# Investigating Collagen Fiber Self-Assembly within Tunable Hydrogel Matrices

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## Introduction

There exists a gap in the current understanding of how collagen self-assembly is regulated within environments of varying viscoelasticity. It has been well established that solid tumor cancers change the viscoelasticity of tissue, rendering them stiffer and more fibrotic than surrounding healthy tissue. This is caused by the overproduction of collagen, the main component of the human extracellular matrix, by solid tumor cells. This fibrotic tissue then upregulates tumor cell growth, spreading and adhesion, as well as impacting further collagen formation and subsequent self-assembly. To better understand the viscoelastic conditions in which collagen assembly is regulated, a cell-free biomaterial-based approach can be taken, using collagen embedded within 3D interpenetrating polymer network (IPN) hydrogels as an in-vitro model for human tissue. This study specifically uses alginate hydrogel, which is a biocompatible, biphasic polymer network. What makes this hydrogel a valuable in-vitro model is its mechanically tunable nature; using both unmodified alginate and alginate modified with click chemistry groups, significant modifications to the hydrogel's final mechanical properties can be achieved. The adjustable nature of this hydrogel makes it an effective platform to study the assembly of collagen fibers within different 3D viscoelastic conditions.

## Methods

Unmodified alginate and alginate (1.5 wt%) modified with click chemistry groups norbornene (Nb) and tetrazine (Tz) in varying ratios (0:0, 1:1, 1:2, 2:1) was mixed with 30mM of precipitated calcium carbonate (PCC) and type I collagen derived from rat tail (4 mg/mL) to prepare the hydrogels. These gels were maintained at 4°C to prevent collagen assembly prior to gelation. Within the matrix, spontaneous reactions occur between Nb and Tz molecules that introduce covalent crosslinks to the network, alongside the ionic crosslinks between PCC and alginate, thereby enhancing the crosslink density of the matrices.

SHG Imaging Preparation: The gels were plated and kept at 4°C, during which covalent and ionic crosslinking within the matrix occurs, while there is a negligible level of collagen assembly. After gelation, the samples were put in an incubator at 37°C for one hour to allow for collagen assembly. They were then submerged in buffer to prevent desiccation of the hydrogels and were left in the incubator at 37°C overnight for further assembly. The samples were subsequently imaged using second harmonic generation imaging. Once imaged, collagen assembly was quantified by calculating the area of the image covered by the assembled collagen fibers.

Rheometer Preparation: Once the gels were initially prepared at 4°C, they were immediately pipetted onto the measuring stage of the rheometer. Using a 20mm cone-plate geometry, the samples underwent shear oscillation. Two tests were conducted: a time sweep and a frequency sweep at 4°C to determine their mechanical properties like storage modulus and tan(δ).

