal or coronal planes of the sample. The containers were sealed with screw-tightened lids during scanning to minimise dehydration.

In view of different possibilities for the change in structural geometry of the sample or loss of material during the decalcification process, a post-decalcification MRI scan was also performed with the same pre-decalcification scanning parameters in place. The objective was to minimise the geometrical discrepancies between the scanning and the histology procedures.

MR Imaging and Analysis

MR imaging was performed on a 9.4T scanner having a 89mm internal diameter vertical bore (AVANCETM II 400 MHz laboratory NMR system, Bruker, Switzerland) at a temperature of 22 ± 0.5 °C The RF coil is; 30 mm diameter, birdcage, 1H coil. 3-dimensional (3D) spin echo (SE) and gradient echo (GE) acquisition techniques were used to achieve high spatial resolution with isotropic voxel dimensions. Given the long acquisition times only one pulse sequence was applied to each sample to avoid excessive dehydration of the fresh specimens. The first sample (C7T1) was imaged using a 3D SE multislice-multiecho pulse sequence (MSME) and the second sample (T2T3) was imaged using a 3D spoiled GE pulse sequence (Fast Low-Angle Shot – FLASH). The SE and GE-based pulse sequences provide complementary information: the SE pulse sequence was chosen as the use of multiple 180° RF refocusing pulses minimises the effect of magnetic field homogeneities induced by the differing magnetic susceptibilities of the different tissue components, therefore resulting an overall higher signal-to-noise ratio. The absence of the 180° refocusing pulse in the GE sequence results in signal

intensities that are influenced by variations in magnetic susceptibility (known as susceptibility weighted or $T2^*$-weighted contrast). The absence of the 180° RF pulse and the use of a low flip angle excitation pulse allows a much shorter repetition time to be used for the GE pulse sequence in comparison to the SE pulse sequence, enabling a much higher acquisition matrix to be acquired within a comparable acquisition time.
The image acquisition parameters for each pulse sequence are given in Table 1.

Although the SNR is not an absolute measure for quality of biomedical images, it is the most popularly used quantitative method both for assessing the quality of images and for evaluating the effectiveness of image enhancement and signal processing techniques [9i]. When the magnitude images are calculated from a single set of complex raw data and the spatial distribution of noise is homogeneous, the commonly used technique is based on the signal statistics in two separate regions of interest (ROIs) from a single image: one in the tissue of interest to determine the signal intensity, and one in the image background to measure the noise intensity [10,11]. Therefore this method was selected, using a similar approach to several published studies [12,13]. In order to compare the image quality from the two MR sequences, the SNR was measured using image processing software (ImageJ Version1.45s, NIH, USA). Separate rectangular areas were selected within the AF, NP and in the vertebral bone respectively. These areas were copied through the image stack, and the consecutive slices were checked to ensure that selected areas were within the corresponding region under consideration. The mean value of the signal intensity for each of these selected areas was calculated. The largest possible rectangular region outside the IVD in the image background (air) was also selected, and the standard deviation of the background noise intensity was calculated. The SNR for each of the areas was then determined by dividing its mean signal intensity by the standard deviation of the background noise intensity.

The contrast-to-noise (CNR) ratio was calculated as

$$CNR_{a,b} = (S_a - S_b)/sd_{\text{background noise}}$$

where S denotes signal intensity, 'sd' denotes standard deviation, subscripts a and b denote corresponding tissue constituents. The Relative Contrast (ReCon) was calculated as

$$ReCon_{ab} = [(Sa - Sb)/ (Sa + Sb)]x100$$

For each sample, five different images were randomly chosen for SNR, CNR, and relative contrast measurements. The mean value based on the values of the five images was finally obtained.

Comparison MR Image Stack with Histology

After the initial scanning, the C7-T1 sample was fixed in 10% (v/v) neutral buffered formalin. It was then transferred to 100 ml ethylenediaminetetraacetic acid (EDTA) of pH 7.0-7.4, for decalcification.[14] The sample was continuously shaken and the EDTA solution was replaced daily for a period of seven days, and subsequently every week for a minimum of 16 weeks. The specimen was imaged using micro-computed tomography (Scanco, microCT100, Switzerland) with a hydroxyapatite phantom for bone mineral density calibration, in order to confirm that complete decalcification had been achieved.

