CHANGES IN MINERAL CONTENT AND MECHANICAL PROPERTIES OF CALCIFIED CARTILAGE IN KNEE DURING AGING

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Introduction

The bone-cartilage interface in joints is a region of high biomechanical importance that connects two strongly dissimilar tissues and must withstand substantial loads. A layer of calcified cartilage (CC), joining non-calcified cartilage (NCC) to subchondral bone (SB) is present to solve these challenging tasks. This junction is of high clinical relevance, being the target of age-related degeneration and osteoarthritis [1]. Despite its recognized importance in the medical setting, CC has received relatively little attention from the biomechanics community in comparison to NCC and bone, due to its small size and high heterogeneity.

In a previous work, we have analyzed morphological changes of subchondral bone in the tibia of adult and old rats, highlighting age-related trabecular bone loss accompanied by a marked increase in the thickness of the subchondral plate [2]. Here, we further investigate composition-mechanical function relationships in CC during aging by assessing the mineral content, with quantitative backscattered electron imaging (qBEI), and the mechanic properties, with nanoindentation (nIND).

Methods

Proximal tibia specimens from adult and old Wistar rats (3 and 15 months, 5 specimens per group) were dehydrated, embedded in resin, and cut to expose coronal sections. After surface polishing, two-dimensional mineral content maps were obtained with qBEI at 0.88 µm pixel resolution (Fig. 1A, B). Mineralized cartilage was manually segmented from bone following the cement line (CL, highlighted in Fig. 1). Adult and old samples were compared in terms of mean calcium content and frequency distribution of mineral content. The spatial increase in calcium content across the NCC-CC interface on the medial and lateral tibial plateau was characterized using at least 20 profile lines per sample. Reduced elastic modulus (Er) and hardness (H) were measured across the NCC-SB interface using nIND (Berkovich tip, 400 nm penetration depth, 4 µm spacing between indents).

Results

qBEI images reveal clear differences in CC between adult and old rats (Fig 1A, B). In older samples, CC was about 6.3% thicker and several chondrocyte lacunae were calcified. The mean Ca content increased with age for SB and CC (7.26% and 11.41%, respectively). In CC, the lateral plateau showed lower Ca content with respect to the medial plateau in both groups (2.1% and 3.14% for adults and elderly, respectively). Mineralization heterogeneity was quantified as full width at half maximum of the frequency distributions, measured within regions of interest having similar size. Heterogeneity was higher for CC and, with aging, decreased in cartilage and increased in bone (Fig. 1D). Spatial variations in mineral content across the NCC-CC interface indicate a very sharp increase over a very tiny region of less than 10 µm in width and nIND results had a similar trend (Fig. 1C). With aging a peak in Ca content appeared right at the NCC-CC interface and slightly decreased towards CC.

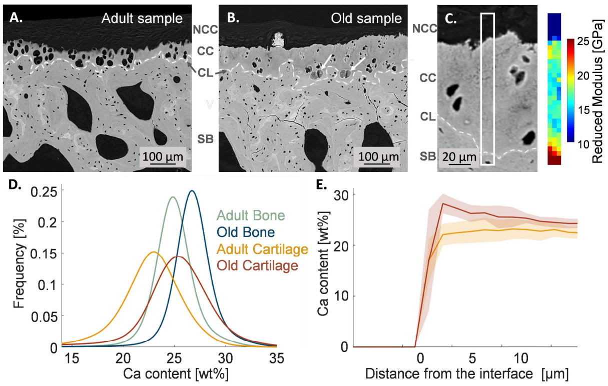

Fig. 1: qBEI image of adult (A) and old (B) samples with calcified lacunae highlighted by the arrows. (C) Zoom on qBEI maps showing the region probed by nIND (white box) and corresponding map of elastic modulus (Er). (D) Frequency distribution of Ca content of young and old bone and cartilage. (E) Evolution of mineral content across the NCC-CC interface (full lines represent mean values and shaded areas the standard deviation interval).

Discussion

This study reveals age-related variations in CC and SB of tibiae in rats. In CC, the observed thickening is likely due to the calcification of non-mineralized cartilage with aging [3]. The calcified lacunae may also suggest a decreased biological activity within old CC. A higher degree of mineralization together with the peak in mineral content at the NCC-CC interface observed in the old sample may lead to stress concentrations, and may trigger crack formation and failure in this region. Ongoing work focuses on the local correlation between mineral content and mechanical data for an improved understanding of the impact of tissue aging on material-level properties of CC. This knowledge may be fed into multiscale computational models of the joint.

References

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