Published in final edited form as:
Conf Proc IEEE Eng Med Biol Soc. 2008 ; 1: 3434–3437.

Application of Raman Scattering to the Measurement of Ligament Tension

M.W. Winchester,
M.W. Winchester is with the University of Mary Washington, Fredericksburg, VA 22401 (email: mwinc2fi@umw.edu)

L.W. Winchester [Senior Member, IEEE], and
L.W. Winchester is with CW Optics, Inc, Yorktown, VA 23690 (phone: 757-872-4000, email: LWW@cwoptics.com)

N.Y. Chou
N.Y. Chou is with CW Optics, Inc, Yorktown, VA 23690 (phone: 757-872-4000, email: NYC@cwoptics.com)

Abstract

More marginal results and complications occur as a result of knee ligament surgery than of other common surgical procedure. Long-term success rates of anterior cruciate ligament reconstruction range between 75 and 90%. The goal of knee surgery is to restore the normal kinematics of the knee. If the tension is too high, the range of motion of the joint is restricted, resulting in abnormal stresses on the articular cartilage and the meniscuses, and interfering with the revascularization of the graft. The use of Raman spectroscopy for the measurement of tension in ligaments and tendons is described. Measurements of the Raman spectrum demonstrate that the Raman frequencies shift with applied tension.

I. INTRODUCTION[1]

The knee joint is one of the most complex joints in the body, leaving it liable to an array of injuries. In 2003, knee problems resulted in 19.4 million visits to physicians' offices and was the most common reason for visiting an orthopedic surgeon [1]. The most common contact injury of the knee is the anterior collateral ligament (ACL) rupture [3]. Injury to the ACL without injury to any other ligament occurs in approximately 60% of all ACL injuries. Between 15 and 25% of patients undergoing ACL surgery experience results that are less than optimal [3]. Ligaments and tendons both display nonlinear elasticity. A typical stress/strain curve for a ligament or tendon displays a linear region but also a nonlinear yield and failure region, ending in loss of tension resulting from a tear in the tissue. The relationship between stress and strain, however, is not constant with time.

One of the most difficult challenges facing orthopedic surgeons during ligament surgery is setting the correct ligament tension [4]. If the ligament is too loose then the knee joint will wobble and be unstable. If the tension is too tight, then the range of motion of the knee joint will be reduced. Finding the correct tension of knee ligaments is crucial to the success of the operation. The method presented in this paper uses Raman spectroscopy to evaluate tension on animal tendons.

II. BACKGROUND

Ligaments are dense connective tissue consisting mostly of collagen and are longitudinally aligned in the direction of stress allowing the collagen fibers to slide across one another for extensibility. Ligaments respond to stresses at a microscopic level over time and influence the laxity, stresses, and kinematics of motion. The anterior collateral ligament has a basic structure of fascicular subunits with larger functional bands. It has a core of tension-carrying fibrous collagen surrounded by blood vessels, nerves, and fibroblasts. There are also various amounts of folding or “crimp”, a sinusoidal pattern in collagen fibrils of the ACL [4]. The collagen fibers in the ACL increase in length by straightening the crimps when resistance decreases in the knee.

Collagen is the major protein component of connective tissue. It has a triple-helix structure, stabilized by hydrogen bonding between the polypeptides. Collagen contains about 33% glycine, which allows for close packing of the triple helix, 21% proline and hydroxyproline, which help stabilize the molecule, and 11% alanine. Collagen’s precursor molecule, tropocollagen, has a molecular weight of about 300 KD and a length of 300 nm. The tropocollagen molecules are arranged in a quarter-stagger conformation to form microfibrils, which then aggregate to form the connective tissue. The stability of collagen is attributed to the intra- and inter-molecular crosslinks, which also impart the tensile strength to the fibrils.

A. ACL Reconstruction

The maximum load and stiffness measured from ACLs obtained from young donors are 1725 ± 269 N and 182 ± 33 N/mm, respectively [5]. Two major options are available when deciding what to repair the ACL with: prosthetic ligaments or autografts. The strength of the graft is a major consideration in graft selection. Beynnon et al. [6] found that in order to approach the strength of a normal ACL, either the large bone-patellar tendon-bone graft or the multiply-looped hamstring tendon graft should be used. Butler [7] and McFarland et al. [8] suggested that all tendon tissues lose a considerable amount of strength during the early healing period. Ballock et al. [9] showed that proper placement of the tunnels in the femur and tibia during ACL reconstruction can minimize permanent stretching of the graft.