The specimen was then sectioned into two halves through the marked mid anterior-posterior line. The resulting right and left halves of the sample were processed and embedded separately in paraffin wax. Using a Leica RM2255 microtome (Leica Microsystems, Milton Keynes UK) 5µm sections were obtained parallel to the anterior-posterior direction such that their orientation was the same as the imaging plane of the MRI. A selection of sections were stained with haematoxylin & eosin (H&E) and scanned using a slide scanner (Aperio AT2 -Leica Microsystems, Milton Keynes UK)) with a 20 x objective lens. The digital micrographs were sequentially arranged so that the complete stack represented images from the right lateral margin through to the left lateral margin of the sample.

Since there was potential for changes in the structural geometry of the sample or loss of material during the decalcification process, a post-decalcification MRI scan was also performed using the same scanning parameters (except the number of averages). The objective was to minimise the geometrical discrepancies between the scanning and the histology procedures.

From the post-decalcification MR image stack which consisted of sequential sagittal images between the right and left lateral margins of the specimen, the images representing the sagittal section of the sample at 0.2, 0.4, 0.6, and 0.8 proportional distances between the right and left lateral margins were selected. These were then compared with similarly selected histological micrographs using landmark features and quantitative measurements. (Figures 1-3)

Results

The SNR and CNR values obtained in order to quantitatively compare the images between the different sequences and under different conditions are given in Tables 2 and 3. In all scans, the nucleus produced the best signal. Therefore, ReCon values were calculated in relation to the signal intensity of the nucleus, and are shown in Table 4. The SNR values obtained with SE imaging were higher than those for GE images. It was also evident that when comparing the pre and post-decalcification images, while the SNR values for the post-decalcification images were better, the relative contrast remained largely unchanged except for the vertebral bone marrow (Figure 4A and 4B). This was likely to be due to the tissue hydration taking place during the decalcification process. The relative contrast between different constituents under GE was relatively poor.

The GE images across the anterior AF of the T2T3 IVD showed the highly organized AF structure comprising of collagen fibres oriented at alternating directions with successive layers. (Figure 4C and 4D) The angle between these alternating fibre directions was measured in ImageJ software by drawing line segments parallel to the fibre directions. As shown in Figure 5, this angle was found to be 59°.The MRI-histology comparison is presented in Table 5 for confirmation of the features seen on the MR images.

Discussion

Anatomically, the intervertebral disc exhibits three-dimensional structural characteristics. Using two-dimensional projection imaging to study these characteristics is not only challenging but can lead to erroneous interpretations. Recent advances in MRI hardware, software and pulse sequence design have provided the capacity to acquire 3D volumetric MRI scans of the human spine with

187 nearly isotropic resolution under 0.5 mm^3 (e.g. 3D FSE), compared to traditional 2D
188 scans acquired with inter-slice gaps of around 3mm. Automatic segmentation
189 algorithms have been developed [9] that extract detailed 3D representations of the
190 IVDs from volumetric MRI. The introduction of phased array coil technology with an
191 increased number of coil elements [15], and increased field strength, both of which
192 increase the baseline SNR, are particularly important for studies that are notoriously
193 SNR deprived, for example, in the AF region of the IVD. Even though ultra-high-
194 field strength MR scanners are not yet common in the current clinical setting, there
195 is an upward trend in terms of field strength, allowing higher resolution imaging as
196 well as an increased SNR for the acquired images. This opens up the possibility
197 of capturing structural details that previously had not been possible to distinguish in
198 vivo. In addition, small bore ultra-high field strength MRI scanners are increasingly
199 being used in laboratory settings for in vitro studies. The aim of this study was to
200 examine the potential of ultra-high field MRI to visualise the substructure of the
201 IVD. In order to confirm the details captured, histology of the same sample was
202 carried out in a manner which allowed the comparison of selected MRI slices with
203 corresponding histological sections.

