

# Exploring the Mechanical Role of COMP & Other Small Molecules in a Cartilage Model: A Rheological Approach

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**INTRODUCTION:** Articular cartilage is a complex tissue with a dense extracellular matrix (ECM) consisting of collagen, aggrecan, hyaluronan, and small molecules like cartilage oligomeric matrix protein (COMP) and other small molecules (e.g., decorin, matrilin). While the role of COMP as a biomarker for osteoarthritis (OA) is well-established [1], its functional contribution to cartilage health is less clear. Previous studies have indicated that COMP may be involved in collagen fibrillogenesis, chondrocyte proliferation, and the mechanical strength of tendons [3, 4]. It has been shown that this pentameric glycoprotein binds with multiple ECM proteins, including collagen and proteoglycans [7,8]. However, the specific impact of COMP on the mechanical properties of articular cartilage, has not been elucidated.

In this study, we employed oscillatory shear testing using a rheometer with collagen gel models of cartilage to investigate the role of COMP and other small molecules in modulating the mechanical properties of the tissue. In particular, we examined the shear stress, strain, yield point, storage ( $G'$ ) and loss ( $G''$ ) moduli in collagen gel models of cartilage of various complexity. We hypothesized that COMP, in conjunction with other small molecules, plays a pivotal role in influencing the mechanical properties of collagen gel models of cartilage.

**METHODS:** A total of 24 cylindrical gels (8mm diameter, 2mm thickness) of various complexity were prepared using 8-well chamber slides. There were four different gels (N = 6 per group): 1) Collagen-only, 2) Collagen+ aggrecan, 3) Collagen+ aggrecan+ hyaluronan, and 4) Collagen+ aggrecan+ hyaluronan + COMP. The collagen gel consisted of 3 mg/ml type I collagen from rat tail tendons in 10 mM HCl (Type I collagen forms stronger gels than type II collagen). Aggrecan proteoglycan (1 mg/ml) from bovine nasal cartilage. Hyaluronan (200  $\mu$ g/ml) from human umbilical cord. COMP (0.5  $\mu$ g/ml 50% pure), was a 4-hour extract using 50 mM EDTA from bovine MCP articular cartilage. All gels were formed in Tris (25 mM) buffered saline at pH 7.5. Mechanical tests were performed using a stress-controlled Rheometer MCR 302e by Anton Paar, equipped with TruStrain, TruRate, and Tack/Normal Force capabilities. To minimize slippage between the piston and gel samples, profiled measurement systems were employed during shear tests. Each sample was subjected to a compressive strain of 20%. Upon reaching equilibrium, a stepwise series of shear strains ranging from 0.01% to 200% were applied at a frequency of 1 Hz. Yield points were determined using strain-shear stress diagrams, identified at the intercept of two regression lines above and below the point of interest. Both Storage ( $G'$ ) and Loss ( $G''$ ) Moduli were calculated, with the Storage Modulus derived from data points within the Linear Viscoelastic Region (LVE, 3% tolerance). Statistical analysis was conducted using a one-way ANOVA, followed by Bonferroni's multiple comparisons test ( $\alpha=0.05$ ).

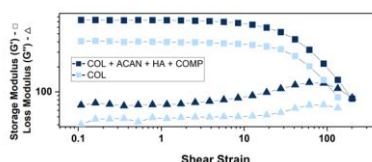
**RESULTS:** Significant changes were observed in the mechanical properties of collagen gels when other ECM molecules were added, accompanied by a notable upward trend in the storage ( $G'$ ) and loss ( $G''$ ) moduli values with increasing gel complexity [Fig 1]. The storage modulus ( $G'$ ) for collagen-only gels stood at  $440.96 \pm 53.83$  Pa and reached  $651.71 \pm 127.82$  Pa, when all four molecules were present [Fig 2]. While the addition of aggrecan and hyaluronan did not significantly alter the yield point, the most transformative change was observed with the inclusion of COMP [Fig 3]. Yield strain increased significantly ( $p < 0.001$ ) from  $11.32 \pm 1.52\%$  to  $18.63 \pm 2.45\%$ , representing a 65% increase [Fig 3A]. Similarly, yield stress soared from  $43.3 \pm 17.3$  Pa to  $112.4 \pm 26.6$  Pa ( $p < 0.001$ ) upon the addition of COMP, marking a 160% increase [Fig 3B].

