Loss of Collagen Orientation Heterogeneity in Moderately Degenerated Human Intervertebral Discs

Marc van Rijsbergen, Msc¹, Roman Dittmar, MSc², Keita Ito, MD, ScD².

Disclosures: M. van Rijsbergen: None. R. Dittmar: None. K. Ito: None.

Introduction: The collagen fiber orientation of the healthy annulus fibrosus (AF), the outer part of intervertebral discs (IVDs), is generally reported to be 30°, yet this orientation is known to be non-uniform throughout the tissue [1]. It increases from the anterior to posterior location [1], i.e. in circumferential direction, and it also increases from the outer AF (OAF) to inner AF (IAF), i.e. in radial direction [2]. As IVDs start to degenerate, the amount and type of collagen change [3] and likely the collagen architecture is adapted. While it is well established that severely degenerated IVDs suffer from dramatic structural changes, less is known about effects of degeneration on the tissue architecture in moderately degenerated discs. Since collagen organization strongly affects disc functional biomechanics and mechanobiology of disc cellular behavior [2], long-term success of e.g. novel regenerative therapies aiming at treating early degeneration will likely also depend on a thorough understanding of the biomechanical environment they are exposed to. Hence, the aim of this study was to quantify the AF collagen orientation and its spatial distribution in moderately degenerated IVDs.

Methods: Seven moderately degenerated human IVDs (Pfirrmann grade III, levels L1-L2 and L3-L4) from five different donors (all male, mean age 62.8 ± 12 years) were dissected post-mortem following an IRB approved protocol. From each disc, IVD tissue samples containing the AF were dissected from four locations: anterior, right and left lateral, and posterior (n = 7 per location). Samples were split into OAF and IAF specimens by cutting the dissected samples at 2 mm from the disc periphery using a razor blade [1]. Next, 50 μm thick tangential cryo-sections of the OAF and IAF were obtained. Their collagen architecture was visualized without staining using Second Harmonic Generation (SHG) microscopy on a Leica TCS SP5X laser scanning microscope (Aexcitation = 810 nm). Per sample, 80 individual SHG images were acquired and stitched together into one mosaic image covering the entire IAF or OAF cryo-section. Images were then registered utilizing a custom written Matlab script. From this mosaic image, a representative sub-images (approx. 1 x 1.5 mm) were cropped and the primary collagen fiber orientation was determined using a custom collagen fiber tracking algorithm in Mathematica [4]. Also, collagen fiber proportionality was determined as ratio of fibers in the primary vs. other directions. To determine differences in primary collagen fiber orientation and proportionality between locations, blocked two-way analysis of variance (ANOVA; blocked per disc) followed by one-sided paired t-tests (Fisher’s LSD correction) was performed. Statistical significance was assumed for p<0.05.

Results: Mean primary collagen fiber angles were between 15° to 31° (Fig 1B) throughout the AF. No statistically significant differences were found between the mean primary collagen orientations of OAF specimens at the various circumferential locations. Still, a trend for increasing mean primary angle from anterior to posterior position was observed (Fig 1A). In the IAF, mean primary angle values were significantly different from each other between anterior and lateral locations (p=0.03). In contrast to the OAF, mean primary angle values in the IAF at lateral and posterior locations were very similar and larger as compared to the anterior location (Fig 1A). In the radial direction, fiber angles did not show strong alterations depending on the position, except for the posterior location where OAF and IAF angles were significantly different from each other (p=0.008). In both the OAF and IAF, mean fiber proportionality was 70 ± 20% (Fig. 1C). OAF samples showed significantly larger fiber proportionality posteriorly than laterally (p=0.03). No other statistically significant differences in proportionality depending on circumferential locations were observed. In contrast, IAF samples showed very similar mean fiber proportionalitys for all circumferential locations and no statistically significant differences were observed. Also, in radial direction, no statistically significant differences in collagen fiber proportionality between the OAF and IAF at the various locations were found.

Discussion: The results of this study provide the first evidence that moderately degenerated IVDs are characterized by a less spatially heterogeneous collagen fiber orientation. No strong collagen fiber gradients in circumferential or radial direction were determined, whereas healthy IVDs have been characterized by clear spatial gradients in collagen orientation. Also, the degree of collagen fiber anisotropy, as measured by the fiber proportionality, did not exhibit large variations in either circumferential or radial direction. We believe that this information will lead to a better understanding of the mechano-biological environment of moderately degenerated discs. Thus, key processes and risk factors involved specifically in early disc degeneration may be better understood, potentially leading to improved therapy. Furthermore, with the advent of novel MRI techniques that provide information on both collagen orientation and anisotropy in IVDs [5], changes to the AF collagen organization may serve as biomarker for improved diagnosis of early IVD degeneration.

Significance: The results of this study provide the first quantitative evidence that moderately degenerated discs are characterized by a more spatially homogeneous collagen fiber orientation in contrast to that reported for healthy IVDs.
Acknowledgments: We thank Prof. Ronald Bleys from the Department of Anatomy Utrecht Medical Center (Utrecht, The Netherlands) for providing cadaveric human lumbar spines. This research was partially funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreements MySpine (No. 269909) and Genodisc (No. 201626).


![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

Figure 1. A) Graphical presentation of primary collagen fiber orientation for OAF and IAF samples at the various circumferential locations. B) Absolute values of primary collagen fiber orientation for OAF and IAF samples at the various circumferential locations. A significant difference in fiber orientation between the anterior and lateral location of the IAF (p<0.05; *) and between OAF and IAF angles at posterior location (p<0.001; ***) was determined. C) Collagen fiber proportionality (in %) for both OAF and IAF samples at the various circumferential locations. A significant difference in fiber proportionality between the posterior and lateral location of the OAF was determined (p<0.03; ***)

ORS 2014 Annual Meeting
Poster No: 1589