204 Two MRI pulse sequences were used in this study: gradient echo and spin echo.
205 Each method has its own advantages as well as disadvantages. For instance, a
206 gradient echo sequence allows faster image acquisition, and is more sensitive to
207 magnetic susceptibility effects compared to spin echo, however in general spin echo
208 provides better SNR for the images.

209 The SNR ratio derived from the acquired images was higher for the SE sequence.
210 This was partly a consequence of the larger voxel size chosen for this sequence.
211 However, the GE (FLASH) sequence, helped by the higher spatial resolution,
212 revealed more information about the IVD constituents. Collagen present in the IVD
213 is generally difficult to image with MRI, hence the use of gadolinium chelates and
214 magic-angle imaging [16] have been suggested to improve visualisation. This study
215 indicates how ultra-high resolution imaging can provide useful structural information
216 of the collagen-rich IVD.

217 The consistently higher SNR values obtained for the respective regions after
218 decalcification (Table 2) clearly suggested that tissue hydration had taken place

219 across the entire sample. However, importantly, when ReCon values were
220 considered (Table 4), the relative contrast between different regions remained
221 relatively the same except with the vertebral bone marrow where ReCon was
222 reduced by nearly 40% from the pre-decalcification value. This indicated that the
223 hydration during decalcification was relatively similar across the disc, but
224 substantially higher when the vertebral bone marrow regions were under
225 consideration. Understandably the bone trabecular structure has the capacity to
226 accommodate more water. Since the hydration was similar across the disc it was
227 not an issue for consistent visualisation of structural details of the IVD.

228 The MRI-histology comparison confirmed that the features seen on the MR images
229 were realistically related to the actual structures. (Figure 1) It was evident from the
230 histology micrographs that lamellae thicknesses vary considerably. (Figure 2) MR
231 and histology images matched well in terms of the lamellae number, shape, position
232 and orientation. However, due to the relatively low number of voxels across each
233 lamella, attempts were not made in this study to measure the thickness of the
234 lamellae. As such, on the MRI images, peaks and troughs of the grayscale profile
235 along a selected length were used only to interpret the lamellae alternation (Figure
236 3).

237 The lamellae architecture demonstrated here, especially the fibre alternating pattern,
238 is not visible in vivo using routine MR imaging pulse sequence protocols and may
239 be impossible to visualise even when using specially optimised imaging protocols in
240 a high field (3T) clinical system. This alternating pattern is also difficult to visualise
241 using normal 2D histological techniques across the whole disc, due to its 3D
242 morphology. The identification of the lamellae structure across the entire AF, the
243 lamellae discontinuities and the visualisation of fibre bundles in the AF were only
244 possible due to the higher spatial resolution adopted in the study. Furthermore it
245 facilitated clearer demarcation of the main IVD regions; the AF, NP and the EP.
246 The technique presented here therefore exhibits the potential for improved 3D
247 structural characterisation. It has important applications in computer aided simulation
248 of tissue structures since better definition of morphology during model development
249 certainly increases the accuracy of the simulation outcomes.

There are also potential applications in minimally invasive procedures and microsurgery, where detailed preparation and surgical planning with the aid of high resolution and three-dimensionally reconstructed MRI data would help individually tailored, safe and precise surgical approaches.

Disc degeneration is associated with biochemical and morphological changes. Previously, planar morphological IVD measures (height, width and shape) have been studied in the context of IVD degeneration and ageing, to understand the symptoms and factors influencing the lower back pain [17]. It is believed that disc injuries such as needle punctures, annular tears or ruptures, contribute towards disc degeneration in the longer term. Intervertebral disc herniation is a major cause of back pain but the underlying mechanisms are still not well understood. Based on animal studies Tampier et.al. [18] has suggested that formation of clefts in weaker areas in lamellae due to intradiscal pressure, and the nucleus material advancing through these clefts towards the periphery. Prime sites for these weaker areas are where lamellae discontinuities exist or lamellae thickness narrows. High resolution 3D volumetric data sets allow improved recognition of any advancement of nuclear material through these sites, thereby facilitating early detection of prospective disc herniation.

