Subchondral Bone Sclerosis Increases In Both Trabecular Bone And Subchondral Plate In Human Knee Osteoarthritis: Relationship With Histology

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Introduction: Osteoarthritis (OA) is a disease of a whole joint. Yet most of the diagnostic methods are focused mainly on cartilage degeneration and erosion. However, there is increasing evidence that the subchondral bone significantly contributes to the pathogenesis of OA. It has even been suggested that first tissue-level changes in OA occur in the subchondral bone before any signs of degeneration in the overlying articular cartilage.

Micro-computed tomography (µCT) is a technique enabling 3D characterization of the subchondral bone morphology. However, there is no data of the 3D morphology and densitometric parameters of human trabecular bone and subchondral plate in relation to the actual histological degeneration of the overlying cartilage. Therefore, the aim of this study was to characterize morphological changes in human tibial trabecular bone and subchondral plate as a function of histological progression of OA.

Methods: 25 osteochondral samples were prepared from 13 OA patients treated with total knee arthroplasty at Oulu University Hospital. Samples were prepared from tibial plateaus which are always extracted during routine total knee endoprosthesis surgery. Tibial plateaus were visually classified into three categories in terms of degeneration of the articular cartilage: 1) most inviable (or intact) cartilage, 2) moderate cartilage degeneration, and 3) partly or fully exposed subchondral bone. A cylindrical osteochondral samples with diameter of 6 mm were prepared from all the visual grades. Samples were stored in phosphate-buffered saline (PBS) for µCT imaging. Osteochondral cylinders were scanned with µCT device at isotropic 27.8 µm voxel size (Skyscan 1172, Bruker microCT, Kontich, Belgium). Images were reconstructed and analysed with software package provided by the manufacturer. Trabecular bone was manually segmented according to histomorphometric principles for bone, where bone branch was considered as trabeculae, if the height of the branch was higher than the contact length to subchondral bone. Region of interest (ROI) was adjusted for subchondral bone plate with bitwise and shrink-wrap functions. Furthermore, the ROIs for tissue mineral density analyses were copied from the thresholded images, including only the tissue/material density, which were calibrated against standard phantoms. After the µCT imaging, cylinders were formalin-fixed, paraffin embedded and sectioned. Sections of 5 µm were stained Safranin O staining method. Histological sections were graded for stage of OA by three independent evaluators according to the standardized OARSI grading system. Final OARSI grade was defined as the average from three evaluators, which was then correlated with bone morphometric properties (figure 1).

Results: All knees had large internal variation in OARSI grades depending on the site. All the morphological parameters showed significant increase with increasing OARSI grade in both trabecular bone compartment as well as in subchondral bone plate. Trabecular bone volume fraction, number and thickness increased 273%, 123% and 54%, respectively, between OARSI grades 1 and 6. At the same time trabecular separation and structure model index decreased by 46% and 70%, respectively. Subchondral plate thickness increased and bone specific surface decreased from 250 µm to 810 µm and 8.1% to 3.1%, respectively, between OARSI grades 1 and 6. Interestingly, we did not observe any changes in tissue mineral density (excluding pores) or porosity of subchondral bone plate between OARSI grades 1 and 6 (figure 2).

Discussion: In the present human tibial sample material, we observed increased sclerosis of subchondral bone as a function of OARSI grade. Trabecular volume fraction also increased together with OARSI grade. This was not only due to an increase in trabecular number but these trabeculae became also thicker and more closely packed leading to more plate-like shape of trabeculae. There was also significant thickening of subchondral bone plate, while reduced bone specific surface with constant porosity indicates of reduced surface roughness that could be also detected in 3D models (figure 1). Lack of correlation of OARSI grade with tissue mineral density is in line with work by Li and Aspden, whereas increase in porosity has been previously reported in Labrador dogs with experimentally induced OA by transection of the anterior cruciate ligament and removal of the medial meniscus. In our study lack of porosity might be due to the used resolution (27.8 µm voxel size), which was 18 µm in the
study by Intema et al. Notably, most advanced clinical high-resolution cone-beam CT devices can provide resolution of 100 µm indicating that the observed findings may be possible to detect also clinically; for example trabecular thickness varied between 130-270 µm and separation between 230-660 µm. As a limitation of this study, the sample material did not include any zero OARSI grades. Thus, true comparison between histologically fully intact and degenerated samples could not be made from this data.

Significance: This study further highlights the importance of subchondral bone changes in OA and also demonstrates that the use of human material from endoprosthesis is feasible. We show here, that these sclerotic changes in subchondral bone appear at similar rate to OA progression, when evaluated by OARSI-grading. However, our observation does not solve the chicken or the egg problem related to involvement of subchondral bone in OA development and further studies are needed to warrant any conclusions of the causality.

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Figure 1. (A-B) Reconstructed 3D model of analyzed bone, where subchondral plate is colored as grey and trabecular bone as orange. (C-D) Safranin-O stained tissue sections from the same samples that were used for OARSI grading. Corresponding areas to uCT analysis readouts indicated with respectively colored bars. OARSI grades were 1.4 for A and C and 4.0 for B and D.