**Results**

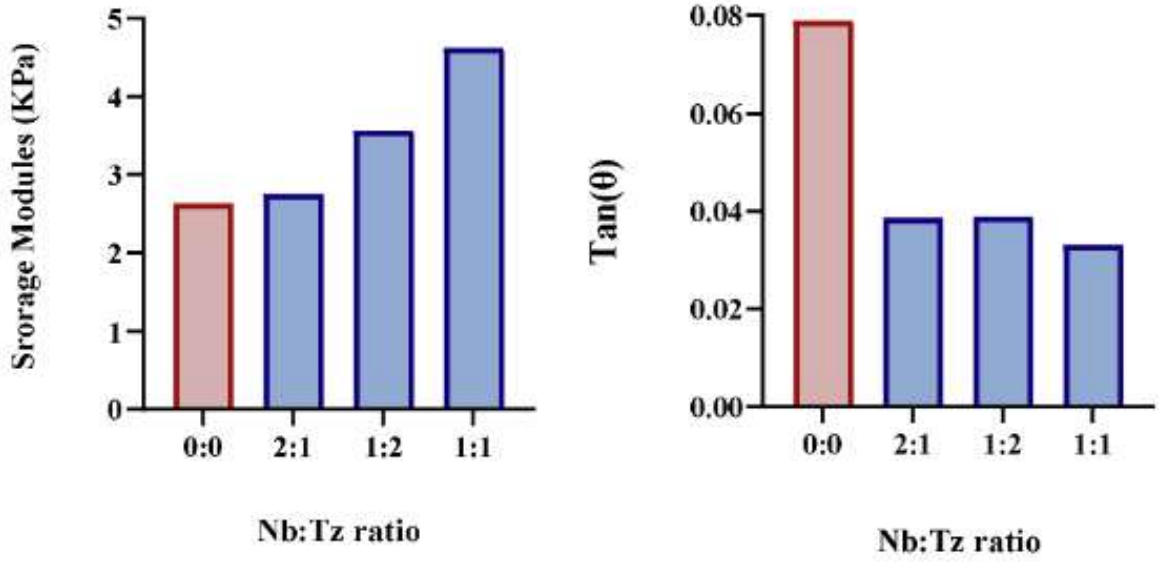


Figure 1: Storage modulus and tan(δ) of 1.5 wt% gels containing collagen and different Nb:Tz ratios at 4°C

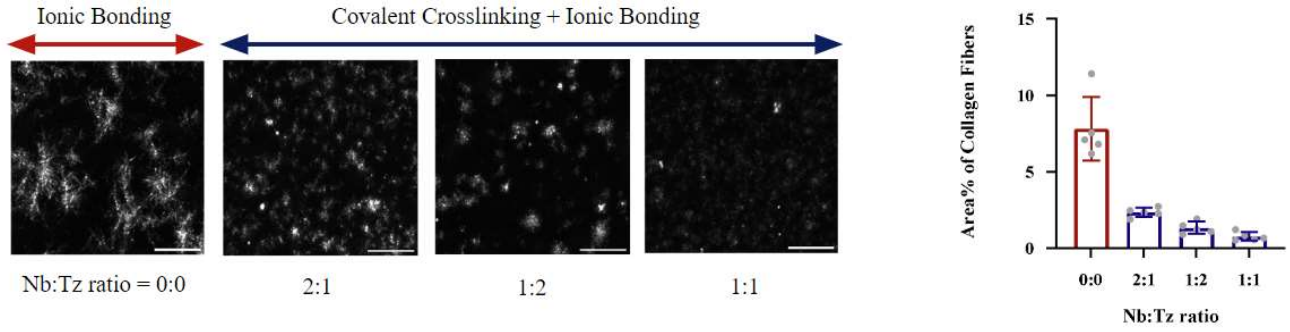


Figure 2: Percentage of image area covered by assembled collagen fibers within gels with varying Nb:Tz ratios

**Discussion**

By modifying alginate with Nb and Tz, a much wider range of viscoelastic conditions can be achieved than when using unmodified alginate hydrogels alone. Covalent crosslinking, which Nb and Tz introduce to the hydrogel system, is a significant regulator of the mechanical properties of ECM. This allows for better modeling of both native and diseased tissues throughout the body, as the gels possess greater structural similarity to ECM.

Figure 1. shows that the storage modulus increased, and tan(δ) decreased when utilizing alginate modified with Nb and Tz, indicating that the gels became stiffer and more solid-like with the higher crosslinking density caused by the introduction of Nb and Tz. Notably, tan(δ) halved between the unmodified alginate gel and samples that possessed Nb and Tz, implying that the covalent crosslinking caused by the Nb and Tz reaction along with ionic crosslinking greatly affected the gel's viscoelastic behavior.

Figure 2. demonstrates that increased crosslink density, which resulted in stiffer, more solid-like gels, fairly impaired the assembly of collagen fibers within the hydrogel matrices. Incorporating the Nb and Tz modified alginate, which produced gels with a lower tan(δ), decreased the area fraction of collagen fibers, suggesting a correlation between tan(δ) and collagen fiber assembly.

## Conclusion

By varying ratios of norbornene and tetrazine added to alginate hydrogels, several different gel chemistries with biomechanical similarity to native and diseased ECM can be achieved. Embedding collagen within these gels allowed for collagen assembly to be imaged and quantified, from which an apparent correlation between the tan(δ) of the hydrogel and the magnitude of collagen assembly within the gel was found. This method of using collagen embedded within different hydrogel environments can provide a more effective platform to study the impact of collagen fibers on the growth of tumor cells in future research. This would better enable the in-vitro examination of tumor malignancy progression under various viscoelastic conditions, similar to what would be found in-vivo.

## Acknowledgements

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