Adverse spinal loading is thought be a key driver for early disc degeneration. Although there are studies that outline measurement of regional strains, mostly under axial loading, a matter that is under considerable discussion is the lamellae deformation pattern, particularly the inner layers, at various locations, under different modes of loading (eg. flexion, extension). Here again, the positive results of this study demonstrate the potential of high resolution 3D data sets to expand the current understanding.

This study has highlighted the potential of ultra-high-field strength MRI to obtain higher quality 3D volumetric MRI data-sets for better structural characterisation. The extra-long acquisition times seen here pose a major limitation and a challenge for clinical translation of the method.

However, the technical development of MRI is still continuing and improvements in hardware and detector systems are paving the way for more efficient image acquisition. Recent technical and methodological developments such as parallel MRI techniques [19] and advances in signal processing [20] are resulting significantly reduced acquisition times and have potentially opened new avenues for the clinical application of ultra-high-field MRI. It is possible that the acquisition time could be reduced by increasing the echo train length. Further work is needed to investigate whether it is possible to identify the IVD lamellae architecture in vitro through optimisation of pulse sequences to obtain sufficient spatial resolution in a clinical 3T MR system. If so, the findings could be compared with larger scale MR imaging methods which may enable characterisation of the IVD and could potentially be translated to in vivo imaging.

In summary, the positive results of this study demonstrate the prospects of ultra-high field strength MR to non-destructively characterise the 3D structure of the IVD. This has applications in pre-clinical testing, for example in imaging in vitro specimens before and after simulated interventions or loading regimes to examine changes in substructure. In the longer term, if higher field strength systems become more widespread clinically, then their capacity to visualise the disc substructure mean they have the potential to become an important diagnostic tool.

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References

306 1. Macfarlane GJ, Thomas E, Croft PR, et.al. Predictors of early improvement in
307 low back pain amongst consulters to general practice: the influence of premorbid
308 and episode-related factors. *Pain* 1999; 80: 113–119.
309 2. Freemont AJ, Watkins A, Le Maitre C, et.al. Current understanding of cellular
310 and molecular events in intervertebral disc degeneration: implications for therapy, *J*
311 *Pathol* 2002; 196: 374–379.
312 3. Roberts S, Evans H, Trivedi J, et.al. Histology and pathology of the human
313 intervertebral disc. *J Bone Joint Surg Am* 2006 Apr;88 Suppl 2:10-4.
314 4. Bernstein MA, Huston J, Ward HA. Imaging artefacts at 3.OT. *J Magn Reson*
315 *Imaging* 2006;24:735-46.
316 5. Gutberlet M, Noeske R, Schwinge K, et.al. Comprehensive cardiac magnetic
317 resonance imaging at 3.0 tesla —feasibility and implications for clinical applications.
318 *Invest Radiol* 2006;41:154-67.
319 6. Schindera ST, Miller CM, Ho LM, et.al. Magnetic resonance (MR)
320 cholangiography —quantitative and qualitative comparison of 3.0 Tesla with 1.5
321 Tesla. *Invest Radiol* 2007;42:399-405.

7. Schmidt GP, Wintersperger B, Graser A, et.al. High-resolution whole-body magnetic resonance imaging applications at 1.5 and 3 Tesla —a comparative study. *Invest Radiol* 2007;42:449–59.

8. Kuo R, Panchal M, Tanenbaum L, et.al. 3.0 Tesla imaging of the musculoskeletal system. *J Magn Reson Imaging* 2007;25:245–61.

9. Neubert A, Fripp J, Engstrom C, et.al. Automated detection, 3d segmentation and analysis of high resolution spine MR images using statistical shape models. *Phys Med Biol* 2012, 57(24):8357-76.

10. Kaufman L, Kramer DM, Crooks LE, et.al. Measuring signal-to-noise ratios in MR imaging. *Radiology* 1989;173:265–267.

