

# VARIATION OF COMPRESSIVE AND SHEAR PROPERTIES OF ALGINATE GEL AS A FUNCTION OF SODIUM AND CALCIUM CONCENTRATIONS UNDER PHYSIOLOGICAL CONDITIONS

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## Introduction

Alginate hydrogels crosslinked with  $\text{Ca}^{2+}$  have been widely used in many biomedical applications, including as synthetic extracellular matrices for articular cartilage [1-3]. In this scaffold system, alginate plays an important role in transmitting mechanical signals to the chondrocytes and resultant matrix formation. Therefore, it is important to quantify the mechanical properties of alginate gel under physiologically relevant ionic conditions [4]. The goal of this study is to quantify the variation of compressive and shear properties of alginate gel as a function of sodium and calcium concentrations. These parameters will be critical in functional cartilage tissue engineering applications utilizing alginate-based scaffolds.

## Materials and Methods

**Sample Preparation:** 2% alginate solutions were made from low-viscosity sodium salt alginic acid (A2158, Sigma). It was purified by sterile filtration and then gelled in 0.05M  $\text{CaCl}_2$  solution for 1.5 hours in a cylindrical mold (10mm diameter $\times$ 1.6mm thickness). The gels were then immersed in a solution with various NaCl and  $\text{CaCl}_2$  concentrations. In study A,  $\text{CaCl}_2$  concentration was kept constant (1.8mM) and the NaCl concentration varied from 0.1M, 0.15M to 0.2M. In study B, NaCl concentration was fixed at 0.15M, and the  $\text{CaCl}_2$  concentration changed from 1mM, 1.8mM to 4mM. (n=4 for each solution)

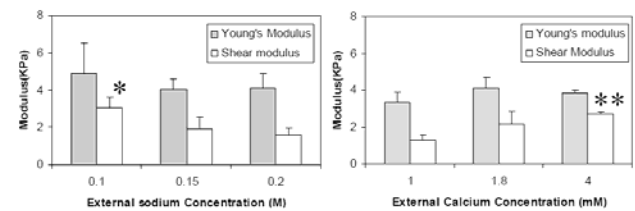
**Mechanical Testing:** After 15-20 hours stabilization in the solution, the diameter of each sample was measured with a stereomicroscope (model PP&E 56939; Bausch and Lomb). The mechanical tests were performed on a shear-strain controlled rheometer (ARES-LS1, TA instruments). The sample was placed between two flat porous platens and immersed in the same storage solution. First, a normal tare load of 0.02N was registered. The normal force was recorded after 30 minutes. Then a 15% normal strain was applied, and the normal force was again determined after another 30 minutes. The Young's modulus ( $E_{eq}$ ) was calculated. A step shear strain of 0.01 radian was applied and followed with a 40-minute shear-stress relaxation. The equilibrium shear moduli ( $G_{eq}$ ) were then calculated. A dynamic shear test with the same strain amplitude was performed over frequencies ranging from 0.01rad/s to 100 rad/s on a logarithmic frequency sweep.

**Statistical Analysis:** The effects of varying sodium and calcium concentrations on mechanical properties were assessed using analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc tests ( $\alpha = 0.05$ ).

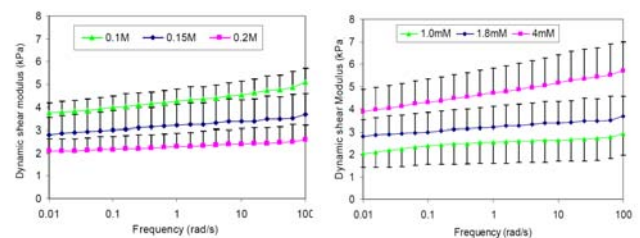
## Results and Discussion

It was found that  $G_{eq}$  of the alginate hydrogel was strongly dependent on the concentrations of sodium and calcium ion in solution ( $p < 0.05$ ). In contrast, the effects of ion concentration on  $E_{eq}$  were not significant ( $p > 0.05$ ,

Figs. 1a & 1b). Specifically, the values for  $G_{eq}$  decreased about 50% when  $[\text{Na}^+]$  increased from 0.1M to 0.2M, and increased more than one-fold when  $[\text{Ca}^{2+}]$  increased from 1mM to 4mM. The magnitude of dynamic shear modulus ( $G^*$ ) increased linearly with the logarithm of frequency. Both  $G^*$  and phase shift angle (not shown) decreased with increasing  $[\text{Na}^+]$  and increased with  $[\text{Ca}^{2+}]$  ( $p < 0.0001$ , Figs. 2a & 2b). It is important to note that the cell culture medium measures a  $[\text{Ca}^{2+}]$  of 1.8mM, while  $[\text{Ca}^{2+}]$  in synovial fluid has been reported to be about 4mM [5]. The observed changes in alginate hydrogel mechanical properties may have an effect on the mechano-transduction of chondrocytes as the matrix can modulate cellular deformations under external loads.



**Figs.1a, 1b** Effects of external sodium and calcium concentrations on the equilibrium shear modulus and Young's modulus; n=4, \*significant compared to  $[\text{Na}^+]=0.15\text{M}$  and  $0.2\text{M}$  ( $P < 0.05$ , SNK), \*\*significant compared to  $[\text{Ca}^{2+}]=1\text{mM}$  ( $p < 0.05$ , SNK).



**Fig. 2a** Dynamic shear moduli of alginate gel in the solution of 1.8mM  $\text{CaCl}_2$  and various NaCl concentrations. **Fig. 2b** Dynamic shear moduli of alginate gel in 0.15M NaCl and various  $\text{CaCl}_2$  concentrations.

## Conclusions

This study has provided critical information on the fundamental viscoelastic properties of alginate gels under physiologically relevant conditions. Findings from this study will enhance our understanding of the micro-deformational environment of chondrocyte cultured in alginate scaffolds. The effects of cell culture on the mechanical properties of the hydrogel matrix will be examined in future studies.

## References

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