CHARACTERIZATION OF THE OSTEOCHONDRAL INTERFACE IN YOUNG HEALTHY MICE

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Introduction

Bone–cartilage interfaces in articulating joints consist of three main layers made of dissimilar materials. On one side, the more compliant articular cartilage (aC) made of water, proteoglycans and Type II collagen. On the other side, the stiffer subchondral bone (SB) made of Type I collagen reinforced by mineral crystals. Between them, there is a layer of mineralized cartilage (mC), which plays a crucial role in load transmission and which is also associated with joint diseases such as osteoarthritis (OA). Despite its importance, mC has received limited attention. To provide reference for future work on post-traumatic OA, this study explores the spatial distribution of mineral content and mechanical properties at the osteochondral junction, with a specific focus on mC.

Methods

After dehydrating and PMMA embedding, 5 knee joints of 10 weeks-old male C57Bl/6 mice were cut, grounded and polished to expose the frontal section of the joint. Mechanical properties were probed with nanoindentation (nIND, 4620 indents) using a Berkovich tip. Firstly, displacement-controlled nanoindentation (200 nm penetration depth) with a trapezoidal load function (8s-15s-8s, loading, holding, unloading) was used to characterize the osteochondral junction [1]. Secondly, to investigate load-rate sensitivity, load-controlled nanoindentation was performed (750 µN maximum load, penetration depth ~160 nm) with a quasistatic load function (8s-15s-8s) as well as a "high speed" triangular load function (1s-1s). On the same locations, quantitative and high-resolution backscattered electron imaging (qBEI and hrBEI) were performed to quantify mineral distribution and to locate the indents. Local gradients in composition were explored at the tidemark and around chondrocytes lacunae.

Results

In general, mC was less mineralized and, consequently, less stiff and hard than SB (Fig.1). Indentation modulus showed a higher correlation with hardness than with

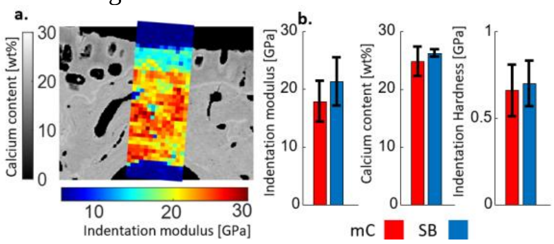

Fig. 1: a) Superimposed spatial maps of mineral content and indentation modulus. b) Average mineral content, indentation modulus and hardness in mC and SB.

calcium content (Spearman's correlation coefficient of 0.89 and 0.50, respectively; $p<0.0001$). A moderate loading rate sensitivity was detected in mC: increasing the loading rate by a factor of 8 increased the measured indentation modulus and hardness by 16% and 25%, respectively. A steep increase in mineral content occurring over a width of about 5 µm was observed between aC and mC (Fig. 2a). For several chondrocyte lacunae, the mineral content was higher in the first 3-4 µm around the lacunae and it reached a plateau between 5 and 10 µm from the border (Fig. 2b).

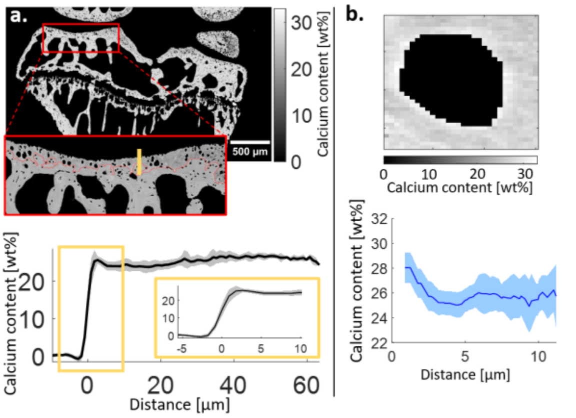

Fig. 2: Local analysis of mineral content at: a) the tidemark and b) around chondrocyte lacunae.

Discussion

SB and mC exhibit different mineral content and local mechanical properties. The effect of loading-rate is higher on hardness than on modulus, and it is likely influenced by sample dehydration with ethanol, which may affect hydrogen bonds between collagen and mineral and within collagen [2]. The tidemark region is much steeper than at the osteochondral junction of larger animals such as rats and sheep, suggesting a correlation with animal size [3]. The "halo" of mineral content around chondrocyte lacunae may indicate some kind of mineral storage there; whether this process is controlled by the cells remains to be understood.

References

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Acknowledgements

This project has received funding from the FWO and F.R.S. FNRS under the Excellence of Science (EOS) program (EOS No. 40007553).