

Exploring the 3D architecture of native and stained human intervertebral discs through micro-CT

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Background incl. aims

The disruption of normal intervertebral disk (IVD) structure and its subsequent degeneration significantly contributes to the onset of low back pain [1]. To date, the human IVD 3D microstructure characterization has been confined to imaging the annulus fibrosus (AF) [2] and the subchondral endplate [3]. Our study aimed to propose a step-wise approach for the complete morphological investigation of human IVDs at various tissue processing stages using a non-destructive micro-CT analysis. This approach allowed detailed 3D visualization of distinct IVD anatomical components: nucleus pulposus (NP), AF, and the orientation of collagen fibers. Additionally, the method provided a precise quantification of lesions and calcifications within the disk, which affect the integrity of its microstructure and thus its functional and biomechanical characteristics [4].

Methods

The IVDs ($n = 14$) were examined through the use of the micro-CT EasyTom XL Ultra 230–160 micro/nano-CT scanner (RX Solutions, Chavanod, France) with a voxel size of 40 μm . Each sample was scanned label-free (both frozen and thawed) and after formalin fixation and staining of the tissue in an iodine-based contrast agent solution. The calcifications and lesions present in the tissue were determined with an automatic segmentation approach using the software Avizo (Thermo Fisher Scientific, MA, USA). For each processing stage, the calcification and lesion volume fractions were compared considering the entire disc and selecting the AF region. In addition, the degree of contrast enhancement has been defined calculating the Hounsfield Units (HU) relative to the acquired CT values.

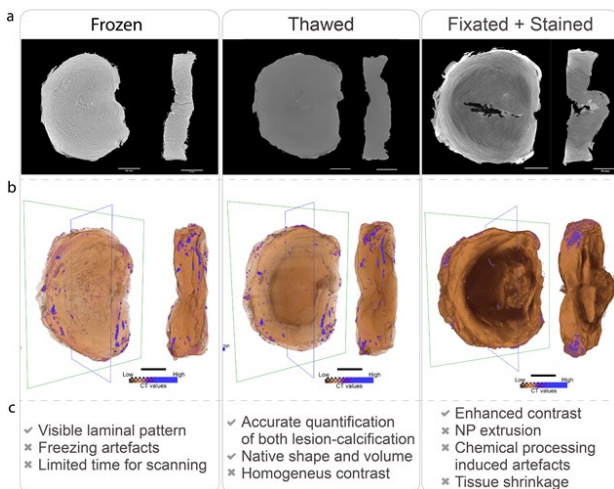
Results

Thawed samples allowed an accurate quantitative analysis of lesions and calcifications present in the native IVD. Additionally, the analysis of frozen samples provided valuable insight into the alternate laminal pattern of the AF. Iodine staining, while providing a homogeneous increase in contrast and simultaneous visualization of the IVD anatomical components, was time-consuming (2 weeks of staining) and resulted in the extrusion of the NP, leading to an increase in lesion volume fraction. The contrast measured in HU increased between the different preparation steps.

Conclusion

The examination of frozen, thawed, and chemically fixed-stained IVD tissues using micro-CT revealed distinctive microstructural information, presenting different benefits for each stage. The systematic methodology used in this study had the aim to define the trade-off between contrast enhancement and processing artefacts. A label free imaging of the IVD represents a valuable option for a 3D morphological investigation of the sample, especially when combined with biomechanical testing due to its non-destructiveness thus preserving the original stiffness and geometry.

Graphic:



Keywords:

Micro-CT, IVD, 3D-anatomy, X-ray imaging

Reference:

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