B. Graft Tension

The purpose of tensioning the graft is to establish and maintain normal stability of the joint by eliminating wobble and restoring movement to the normal range. In a randomized, short-term study, Yasuda et al. [10] demonstrated that a relatively high initial ACL tension (up to 80 N) reduces the postoperative anterior laxity of the knee. Harner et al. [11] suggested that graft fixation with a full extension may over-constrain the knee, and fixation at flexion and an anterior tibial load best restored normal knee biomechanics. Studies on the effect of graft tension and on the restoration of the knee’s range of motion and laxity performed on cadaveric knees reveal conflicting results. Burks et al. [12] reported that the restoration of anterior laxity require tensions between 25 and 180 N applied at a flexion angle of 22.5°. Gertel et al. [13] showed that initial forces in a graft are greater near extension when tension is applied to the graft from its proximal end with the knee at 30° if flexion. Eager et al. [14] found that anterior laxity returns close to normal (less than 1 mm difference), but grafts with high initial tension and low stiffness caused a tibia posterior sublaxation with respect to the femur in the unloaded state. Using a Hall effect strain transducer in vivo, Beynnon and Fleming [15] measured a peak strain of 4.4% in human ACL.

C. Raman Scattering

The Raman effect is the scattering of electromagnetic radiation by matter with a change in the frequency of the radiation and a change in energy level of the matter. Raman scattering occurs

when there is an inelastic collision between an incident photon of frequency v_0 and a molecule in its initial state E_m. Following the collision, the transition of the molecule to state E_n generates a photon of frequency *v*. The difference between Raman scattering and fluorescence is illustrated in the energy level diagram shown in Fig. 1. Here it is assumed that initially the molecule is in its ground electronic state and there are four vibrational levels in both the ground electronic state and the first excited state. W hen a photon is incident on the molecule, both Raman scattering and fluorescence may occur. The Raman scattering processes, A1, A2, S1, and S2, all involve a virtual state of the molecule. A1 and A2 are anti-Stokes Raman scattering with vibrational quantum number changes of v = 1 and 2, respectively, and these processes result in the final state of the molecule being at a lower energy level than the initial state (usually the ground electronic state). S1 and S2 are Stokes scattering processes where the final state of the molecule is at a higher energy level than the initial state. Fluorescence may involve many transitions as shown in the figure. However, the fluorescence transitions involve only actual states of the molecule. Unlike fluorescence, the Raman spectrum shows distinct spectral lines and the lifetime of the Raman transition is in the order of 10^{-12} s, much shorter than that of the fluorescence lifetime (~10^{-8} s). Fluorescence can only occur within the absorption band of the molecule - the energies of both the absorbed and emitted photons must correspond to energy differences between states of the molecule. Each of the two transitions in fluorescence is a first-order radiative process and is described by matrix elements linking the two molecular states. Raman scattering, however, is a second-order radiative process and can occur for an incident photon of any energy. If a photon of frequency v_0 is incident on a molecular system in state E_m, scattered radiation at frequencies *v* is given by:

$$v = v_0 - (E_n - E_m)/h \tag{1}$$

where h is Planck's constant. The state E_n may be higher or lower than E_m. The Raman shift, defined as $|v_0 - v|$, is characteristic of the molecule being probed by the incident radiation. Selection rules based on symmetry of the molecular species are used to determine which vibrations of the molecule are Raman-active.

III. MATERIALS AND METHODS

The source of mink and rabbit tissue was the Carolina Biological Supply Company. The cadavers were dissected at CW Optics in order to obtain tendon and ligament samples. The mink were used initially to test the measurement protocols. Mink ligaments and tendons were much smaller than those of the rabbit. Tissue was isolated by dissecting the cadaver and placed in saline. The tissue was then secured between the jaws of the sample holder and the lead screw used to remove the slack in the tissue without putting tension on the sample. Three different experiments were then performed using different samples.

