High Resolution Characterization Of Intervertebral Disc Degeneration Using Equilibrium Partitioning Of An Ionic Contrast Agent Micro Computed Tomography (EPIC)-µCT

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Disclosures:

Introduction: Several small animal models are currently employed to study intervertebral disc (IVD) degeneration both in vivo and in vitro. Magnetic resonance imaging (MRI) techniques such as delayed gadolinium-enhanced MRI (dGEMRIC) and T1-rho MRI are capable of highly sensitive and quantitative characterization of structural and biochemical changes within human IVDs, but their use within small animals IVDs is limited by their low resolution. IVD tissue lacks the x-ray attenuation properties to facilitate high resolution imaging by µCT. Equilibrium partitioning of an ionic contrast agent-enhanced µCT (EPIC-µCT) can provide contrast enhancement in cartilaginous tissues by interacting negatively with sulfated glycosaminoglycans (sGAGs). This has been shown to provide high resolution data of sGAG distribution within articular cartilage [1]. This study aimed to employ EPIC µCT to map degenerative changes of the rabbit IVD both in vitro and in vivo.

Methods: The incubation of IVDs in ioxaglate contrast agent was optimized between dilutions of 40%, 50%, and 60% (v/v) in phosphate buffered saline containing protease inhibitors, and between incubation times of 3, 12, and 24 hours at 25°C. The optimal incubation parameters were determined to be 50% ioxaglate for 24 hours; all further scans were carried out using these parameters. In Phase I, in vitro enzymatic digestion of the nucleus pulposus (NP) of lumbar IVDs was undertaken. Lumbar spines from four healthy New Zealand White rabbits were excised and individual spinal segments isolated. 5 IVDs were injected with 10 μL of 50 Units/mL papain and incubated in PBS (pH=7.4) at 37°C for 24 hrs. 5 IVDs received injections at 0 and 24 hrs and were incubated for a total of 48 hours; 5 more IVDs were injected at 0, 24, and 48 hrs and were incubated for a total of 72 hours. Five control IVDs were incubated without papain injection. For imaging, samples were incubated in ioxaglate using optimized parameters and scanned using µCT (Triumph Trimodality, Gamma Medica, Salem, NH) with 100µm isotropic voxels, 1x1bin, 5 frame averaging, 2.0 zoom factor, at 60 kVp and 470 µA. IVDs were then fixed in formalin, reincubated with contrast agent, imaged again, and processed for histologic analysis of sGAGs with Safranin-O staining. Mean attenuation in the whole disc, nucleus pulposus (NP), and annulus fibrosus (AF) was calculated in Hounsfield Units (HU) using ROI-processing in Matlab (2013a, Mathworks, Nattick, MA). Histology was graded on a 4-point scale (0 = healthy, 4 = severely degenerative) in a blinded fashion. In Phase II, twelve New Zealand White rabbits underwent annular puncture and percutaneous nucleotomy in nonadjacent lumbar discs (randomized to L2-3 and L4-5) using an 18-gauge needle to induce degeneration. Control discs were above and below treated discs. Six rabbits each were euthanized after 3 and 6 weeks. Punctured, nucleotomized, and control IVDs were excised, imaged, and analyzed as described above.

