High-resolution non-destructive 3D quantitative imaging of the cartilage subarchitecture

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Introduction: Preclinical efficacy of novel therapies for cartilage repair are commonly assessed by immunohistochemistry, which has a high discriminative power but is destructive, labor intensive and only provides 2D data. EPIC-CT (equilibrium partitioning of an ionic contrast agent via microfocus X-ray computed tomography) has been employed for imaging and quantification of cartilage structure and composition[1-3], but its limited spatial resolution excludes separate analysis of architecture and composition of the calcified and non-calcified cartilage. We describe an improved non-destructive 3D imaging method, named contrast-enhanced nanofocus X-ray computed tomography (CE-nanoCT), that permits imaging the subtissue architecture of cartilage in small animal models at unprecedented contrast and spatial resolution.

Materials and methods: Knee joints were dissected from adult mice and the joints capsules were dislocated to expose the tibial and femoral cartilage layers. There were untreated knee joints, joints that were treated *ex vivo* with papain [Sigma Aldrich] to enzymatically digest the non-calcified cartilage layer only[4], and joints that were harvested from mice that underwent an 8 week *in vivo* period after medial meniscus destabilization surgery[5], serving as a model for osteoarthritis[6]. All knee joints were first imaged with CE-nanoCT (isotropic voxel size 2 µm). The contrast agent used was Hexabrix® 320 [Guerbet Nederland B.V., The Netherlands]. The volume and thickness of the non-calcified and calcified cartilage were calculated in 3D using CTAn [SkyScan NV, Belgium]. After nano-CT imaging, the joints were fixed, decalcified and embedded in paraffin. 5 µm sections were stained with Safranin-O and counterstained with Fast Green.

Results and discussion: The histological sections (Figs. 1a and 1d) and the corresponding CE-nanoCT images (Figs. 1b, 1c, 1e and 1f) of the untreated and papain-treated femoral condyles showed that after papain treatment the porous calcified cartilage layer was still clearly visible, whereas the dark-gray non-calcified layer had disappeared, thus confirming the unprecedented discriminative power of CE-nanoCT enabling discrimination of subcartilage architecture. After meniscus destabilization, both a decrease in sGAG content in the non-calcified cartilage layer and chondral lesions were noticed. CE-nanoCT is thus able to distinguish between an alteration of cartilage composition and cartilage lesions. Statistical analysis revealed a significant decrease in volume and thickness of the non-calcified cartilage, but leaving the underlying calcified cartilage mostly unaffected.

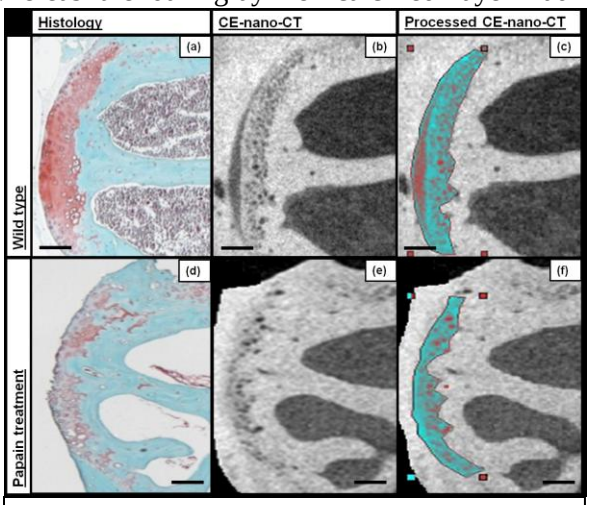

Figure 1: (a, d) A representative coronal histological section (SafO/Fast green staining) through a femoral condyle, (b, e), the corresponding CE-nanoCT sections and (c, f) the same CE-nanoCT section with the traced regions of interest (i.e. total (non-calcified and calcified) hyaline cartilage) of (a – c) an untreated mouse condyle and (d – f) a condyle treated *in vitro* with papain. Scale bars are 200 µm.

Conclusion: CE-nanoCT can reveal differences in structure, composition and quality between calcified and non-calcified cartilage in the knee joints of small animal models. This technique can provide novel insights in preclinical research by revealing and quantifying in a non-destructive 3D manner pathological differences.

References:

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