# ANALYSIS AND BIOMECHANICS OF TYRAMINE-CROSSLINKED HYALURONAN HYDROGELS

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## INTRODUCTION

Hyaluronan (HA) is a hydrophilic glycosamino-glycan (GAG) that is a major component of most extracellular matrices [1]. This unique biopolymer is found in most of mammalian tissues and body fluids. Hydrogels prepared from chemically modified HA possess considerable potential in tissue engineering due to their ability to mimic desirable biological and physiochemical properties. In this study, HA is chemically substituted with tyramine (TS-HA) through conventional carbodiimide chemistry in which the carboxyl groups within the glucuronic acid residues of the HA are reacted with the amine group of tyramine to form an amide bond. Enzymatic cross-linking of the purified TS-HA is initiated through introduction of very dilute hydrogen peroxide ($H_2O_2$) in the presence of horseradish peroxidase (HRP) to form tyramine-based HA (TB-HA) hydrogels. This study described the peroxidase catalyzed oxidation of tyramine to dityramine (cross-linking) as characterized by amino acid analysis (AAA) and fluorescence spectroscopy (FS) as well as the mechanical properties of various concentrations of TB-HA hydrogels appropriate for tissue engineering orthopedic applications.

## METHODS

### Dityramine Preparation:

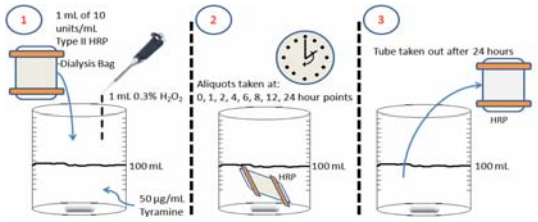


**Figure 1:** Step 1: Tyramine cross-linking begins in the presence of HRP and $H_2O_2$. Step 2: Monitor enzyme-catalyzed conversion of tyramine to dityramine. Step 3: Stop reaction by removing dialysis bag.

AAA of dityramine at the various time points was performed by cation exchange chromatography at the University of Oklahoma, Molecular Biology-Proteomics Facility. FS was recorded using a Spectra MaxGeminiXS spectrophotometer and employing excitation wavelength of 260 to 305 nm with 5 nm increments.

### Confined Compression Tests:

An Instron 5543 machine and custom built confining chamber with porous platen was used to apply compression to TB-HA hydrogels of concentrations 6.25, 12.5, 25, 50, and 100 mg/ml with a series of stress relaxation measurements.

## RESULTS AND DISCUSSION

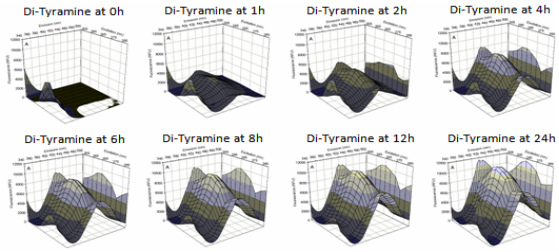


**Figure 2:** Time course of dityramine formation from tyramine by peroxidase/peroxide reaction as monitored by fluorescence measurement.

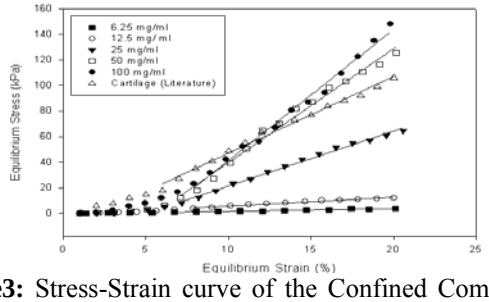


**Figure3:** Stress-Strain curve of the Confined Compression Tests.[2]

The FS data (Figure 2) show increasing fluorescent signal ($\lambda_{ex}$ = 285 nm; $\lambda_{em}$ = 415 nm) with time reflecting increased production of dityramine from tyramine with time. This serves to illustrate the utility of this assay in determining the degree of cross-linking within TB-HA hydrogels. This increase in fluorescence correlates with a decrease in tyramine as measured by AAA (data not shown). The confined compression results (Figure 3) demonstrated that the 50 mg/ml and 100 mg/ml samples of TB-HA have aggregate moduli which are equal or exceed that of articular cartilage values reported in the literature. The results of this study demonstrate our ability to chemically and mechanically characterize dityramine cross-linked hydrogels and generate properties similar to that of natural tissues.

## REFERENCES

1. Keun Su Kim, et al., *Spine*. **30**:33-7, 2008.
2. Jorvelin J S, et al., *J. Biomechanics*. **30**:135-41, 1997.