7th World Tribology Congress, WTC 2022
July 10-15, 2022, Lyon, France

# Zinc Distribution in Articular Cartilage and Potential Implications on Structural Role

Spencer Fullam[1)*], Tom Schmid[1)], Catherine Yuh[1)], Benjamin Witt[1)], and Markus A. Wimmer[1)]

[1)]Laboratory of Tribology, Department of Orthopedic Surgery, Rush University Medical Hospital, Chicago, USA
*Corresponding author: spencer_fullam@rush.edu

To better understand the role of cations in the solid phase of articular cartilage, we utilize Laser Ablation ICP-MS and ICP-OES to map the distribution of ions across tissue depth. We found that the concentration of Zinc aligns with transition zones of collagen fibril alignment as reported in the literature. We therefore speculate that Zn-dependent protein/protein crosslinks, like COMP/collagen bonds, are especially important to the resistance of tissue to shear stress.
**Keywords:** Cartilage, Zinc Distribution, Ionic Crosslinking

## 1. Introduction

While multi-phasic material models of cartilage incorporate the flux of charged ions in tissue interstitial fluid, little is known of how these ions affect the strength of the solid protein matrix. However, investigating these protein/ion interactions is a required step to understanding the fundamental damage mechanisms that ultimately yield cartilage failure. We present our work subjecting bovine articular cartilage to axial load and reciprocating shear with a migrating contact area while immersed in solution. By then scanning tissue sections with Laser-Ablation ICP-MS, we look to the relative distribution of cations across tissue depth, and with ICP-OES, we look to tissue absolute concentrations.

## 2. Methods

Cartilage plugs were extracted from the femoral condyles of immature bovine stifle joints. Plugs were cut to 2.5-3mm thickness to ensure no bone remained attached. Samples were loaded in a custom bioreactor, sitting in bath of 37C normal saline with an addition of 10mM of CaCl2. Samples were loaded to 40N against a 32 mm diam. ceramic head (Biolox® Forte) then sheared at 1Hz while the center of contact translated 6mm laterally at 0.1Hz. Control samples were kept under the same fluid conditions. After 3h, samples were cut in half to capture subsurface areas that have been directly in contact with the ceramic ball.

### 2.1. Laser Ablation ICP-MS of sections

The halves were formalin fixed, paraffin embedded, and cut to 10 µm thick sections onto quartz microscope slides. After deparaffinization, 2 sections were scanned by LA-ICP-MS (193nm ESI laser and Thermofisher iCAP TQ ICP-MS, 20um pixel size) by Dr. P Telouk in Lyon, France. Saffrin-O/Fast green stains were performed on adjacent sections.

### 2.2. ICP-OES of bulk samples

Additional cartilage plugs were extracted from nearby areas on the same condyles. For each bulk tissue sample, wet weights were recorded, then samples were digested by 6N HCl. After dilution, 12 samples were run through ICP-OES (Perkin-Elmer Model Plasma 2000) by Dr. J Kunze in Hamburg, Germany.

## 3. Results

LA maps of Zn (Fig. 1) revealed a distribution across depth – with an initial strong band 60-120 µm from the surface, followed by another strong, yet wider band 600-900µm from the surface. In the deep zone, Zn was also heavily concentrated near cartilage canals (arrows in Fig. 1). No damage was noted on the tissue that underwent shear. ICP-OES showed average Ca, Mg, and Zn levels of 899.3, 271.5, and 2.0 ppm respectively. Zn levels were near the detection limit of the machine.

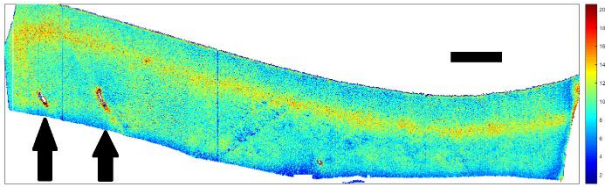

Figure 1: Distribution of Zn across the tissue depth. The top of the image is the articular surface, and the bottom is closer to the bone. Scale bar is 1mm and color scale in ppm.

## 4. Discussion

The high spatial resolution of LA-ICP-MS revealed a wealth of new details on Zn distribution in articular cartilage. Notably, the bands of Zn deposits align well with the superficial-to-middle and middle-to-deep transition zones of immature bovine cartilage [1]. The superficial-to-middle transition zone is the region of greatest energy dissipation, but stiffens under large shear loads, lowering the chance of shear-induced catastrophic delamination. Zn has been identified as being most effective in binding COMP, a cartilage matrix molecule, to collagen [2]. As these molecules bind the fibers, it may provide stabilization to the fibrillar network [3] that manifests as micro-scale stiffening following articular loading [4]. IHC is currently underway to stain our tissue for COMP.

## 5. References

[1] Durney, K. et al., J Biomech 107:109852, 2020.
[2] Rosenberg, K.et. al. J Biol Chem 273:20397, 1998
[3] Nagel, T. et al. JMBBM 22: 22-29, 2013
[4] Yuh, C et al. JMBBM 113:104113, 2013