

# Calcified Cartilage Mineral Maturity Decreases with Early Osteoarthritis in the Human Knee Joint

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**INTRODUCTION:** Calcified cartilage (CC) is a highly mineralized tissue located as a separate layer between articular cartilage and subchondral bone (SCB). The mineralization in the CC offers a gradual shift from the soft uncalcified cartilage to the harder SBC and helps to stabilize their interface. Also, CC functions as a solute diffusion barrier and modulates load transmission between cartilage and bone [1]. Further, it has been suggested that novel therapeutics should target this bone-cartilage interface [2]. Still, there is little information on how different osteoarthritis (OA) stages affect the bone-cartilage interface. Thus, we aimed to analyze the organic and mineral compositions of the CC and the SCB at different OA stages via Fourier-transform infrared spectroscopy (FTIR).

**METHODS:** Knee joints from human cadavers ( $N = 5$ , age range 47–70) were obtained from a biobank (Science Care, USA) under ethical permission (the Research Committee of the Northern Savo Hospital District, 134/2015). Osteochondral plugs ( $n = 34$ ,  $d = 4$  mm) were extracted from the lateral and medial sides of tibial and femoral joint surfaces. The plugs were OARSI-graded, formalin-fixed, ethanol-dehydrated, and embedded in polymethylmethacrylate (PMMA). Uncalcified 3  $\mu\text{m}$  thick longitudinal sections spanning from the cartilage surface to trabecular bone were cut from the plugs and placed on BaF<sub>2</sub> windows for high-resolution FTIR microspectroscopy (FTIR-MS) imaging. Focusing on the cartilage-bone interface, horizontal areas extending from the deep zone of the cartilage to the SCB were imaged with an Agilent Cary 620/670 FTIR-MS system in transmission mode (80 scans per pixel, spatial resolution 1.1  $\mu\text{m}$ , spectral resolution 4  $\text{cm}^{-1}$ ). The spectra were de-noised (principal component analysis, 15 components) and baseline-corrected to zero. Afterward, a section-specific separately measured PMMA spectrum was subtracted from each spectrum. The three separate layers (uncalcified cartilage, CC, and SCB) were segmented from the spectral maps with k-means clustering utilizing the 900–1720  $\text{cm}^{-1}$  spectral region (Figure 1A). K-means clustering is an unsupervised algorithm that groups the spectra into groups based on spectral similarities [3]. The collagen and phosphate contents were calculated using a numerical integration over the amide I peak (1585–1720  $\text{cm}^{-1}$ ) and the phosphate peak (900–1200  $\text{cm}^{-1}$ ), respectively. Additionally, the degree of tissue mineralization (phosphate/amide I area ratio), collagen maturity (1660/1690  $\text{cm}^{-1}$  peak ratio), mineral maturity (1030/1020  $\text{cm}^{-1}$  peak ratio), and new mineral deposition via acid phosphate substitution (1127/1096  $\text{cm}^{-1}$  peak ratio) were evaluated for the CC and SCB [4]. For the SCB, an area of the same thickness as the CC was used in the analyses. Based on the OARSI grade, the samples were pooled into healthy (OARSI 0,  $n = 5$ ), early OA (OARSI 1,  $n = 15$ ), and OA (OARSI 2–4,  $n = 14$ ) groups. A linear mixed-effects model that accounts for the different joint surfaces of the same subject (tibia/femur) was used to compare the parameters between the groups for the CC and SCB separately. The level of statistical significance was set to  $\alpha = 0.05$ .

**RESULTS:** In the CC, mineral maturity was lower in both early OA ( $p = 0.047$ ) and OA ( $p = 0.021$ ) groups compared to the healthy group (Figure 1B). No differences in mineral maturity were observed for the SCB. Similarly, no differences between the groups were found for the other parameters (including amide I and phosphate) in the CC or the SCB.

**DISCUSSION:** Increased mineral maturity has been characterized as the diminution of the hydrated layer surrounding crystals to form well-crystallized apatites [5]. We observed that the mineral maturity in CC was lower within OA samples, with this reduction evident already in the early stages of OA. This suggests that there is a process related to the development of OA which results in less-developed solid cores in the crystals. However, this process is likely not explained by new mineral deposition, which remained similar in all groups. The results encourage further studies to explore the mineralization process in CC and SCB associated with OA progression using a higher amount of samples.

**SIGNIFICANCE/CLINICAL RELEVANCE:** The observed alterations in calcified cartilage composition at the early stage of osteoarthritis provide novel insights into the role of calcified tissues in the mechanisms associated with the initiation and progression of the disease.

**REFERENCES:** [1] Fan et al., *Front. Cell Dev. Biol.*, 9: 659654, 2021. [2] Pesesse et al., *Jt. Bone Spine*, 78:144–149, 2011. [3] Oinas et al., *Sci Rep.*, 6:30008, 2016. [4] Boskey & Pleshko Camacho., *Biomaterials*, 28:2465–2478, 2007. [5] Farlay et al., *J. Bone Miner. Metab.*, 28:433–445, 2010.

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