USE OF RAMAN SCATTERING TO MEASURE LIGAMENT TENSION

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ABSTRACT

Raman spectra of bovine ligaments were measured as a function of tension, using both 658 nm and 785 nm lasers for excitation, to test the hypothesis that ligament tension can be determined from the observed Raman shift. Filters were used to remove extraneous laser lines from the excitation beam and to shield the spectrometer from the Rayleigh-scattered light. A suture was attached to each end of the horizontally-mounted ligament sample, with the incident laser beam impinging at the sample at a 90° angle. The Raman spectrum was measured as a function of stress applied to one end of the sample. Raman lines associated with the collagen backbone and with amino acid residues attached to the backbone were observed. As the strain increases, Raman lines having their origin in the residues shift to higher frequencies whereas those with their origin in the collagen backbone shift to lower frequencies.

INTRODUCTION

- The most common contact injury to the lower extremity is a rupture of the anterior cruciate ligament (ACL).
- Injury to the ACL without injury to other ligaments occurs in 60% of ACL injuries.
- More than 100,000 ACL injuries are repaired annually in the U.S.
- Long term success rate of ACL reconstruction ranges from 75% to 90%.[1]
- Setting proper tension on the graft is necessary for regaining normal anterior-posterior translation.[2]

Ligament

- Ligaments consist of densely packed collagen fiber bundles (Figure 1).
- There are varying amounts of 'crimp' (a sinusoidal pattern in the tissue) in the collagen fibrils which allows for increasing resistance to increasing loads.
- The fibrils change lengths by straightening of the crimp.
- The maximum load and stiffness of ACLs obtained from young donors are 1,725 ± 269 N and 182 ± 33 N/mm, respectively.

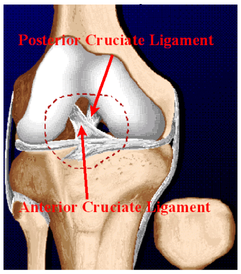

Figure 1. Anterior view of the knee with the patella removed.

Collagen

- Unique triple helix structure with 33% glycine, 21% proline and hydroxyproline, and 11% alanine.
- The cyclic structure of hydroxyproline and proline adds rigidity to the structure.
- The precursor molecule, tropocollagen, has a molecular weight of 300 kD and a length of 300 nm.
- The tropocollagen molecules are arranged in a quarter-stagger configuration to form microfibrils.
- Stability is attributed to both inter- and intra-molecular crosslinks.

BACKGROUND

- Frushour and Koenig[3] determined the band assignments of collagen by comparing the Raman spectra of collagen with gelatin, elastin, and a superposition of amino acids that correspond to the composition of gelatin.
- Dong et al.[4] investigated the temperature effect on the Raman spectra of collagen and observed that as temperature increases, most vibrational modes of collagen shift to lower wavenumbers, some to higher wavenumbers, some disappeared, and some unaffected.
- Wang et al.[4] studied the Raman spectra of a single collagen fibril from a rat tail as a function of strain and found a decrease in Raman frequency in modes associated with the collagen backbone.

EXPERIMENTAL METHOD

- Figure 2 shows a diagram of the experimental setup.
- Filter 1 (laser line filter) was used to eliminate extraneous laser beam. Filter 2 (notch filter) was used to reduce the intensity of the Rayleigh-scattered light.
- Strain (fractional change in length), not stress (force), was used as a variable.
- The ligament was fastened between two metal rods using sutures. The strain was adjusted by moving one of the bars.
- Raman spectra were obtained at several points on the sample under stress. Figure 3 shows a sample spectrum with the 785 nm excitation.

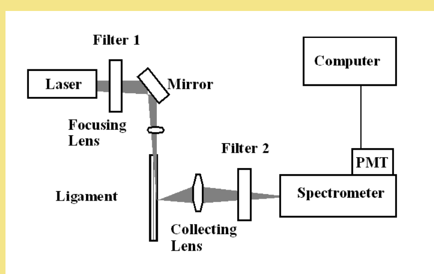

Figure 2. Diagram of the experimental apparatus.

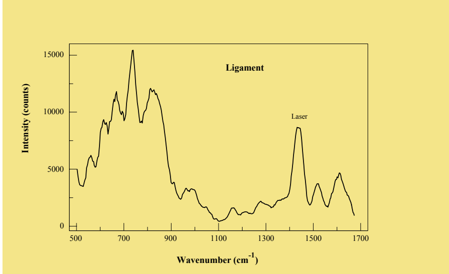

Figure 3. Raman spectrum of a bovine ligament obtained using a 785-nm laser.

RESULTS AND DISCUSSION

- Fluorescent intensity was lower at longer wavelength (785 nm).
- Both positive and negative changes in Raman shift as a function of strain were observed (Figure 4).
- Vibrations associated with amino acid residues exhibit a Raman shift to higher frequencies with increasing strain.
- Vibrations associated with the collagen backbone exhibit a Raman shift to lower frequencies with increasing strain.
- The change in the Raman frequency is linear with increasing strain, consistent with the Hook's Law (stress is proportional to strain).

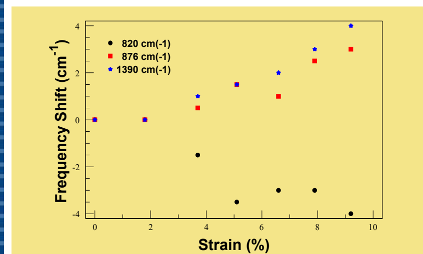

Figure 4. Comparison of Raman frequencies of three vibrations as a function of strain.

CONCLUSION

Preliminary results from measurements of a bovine ligament are consistent with those reported in the literature (Dong et al.[4] and Wang et al.[5]). The decrease in Raman frequency is associated with the elongation of collagen backbone. This supports the hypothesis that ligament tension can be determined from measurements of the Raman shift.

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ACKNOWLEDGMENTS

This work was funded in part by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, under grant 1R43AR053791-01.