Multiscale assessment of the osteochondral interface width in the femoral head

[1]Campbell, S E; [1]Hurley, D C; +[2]Ferguson, V L
[1]National Institute of Standards and Technology, Boulder, CO 80305
+[2]Department of Mechanical Engineering, University of Colorado, Boulder, CO 80301
Virginia.Ferguson@colorado.edu

INTRODUCTION:

A thin (~10 μm to 100 μm) region of articular calcified cartilage (ACC) anchors stiff bone to the significantly more compliant (~1.5 MPa) hyaline articular cartilage (HAC). Although this bone-cartilage, or osteochondral (OC), interface resists remarkably high shear stresses and rarely fails, its mechanical properties are largely unknown [1, 2]. Further, the mechanisms by which loads are graded between the stiff ACC to the overlying soft HAC are poorly understood.

Quantitative backscattered electron (qBSE) imaging of the OC interface in the human femoral head [1] and distal equine third metacarpal [2] showed variable mineralization patterns. Visible tidemarks often demonstrate a mineral gradient from high mineral volume fraction (in ACC) to a low fraction adjacent to the HAC. Mineralizing ACC appears to contain individual spherulites (or calcospherites) which are sometimes identified at distances of several micrometers into the HAC [1]. In other cases, the interface between the ACC and HAC appears to be abrupt with no such mineralized transition.

The functionality of this robust interface may depend on the width of the transitional region between the mineralized (ACC) and unmineralized (HAC) cartilage. Here, we seek to examine this transition by quantifying the width of the interface between the two tissues. We combine nanoindentation (NI) and atomic force microscopy (AFM) methods to quantify the abruptness of the mineralized interface between ACC and HAC. Backscattered electron imaging (BSE) imaging is applied to characterize mineralization within the regions subjected to NI.

METHODS:

A femoral head from a ~6 month old New Zealand white rabbit was embedded in poly(methyl methacrylate) and cut in half with a low speed saw. One half section was polished to 0.05 μm finish and carbon coated, and the other section was faced with an ultramicrotome. Indent arrays were imaged *via* BSE, which enabled semi-quantitative assessment of mineralized tissues [3], identification of HAC versus ACC, and to identify regions for subsequent analysis [1].

Nanoindentation arrays were placed on both sections traversing the interface region from the ACC into the HAC. The "coarse" polished surface was tested with multiple rectangular arrays (~20 μm spacing; 750 nm max depth; conicospherical tip R = 5 μm) in (1) a region of the joint surface that is known to be heavily loaded in compression and (2) in the fovea, a region that is seldom loaded during normal ambulation throughout adulthood. The "fine" ultramicrotomed surface was subjected to nanoindentation (~3 μm spacing; max load = 2 mN with depth ~300 nm; Berkovich tip). Contact resonance force microscopy (CR-FM), which measures the frequency and quality factor of the atomic force microscopy's (AFM) cantilever's vibrational resonance in contact mode, was used to determine the relative storage modulus M' and loss modulus M'' with 300 nm spacing. The width of the transitional region was defined as the distance between (mineralized $M' - \sigma$) and (cartilage $M' + \sigma$), where σ is the standard deviation in the average value of M'

RESULTS:

Nanoindentation measurements on the "coarse," polished surface showed that the transitional region varied with anatomical position. The width of this transitional region was estimated at ~120 μm to 200 μm in the compressively loaded region and ~60 μm within the fovea. In this same sample, the indentation modulus M also varied with position: ACC in the compressively loaded region yielded $M \approx 13$ GPa, and the fovea yielded $M \approx 15$ GPa. Complementary measurements with qBSE indicated a lower mineral content in the ACC compared to the bone.

More shallow nanoindentation measurements on the "fine," ultramicrotomed surface produced modulus values that ranged from that in ACC [M = (21.0 ± 2.9) GPa] to that in HAC [M = (5.7 ± 0.4) GPa] across a narrow transition that spanned a width of ~3 μm to 9 μm wide. Neighboring regions examined via CR-FM indicated a narrower (~3 μm) interface with a significantly lower storage modulus [M' = (10.5 ± 1.3) GPa], demonstrating the importance of testing at multiple length scales. Furthermore, CR-FM revealed a 22 % increase in the viscoelastic loss tangent (tan δ) from ACC to HAC.

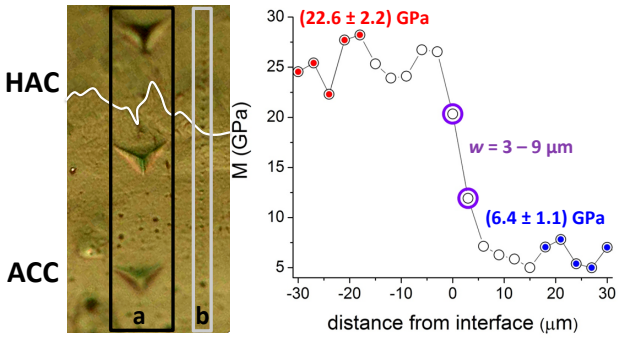

Figure 1. Left: "Fine," ultramicrotomed surface spanning ACC and HAC showing (a) 10 mN (black) marker NI and (b) 2 mN NI (gray); CR-FM measurements performed over 60 μm length adjacent to "b" across a white line that demarcates the border between the tissues. Right: variation of NI M across the OC interface showing ACC (red), HAC (blue) and transitional region (purple).

DISCUSSION:

All nanomechanical measurements of the transitional region were likely influenced by the mineralized tissue interface within each sampled volume. Larger volumes, such as applied to the "coarse" surface, would thus indicate a greater interface width than would smaller volumes. While the ability to exactly define the interface width is limited by the spatial resolution of each measurement technique, these results show that the transition between mineralized ACC and unmineralized HAC is abrupt.

The mineral phase seems to be an obvious contributor to transitioning loads across the OC interface; however, these results indicate that no mineralized functional gradient exists between ACC and HAC. Connectivity of collagen fibrils between ACC and HAC, tortuousity of the OC interface, or composition and hydration of the HAC's extracellular matrix may instead serve to transition loads across this dissimilar material interface.

SIGNIFICANCE:

Knowledge of the mechanisms that serve to minimize stress concentrations across the OC interface are poorly understood. An improved understanding of the material and mechanical tissue level properties of the OC interface is needed to assess how this region may contribute to degeneration with osteoarthritis and to advance our ability to design successful materials for cartilage repair *in vivo*.

ACKNOWLEDGEMENTS:

The authors acknowledge funding sources including: NSF CAREER Award #1055989, NRC Postdoctoral Fellowship for SEC, and the Innovative Grant Program at the University of Colorado at Boulder. Thank you also to Rebecca Pak for collection of initial NI data. Mention of commercial products does not imply endorsement by NIST. Partial contribution of NIST; not subject to copyright in the US.

REFERENCES:

1. Ferguson, V.L., *et al.*, J of Anat, 2003. **203**(2): p. 191-202.
2. Ferguson, V.L., *et al.*, Eur Cell Mater, 2008. **16**: p. 40-6.
3. Campbell, S.E., Ph.D. Dissertation, University of Colorado: Boulder, CO, 2010.