A Novel DTI-Histology Based 3D Model of the Annulus Fibrosus Microstructure Viewed in the Light of Evolutionary Medicine

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Background: Low back pain is an extremely common condition, far from being fully understood. Understanding of the intervertebral disc (IVD) most basic mechanics is a necessity on the path to a more effective medicine. Recent year's publications have begun to defy the simple classic description used to describe the annulus fibrosus (AF) as a set of concentric continues rings around the center nucleus and instead present a highly anisotropic, complex structure. DTI has been widely used to view and quantify biological tissue and thus can be effectively used in order to further reveal the 3D structure of the AF. Previous studies have shown feasibility of DTI of the AF, but have not yet been successful in capturing its 3D microstructure. In this work we used DTI to further reveal the morphological 3D microstructure of the AF and to follow its modifications within an evolutionary context, suggesting an explanation for disc hernia.

Methods: DTI was performed on two intervertebral discs harvested from human lumbar spine cadavers on a 7T/30 MRI scanner (Bruker, Germany) using a 20mm surface coil. The MRI protocol included a diffusion tensor imaging (DTI) protocol acquired with a diffusion-weighted spin-echo echo-planar-imaging (EPI) pulse sequence with the following parameters: TR/TE = 4000/20ms, Δ/δ=10/3.5ms, 4 EPI segments and 16 non-collinear gradient directions with b value of 1000s/mm^2. Geometrical parameters were: 10 slices with cubic resolution of 0.28x0.28x0.28 mm^3. In addition a RARE T2 weighted image was acquired with the following parameters: TR/TE =4000/15ms and cubic resolution of 0.072x0.072x0.072 mm^3. The total acquisition time was 41 hours.
Tractography was applied using the principal eigenvectors and FA: the brute force FACT algorithm was used to generate the fiber coordinates, terminating at voxels with FA lower than 0.2 or following tract orientation change higher than 60°.
Histological sections 5μ thick were taken of human and gibbon lumber spine and viewed with polarized light microscopy.

Results and Discussion: Initial results confirm previous findings regarding the anisotropic composition of the AF. While the T2 image (figure 1.A) shows what appears as a general homogenous structure, the fractional anisotropy (FA) map (figure 1.B) obtained from the DTI data presents a much more complex and anisotropic structure. There is a noticeable difference between the anterior and posterior regions of the AF. The higher FA seen in the posterior part implies to the difference between a thinner posterior AF with substantially more lamellae than the anterior section. The histological typical image (figure 1.C) of the highlighted square in figure 1.B confirms the uneven distribution of the lamellae width and points out at the gaps between the lamellae, which prevent them from completing full contours around the IVD as classically described. Instead, images taken from human and gibbon IVDs (figure 1.C and 1.D), show the lamellae start and terminate irregularly with changing lamellae of unequal width and length, having gaps often appearing between them. Most importantly, using DTI fiber tracking, we present for the first time a 3D structure of the AF microstructure (figure 2). This image presents two adjacent layers of lamellae fibers with opposing fiber directions crossing each other, than continuing around the IVD, but terminating much before forming a full circle. Viewing this important structure's architecture in 3D can effectively increase our understanding of its mechanism and its failure points in the human lumbar spine. Obtaining the 3D structure of the AF can, with further research, allow a true and effective comparison of the human AF to that of other primates which can aid in understanding the unique human spine's IVD having to deal with a challenging demands of posture and environment. It has been suggested that the basic structure of the AF, both on a macro and micro scale, has evolved to fit a quadruped animal, hence forth its routine failure in humans. This hypothesis could be effectively tested given the current results.
Conclusion: DTI is a strong and effective tool for characterization of collagen fiber alignment, both on a micro and macro scale and can greatly aid in unraveling some of the mystery behind the complex structure of the AF and subsequently offers the opportunity to better understand the source of suffering from herniated discs and from low back pain in general.

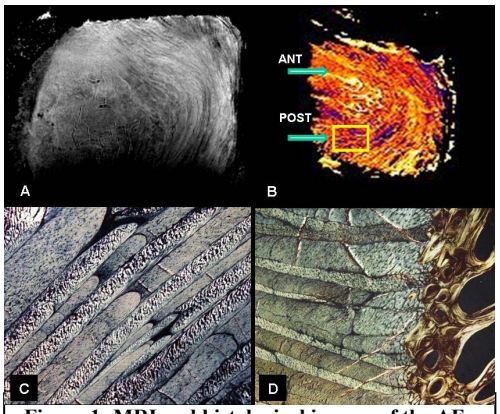

Figure 1: MRI and histological images of the AF: (A) A T2 image of a human lumbar spine IVD disc. (B) FA map obtained from the DTI data. Notice the difference in FA signal between the anterior and posterior parts of the IVD with the yellow highlighted square indicating the lamellae's anisotropic behavior, also viewed histologicaly in figure (C). Notice the substantial gaps between the lamellae appearing in this region. (D) The site of connection between a gibbon's AF fibers and the IVD endplate. Notice the irregular shape of the lamellae and the fact that not all of them terminate at the end plate. The different noticeable colored layers are due to their opposing orientations as they cross each other.

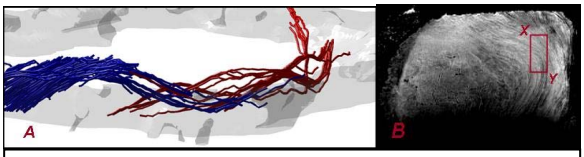

Figure 2: A 3D image of the lamellae architecture as arranged in the human lumbar IVD using fiber tracking. (A) Lamellae collagenous fibers from two adjacent lamella layers going in opposite directions crossing each other to create a complex structure around the nucleus polposus. Fiber tracking was created using two regions of interest (ROI) starting from different points. The closer one colored blue and the further one away colored red. (B) The T2 image shows the AF with the corresponding area selected highlighted in red.