3D High-Resolution Micro-CT Imaging of Nucleus Pulposus with a New Contrast Agent in Preclinical Models of Disc Degeneration

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INTRODUCTION: Intervertebral disc degeneration (IDD) is the primary cause of low back pain, impacting 25% of U.S adults. It is prevalent in 40% of people over age 40 and 80-90% by age 80. MRI is a standard tool for diagnosing IDD but fails to detect early stages of degeneration. The nucleus pulposus is the soft, gel-like center of an IVDs in the spine. It's surrounded by the tougher, fibrous annulus fibrosus, acting as a cushion to absorb shock, distribute forces, and allow flexibility in the spine. Micro-computed tomography (micro-CT) is highly effective for 3D bone imaging but faces challenges in visualizing soft tissues like the intervertebral disc (IVD) due to limited contrast. To date, several contrast agents for IVD micro-CT imaging have been reported, but they have not successfully visualized the IVD effectively. This study explored potassium iodide (KI) and sodium iodide (NaI) as a potential contrast agent for micro-CT imaging of IVDs across various animal models to better understand IDD.

METHODS: We harvested lumbar spines and tails from mice, rats, rabbits, and sheep, fixed them with 10% neutral buffered formalin (NBF), incubated in 25% aqueous KI or NaI solutions for 30 minutes and scanned using micro-CT (Sky Scan 1273) with a resolution of 10 µm. To assess if KI can be used to investigate nucleus pulposus (NP) degeneration, we used trauma induced IDD mice model. We performed caudal needle-puncture-surgery (NPS) with 30-gauge needle on 12-week-old mice (n=10), with adjacent IVDs as sham control. Tails were collected two weeks post-surgery for KI staining. For aging model, we compared IVDs from 18–21-month mice (n=5) to 3-month controls (n=5) and validated our method on rabbit and sheep (n=5 in each group) IVDs. Micro-CT results were compared with histological data from the same sample. To test if KI is specific for NP, we decalcified the NPS mice tails by Immunocal, scanned to make sure the bone mineral contents were removed completely, then we stained the decalcified tail with KI and scanned with micro-CT using above parameters. We used sodium iodide to stain mice tails to test if NaI can donate the Iodine ions to provide contrast to the NP in healthy and degenerated IVDs.

RESULTS: Compared to unstained IVDs, KI-stained IVDs exhibited strong x-ray attenuation comparable to surrounding bone, enabling high-resolution 3D visualization of IVDs in the spine and tails of all animal models (Figure 1). Interestingly, both KI and NaI stained NP only, not AF. Quantitative measures such as IVD height and NP volume showed high reproducibility. IDD was detected in punctured IVDs using KI staining which was comparable to histological findings from the same sample. The results showed a significant reduction in IVD height, NP area, and volume (Figure 4) in the punctured IVD compared to sham IVDs (p=0.014). In aging mouse spines, KI staining revealed varying degrees of degeneration in lumbar IVDs. After scanning, KI was removed by PBS wash for immunostaining, with no additional antigen retrieval step required (Figure 2) showing that the KI stained tissue is compatible with downstream histological and immunostaining procedures. Moreover, decalcified tails after KI staining only showed the NP, removing the bone from the background (Figure 3). This shows KI specifically stains NP in the given staining time.

DISCUSSION: This study presents KI and NaI as rapid, reversible, non-destructive, histologically compatible, and accessible contrast agent for detailed micro-CT IVD imaging in preclinical investigations. Both KI and NaI clearly identified degenerative changes across species and models, and this method is significantly faster (<1 hr) than previously published methods (>24 hr to 2 months).

SIGNIFICANCE/CLINICAL RELEVANCE: Novel contrast agent, KI will revolutionize high-resolution 3D micro-CT imaging of IVDs, offering nondestructive, quantitative insights into disc structure and degeneration. This technique matches histology in accuracy while enabling longitudinal studies, making them critical tools for understanding IVD pathology and developing therapies for low back pain.

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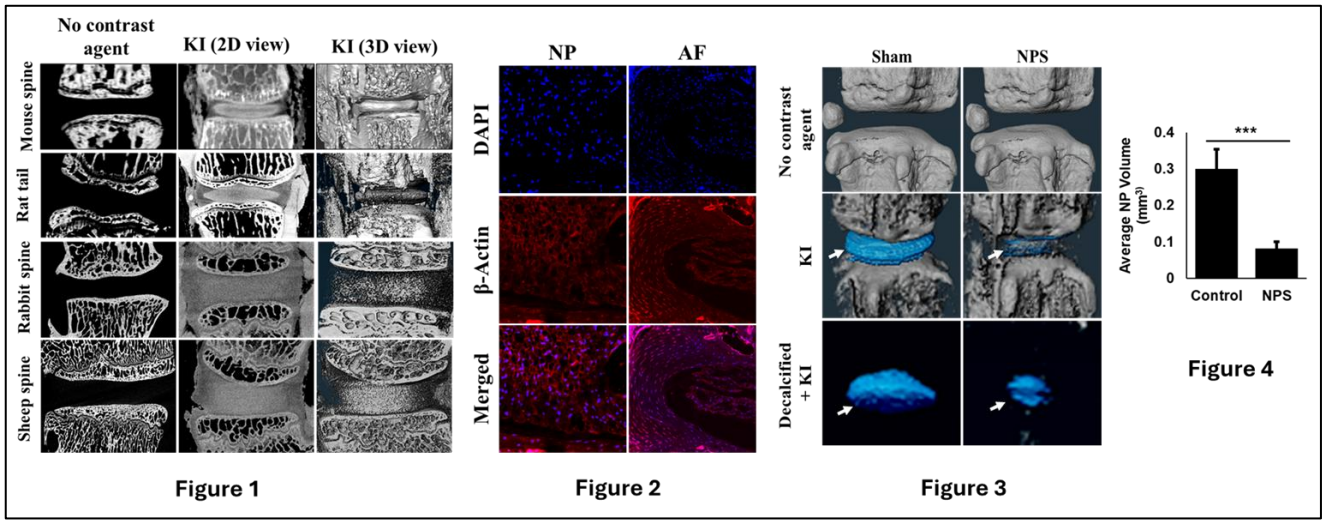

Figure 1: Visualization of NP in mouse spine and large animal IVDs with KI staining and micro-CT.
Figure 2: Immunostaining in disc samples processed after KI staining.
Figure 3: KI visualizes NP in the decalcified mouse tail.
Figure 4: The volume of NP in the sham and NPS IVDs showed a significant loss of NP after needle puncture (Student's t-test, ***p<0.0005).