1. Tension versus elongation

This experiment is basically a demonstration of Hooke's Law - a linear region exists where displacement is proportional to applied force. Besides serving to check the sample holder, this set of measurements defines the linear range (where $T \propto \Delta x$) for the Raman measurements. The separation of the jaws was measured for each movement of the lead screw. The load cell voltage was recorded during the measurement procedure and converted to tension after the measurements. Some experiments were run until the nonlinear region was reached, while others were terminated on the linear region. For those terminated on the linear region, the sample was removed from the sample holder, placed in saline, and remeasured at a later time. The linear range of the ligament or tendon tissues begins when (1) the slack is removed from the sample and (2) crimp is minimized.

Extended measurements were performed to obtain an estimate for the relaxation of the sample with time. Tension was applied to the ligament and monitored with time. These measurements are necessary to evaluate the actual tension in the sample over the sampling interval used to obtain the Raman spectrum.

Repeated measurements of tension versus elongation were performed to obtain an estimate for the hysteresis-like behavior of the samples. The sample was inserted into the sample holder and tension - elongation measurements were performed. The tension was released and the sample removed from the holder. At a later time (ranging from hours to several days) the measurement procedure was repeated.

2. Raman frequency measurements versus tension

After the sample was mounted in the in the tissue holder, the Raman spectrum of the tissue sample is obtained by bringing the probe to a distance of 1 cm (probe focal distance) from the sample. The spectrum integration varied from 35 to 112 seconds, depending on the sample. The sample spectra were obtained in a darkened laboratory with light shields around the instrument. The dark count was removed from the data and the resultant was transferred to a PC for analysis and archiving. The tension on the sample is increased by turning a leadscrew.

IV. RESULTS

The tendons of both minks and rabbits display nonlinear and linear elasticity regions and viscoelasticity. A graph displaying a pair of medial tendons from a rabbit is displayed in Fig. 2. One tendon (thin line) was stretched until failure while the contralateral tendon was stretched to half of the failing strain (7N) and held there for an hour before being relaxed and restretched until failure. The general trend shown in Fig. 2 is seen in the large majority of the tendons. The restretched tendon always experiences higher stress than it did before being held for an hour at half of the failing strain. The control tendon displays a linear region (from 0.3 to 6.0 N) and a nonlinear failure region (from 0.0 to 0.3 N). The maximum load was dependent on the type of tendon examined. The force constant k was determined for the linear region of the stress versus strain curve for both the initial and preconditioned measurements. The values of the force constant k were 70.26 N/cm and 131.97 N/cm for the initial and preconditioned tendon measurements, respectively.

The Raman spectrum from a rabbit tendon is shown in Fig. 3. The Raman lines can be seen sitting on a background of fluorescence. The excitation line was 785 nm at a power of 25 mW. The horizontal axis denotes the frequency shift from the excitation line, measured in cm^{-1}. A notch filter centered at the laser wavelength is used to shield the spectrometer from elastic (Rayleigh) scattering of the excitation line resulting in severely reduced intensity at Raman shifts less than 300^{-1} cm.

The intensity minimum observed at 350 cm^{-1} is due to the transmittance of the notch filter. Two distinct features are observable in the spectrum. They are (1) broad fluorescence starting at the laser wavelength (785 nm which corresponds to a 0 cm^{-1} shift) and decreasing with decreasing wavelength, (2) Raman lines associated with the collagen backbone and with the amino acid residues.

Raman spectra were collected from single tendons as functions of the applied stress. The frequency of the Raman lines associated with the structural backbone of the collagen helices show a decrease in frequency with increasing tension while the Raman lines associated with the residues exhibit an increase in frequency with applied stress.