11. Henkelman RM. Measurement of signal intensities in the presence of noise in MR images. *Med Phys* 1985;12:232–233.

12. Lavdas E, Vlychou M, Arikidis N, et.al Comparison of T1-weighted fast spin-echo and T1-weighted fluid-attenuated inversion recovery images of the lumbar spine at 3.0 Tesla. *Acta Radiol* 2010; 3: 290-295

337 13. Li X, Huang W, Rooney WD, et.al. QIN. Signal-to-Noise Ratio, Contrast-to-Noise
338 Ratio, and pharmacokinetic modelling considerations in Dynamic-Contrast-Enhanced
339 Magnetic Resonance Imaging. *Magn Reson Imaging*. 2012; 30(9): 1313–1322
340 14. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques, 5th
341 Edition, Churchill Livingstone, 2002
342 15. Roemer PB, Edelstein WA, Hayes CE, et.al. The NMR phased array. *Magn*
343 *Reson Med* 1990; 16(2):192–225.
344 16. Bydder GM. New approaches to magnetic resonance imaging of intervertebral
345 discs, tendons, ligaments and menisci. *Spine*, 2002; 27: 1264–1268
346 17. Pfirrmann CW, Metzdorf A, Elfering A, et.al. Effect of aging and degeneration
347 on disc volume and shape: A quantitative study in asymptomatic volunteers. *J*
348 *Orthop Res* 2006, 24(5): 1086–1094.
349 18. Tampier C, Drake JDM, Callaghan JP, et.al. Progressive Disc Herniation, An
350 Investigation of the Mechanism Using Radiologic, Histochemical, and Microscopic
351 Dissection Techniques on a Porcine Model. *Spine* 2007; 32:2869–2874
352 19. Deshmane A, Gulani V, Griswold MA, et.al. Parallel MR imaging. *J Magn*
353 *Reson Imaging.* 2012;36(1):55-72.

354 20. Lustig M, Donoho D, Pauly JM. Sparse MRI: The Application of Compressed
355 Sensing for Rapid MR Imaging. *Magnetic Resonance in Medicine*. 2007; 58:1182–
356 1195.

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Tables

Table 1. Acquisition parameters for the 9.4T images

Table 2. SNR values obtained for the different MR sequences performed at selected conditions of the two specimens. The signal and the noise parameter measurements were based on pixel grey values (intensity).

Table 3. CNR values obtained for the different MR sequences performed at selected conditions of the two specimens. The signal and the noise parameter measurements were based on pixel grey values (intensity).

Table 4. Relative contrast values obtained for the different MR sequences performed at selected conditions of the two specimens. The signal and the noise parameter measurements were based on pixel grey values (intensity).

Table 5. Quantitative comparison of selected MRI slices with correspondingly located histology micrographs

Figures

Figure 1. A selected MRI slice from the post-decalcification scan of the C7T1 IVD and the corresponding histology micrograph. In both images the circles indicate comparative landmarks, vertical arrows indicate height measurements, and the line across the annulus is for lamellae counting.

Figure 2. Magnified image of the region in the histology micrograph in Figure 1, showing the line drawn across the annulus on the virtual slide for lamellae counting.

Figure 3. Greyscale variation plot along the corresponding line drawn across the annulus on the MR image for lamellae counting

Figure 4A. Selected slices from the C7-T1 disc pre decalcification, MSME sequence

Figure 4B. Selected slices from the C7-T1 disc post decalcification, MSME sequence (Supplemental digital content 1 – 10 show additional images for the C7-T1 disc post decalcification)

385 Figures 4C. & 4D. Selected slices from the T2-T3 disc, Flash sequence.
386 (Supplemental digital content 11 – 20 show additional images for the T2-T3 disc)

387 Figure 5. GE (FLASH) image located at the anterior margins of the T2T3 IVD,
388 demonstrating fibre angle measurement

389

390 **Supplemental Digital Content**

391 SDC 1 – 10; Additional images from the C7-T1 disc post decalcification MSME
392 sequence

393 SDC 11 – 20; Additional images from the T2-T3 disc Flash sequence