**DISCUSSION:** The study underscores the substantial impact of COMP and other small molecules on the mechanical properties of collagen gel models of cartilage. While the storage modulus increased (expectedly) with increasing complexity of the gels at a 10% rate, yield strain and stress were only affected by the addition of COMP. The nearly doubling and tripling of stress/strain yield point is even more surprising when considering that COMP was provided at concentration levels more than three magnitudes lower than collagen and aggrecan. Nagel and Kelly [6] suggested that in a crosslinked network collagen fibers would be recruited at a less deformed states, which could explain the shift in yield point. It is a limitation that COMP was not pure and it is possible that the effect is based on other small molecules rather than COMP. Therefore, an important next step will be the detailed analysis of the extract in order to determine its composition. Future studies should aim to validate these findings in human tissue, although similar behavior to bovine tissue is anticipated. Given the significant impact of COMP on the mechanical properties observed in this study, further research is warranted to elucidate the molecular mechanisms underlying these effects.

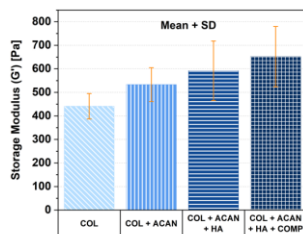
**SIGNIFICANCE/CLINICAL RELEVANCE:** The findings of this study point to a potential mechanical role of COMP in cartilage, which could have implications for understanding the onset and progression of OA disease may offer new avenues for therapeutic interventions.

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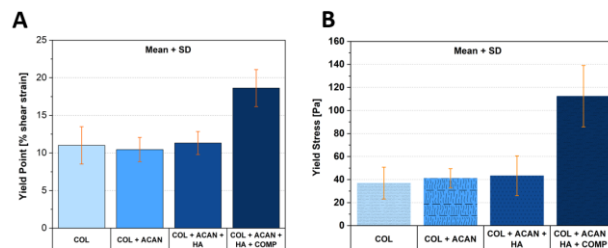
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**Figure 1:** Storage & Loss Moduli Variation in Collagen Gels with ECM Molecules. The graph illustrates the remarkable increase in the storage ( $G'$ ) & Loss ( $G''$ ) moduli when additional ECM molecules are integrated into the collagen gel structure. Values elevated from  $440.96 \pm 53.83$  Pa for collagen-only gels to  $651.71 \pm 127.82$  Pa when all four ECM molecules were included.



**Figure 2:** The Storage Modulus ( $G'$ ) increases with Increasing Gel Complexity: The box diagram highlights the storage modulus for collagen-only gels at  $440.96 \pm 53.83$  Pa, which escalates to  $651.71 \pm 127.82$  Pa when collagen, aggrecan, hyaluronan, and COMP are present



**Figure 3:** A) Impact of COMP on Yield Strain: This box diagram highlights the significant increase in yield strain from  $11.32 \pm 1.52\%$  to  $18.63 \pm 2.45\%$  ( $p < 0.001$ ) upon the inclusion of COMP and other small molecules, marking a 65% increase. B) Comparative Shear Stress Values at Yield Points Across Different Gel Compositions. The graph illustrates a dramatic 160% increase in yield stress from  $43.3 \pm 17.3$  Pa to  $112.4 \pm 26.6$  Pa ( $p < 0.001$ ) upon the incorporation of COMP into the gel matrix, underscoring its pivotal role in enhancing mechanical robustness.