Results: EPIC µCT images show extensive contrast enhancement of the IVD (Fig 1B) compared to non-enhanced CT (Fig 1A). The NP had significantly lower attenuation (320 HU ± 106) compared to the AF (471 HU ± 110, P=.011), demonstrating an inverse relationship between sGAG content and contrast agent uptake within the disc (Fig 1B). Results of the Phase I in vitro digestion demonstrate that EPICµCT is sensitive to molecular changes in the IVD. Papain digestion increased attenuation of the whole IVD 17.6% ± 23 at 24 hrs (P=.29), 20.0% ± 17 at 48 hrs (P=.18), and 23.6% ± 13 at 72 hrs (P=.07), and of the NP by 23.4% ± 40 at 24 hrs (P=.18), significantly by 42.8% ± 34 at 48 hrs (P=.033), and by 37.8% ± 25 at 72 hrs (P=.09). Saf-O stained histology demonstrates decreasing whole-disc staining intensity, confirming the loss of sGAG content due to digestion. Both 24-hr and 48-hr discs had significantly higher histologic grades compared to control discs (24 hr: 1.06 ± 0.68, P = .023; 48 hr: 1.93 ± .64, P < .001). Results of Phase II demonstrate that EPIC-µCT effectively characterizes in vivo IVD degeneration. At 3 postoperative weeks, attenuation of the whole IVD increased by 11.1% ± 14 in punctured discs (P=.10) and significantly by 24.7% ± 13 in nucleotomized discs (P=.042), and attenuation of the NP increased by 17.8% ± 24 in punctured discs (P=.13) and significantly by 54.1% ± 24 in nucleotomized discs (P=.014). At 6-weeks, attenuation of the whole IVD decreased by 0.309% ± 10.0 in punctured discs (P=.96) and increased significantly by 26.2% ± 20 (P=.013) in nucleotomized discs Figure 3A), and attenuation of the NP increased by 9.18% ± 24 in punctured discs (P=.54) and significantly by 29.1% ± 12 (P<.001). The histologic grade of 3-week annular puncture (1.27 ± 1.0) and 3-week nucleotomy (2.5 ± 0.79) animals were significantly higher than matched control discs (0.33 ± 0.31, P <.018 and P <.001, respectively). The histologic grade of 6-week annular puncture (2.00 ± 1.2) and 6-week nucleotomy (2.83 ± 1.0) animals were significantly higher than matched control discs (0.14 ± 0.26, P <.002 and P <.001, respectively). There were significant correlations between disc height (measured on histology) and whole-disc attenuation (R = .701, P <.001), disc height and NP attenuation (R = -.778, P < .001), disc height and histologic score (R = -.639, P <.001), histologic score and whole-disc attenuation (R = -.500, P <.001) and histologic score and NP attenuation. (R = .508, P <.001). Formalin fixation decreased whole-disc attenuation by 19.2% ± 16 (P<.001) and NP attenuation by 17.1% ± 19 (P<.001) across all samples. In addition to decreasing total attenuation, formalin fixation also decreased differences in attenuation between control
and degenerate discs, though major trends remained the same.

**Discussion:** EPIC μCT is sensitive to temporal and spatial differences in sGAG content in rabbit IVDs in both in vitro and in vivo models of disc degeneration. Though in vitro digestion is unable to cause a loss of disc height, higher attenuation due to the loss of sGAG content was measured, and this loss was confirmed with histology. Digestion appeared to level out after 48 hrs, indicating that more extensive digestion or higher resolution may be necessary. In vivo degeneration using the established models of annular puncture and nucleotomy [2] was effectively characterized using EPIC μCT. Both disc height and histologic grading, established metrics of disc degeneration, correlated highly with EPIC μCT attenuation, demonstrating that the loss of sGAG content due to degeneration is accurately measured even with decreasing tissue volume. As previously shown in literature [3], formalin fixation decreased absolute sample attenuation and also decreased differences between groups. In conclusion, EPIC μCT can be utilized as a non-destructive analysis tool for studying disc degeneration and/or regeneration approaches.

**Significance:** EPIC-μCT is a high resolution, contrast-enhancing CT method sensitive to temporal and spatial differences in sulfated glycosaminoglycan content in both in vitro and in vivo models of disc degeneration in a rabbit.

**Acknowledgments:** None

**References:**

![Figure 2. Representative contrast-enhanced μCT colormap of control (A), 6-week puncture (B), and 6-week nucleotomy (C); histology slice of control (D), puncture (E), and nucleotomy (F).](image-url)
Figure 1. Non-contrast-enhanced IVD (A); EPIC contrast-enhanced (B), with the edges of the IVD shown by a dotted line.
Figure 3. Whole-Disc attenuation (normalized to control) of 3 and 6 week in vivo discs (A). Nucleotomized discs exhibited significant increases in normalized attenuation at both 3 and 6