IV. DISCUSSION

The major spectroscopic features of the Raman spectra of collagen were assigned to internal vibrations of individual amino acids, especially hydroxyproline, which is present at a high concentration and serves as a crosslink between the triple helices of the collagen molecule [16]. At Raman shifts smaller than 1000 cm^{-1}, the vibrations between the adjacent carbon atoms of the backbone, and of the hydroxyproline and prolinerings, account for the most prominent Raman features. At larger shifts, Raman lines corresponding to vibrational contributions of molecular subunits such as CH_3, NH_3^+, C-N, and the amide I and III become important. The peptide CONH group exhibits the characteristic Raman frequencies of the secondary amides. Strong Raman lines also can be observed at 1248 cm^{-1} (amide III), 1271 cm^{-1} (amide III), 1451 cm^{-1} (bending of adjacent CH_3 subunits), and 1671 cm^{-1} (amide I).

While investigating the effect of strain on collagen, Wang et al. [17] measured Raman features of a single fiber obtained from a rattail tendon. They observed that with increasing strain, some collagen Raman features (Raman lines at 822, 1166, 1417, 1443 and 1460 cm^{-1}) decrease in frequency whereas others (Raman lines at 879, 952, 1392 and 1672 cm^{-1}) increase in frequency. The Raman shift of the 824 cm^{-1} line is attributed to deformation of the collagen backbone (C-C stretching mode) with increasing tension. The amide groups and the hydroxyproline ring (879 cm^{-1}) reveal the signs of compression due to strain, as indicated by their Raman shifts to higher frequencies. The amide I vibration (1672 cm^{-1}) is characterized by carbonyl stretching and the N-H in-plane bending. The carbonyl group is almost perpendicular to the collagen axis (the direction of strain). It also shows signs of compression due to strain. The amide III vibration (963 cm^{-1}) is characterized by an in-plane C-N vibration which is laterally compressed when the structure is deformed under strain.

As the stress was increased, both positive and negative changes in Raman frequencies reported by W ang et al. [17] were also observed. The decrease in Raman shift with increasing stress is observed in the vibrations associated with the collagen backbone. The vibrations, which exhibit an increase in Raman shift with increasing stress, are associated with amino acid residues attached to the collagen backbone.

Figure 4 depicts the changes in Raman shifts of four Raman-active collagen vibrations 824 cm^{-1}, 879 cm^{-1}, 1417 cm^{-1} and 1672 cm^{-1}, as a function of stress. The 824 cm^{-1} line was assigned to C-C motion of the backbone by Frushour and Koenig [16]. The Raman line at a shift of 879 cm^{-1} is due to an internal vibration of hydroxyproline (most likely in the ring structure). The 1672 cm^{-1} line is an Amide I vibration. The slope of the curve associated with the 822 cm^{-1} line appears to become more negative (steeper) as the stress increases beyond 10N. This is in agreement with the data of Wang et al. [17]. A linear regression analysis was performed on the limited set of data shown in Fig. 4. The resulting coefficients were −0.49, 0.36, −.59, and 0.41 cm^{-1}/N for the Raman lines occurring at 824, 879, 1417, and 1672 cm^{-1} under a condition of zero stress.

V. CONCLUSIONS

The dependence of the frequency shift of the spectral lines of the Raman spectrum of collagen with applied tension demonstrates that Raman scattering is a means of determining the tension in ligaments and tendons.

Acknowledgements

This work was supported in part by NIAMS under grant 1R43AR053791.

References

1. orthoinfo.aaos.org/topic.cfm?topic= 100325, accessed march 27, 2008.
2. Sekiya JK, Ong BC, Bradley JP. Complications in anterior cruciate ligament surgery. Othrop Clin N Am 2003;34:99–105.
3. Zelle BA, Brucker PU, Feng MT, Fu FH. Anatomical double-bundle anterior cruciate ligament reconstruction. Sports Med 2006;36:99–108. [PubMed: 16464119]
4. Amiel, D.; Billings, E., Jr; Akeson, WH. Ligament Structure, Chemistry, and Physiology in Knee Ligaments Structure, Function, Injury, and Repair. Daniel, DM.; Akeson, WH.; O'Connor, JJ., editors. Raven; New York: 1990. p. 77-91.
5. Noyes FR, Grood ES. The strength of the anterior cruciate ligament in humans and rhesus monkeys. Age-related and species-related changes. J Bone Joint Surg 1976;58A:1074–82. [PubMed: 1002748]
6. Beynnon BD, Johnson RJ, Fleming BC, Kannus P, Kaplan M, Samani J, Renstrom P. J Bone Joint Surg 2005;84A:1503–1513.
7. Butler DL. Anterior cruciate ligament: its normal response and replacement. J Orthoped Res 1989;7:910–21.
8. McFarland EG, Morrey BF, An KN, Wood MB. The relationship of vascularity and water content to tensile strength in a pateller tendon replacement of the anterior cruciate in dogs. Am J Sports Med 1986;14t4:436–48. [PubMed: 3541655]
9. Ballock RT, Woo SLY, Lyon RM, Hollis JM, Akeson WH. Use of pateller tendon autograft for anterior cruciate ligament reconstruction in the rabbit: a long-term histologic and biomechanical study. J Orthop Res 1989;7:474–85. [PubMed: 2738766]
10. Yasuda K, Tsujino J, Tanabe Y, Kaneda K. Effects of initial graft tension on clinical outcome after anterior cruciate ligament reconstruction. Am J Sports Med 1997;25:99–106. [PubMed: 9006702]
11. Harner CD, Janaushek MA, Ma CB, Kanamori A, Vogrin TM, Woo SLY. The effect of flexion angle and application of an anterior tibial load at the time of graft fixation on the biomechanics of a posterier cruciate ligament-reconstructed knee. Am J Sports Med 2000;28:460–5. [PubMed: 10921635]
12. Burks RT, Daniel D, Losse G. The effect of continuous passive motion on anterior cruciate reconstruction stability. Am J Sports Med 1984;12:323–7. [PubMed: 6476191]
13. Gertel TH, Lew WD, Lewis JL, Stewart NJ, Hunter RE. Effect of anterior cruciate ligament graft tensioning direction, magnitude, and flexion angle on knee biomechanics. Am J Sports Med 1993;21:572–81. [PubMed: 8368419]
14. Eager P, Hull ML, Howell SM. How the fixation method stiffness and initial tension affect anterior load-displacement of the knee and tension in anterior cruciate ligament grafts: a study in cadaveric knees using a double-loop hamstring graft. J Orthop Res 2004;22:613–24. [PubMed: 15099643]
15. Beynnon BD, Fleming BC. Anterior cruciate ligament strain in-vivo: A review of previous work. J Biomech 1998;31:519–25. [PubMed: 9755036]
16. Frushour BG, Koenig JL. Raman scattering of collagen, gelatin, and elastin. Biopolymers 1975;14:379–91. [PubMed: 1174668]
17. Wang YN, Galiotis C, Bader DL. Determination of molecular changes in soft tissues under strain using laser Raman microscopy. J Biomech 2000;33:483–6. [PubMed: 10768397]

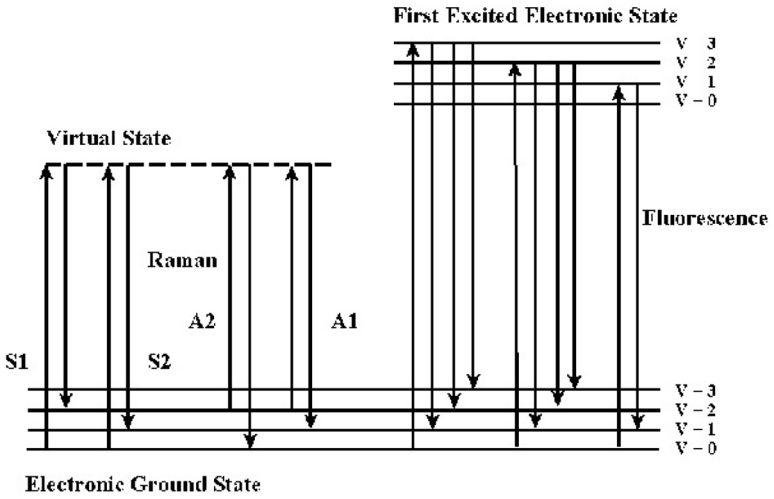

Fig. 1.

Comparison of energy levels in Raman scattering and fluorescence. The intermediate state in raman scattering is virtual while in fluorescence the intermediate state is an allowed state of the system.

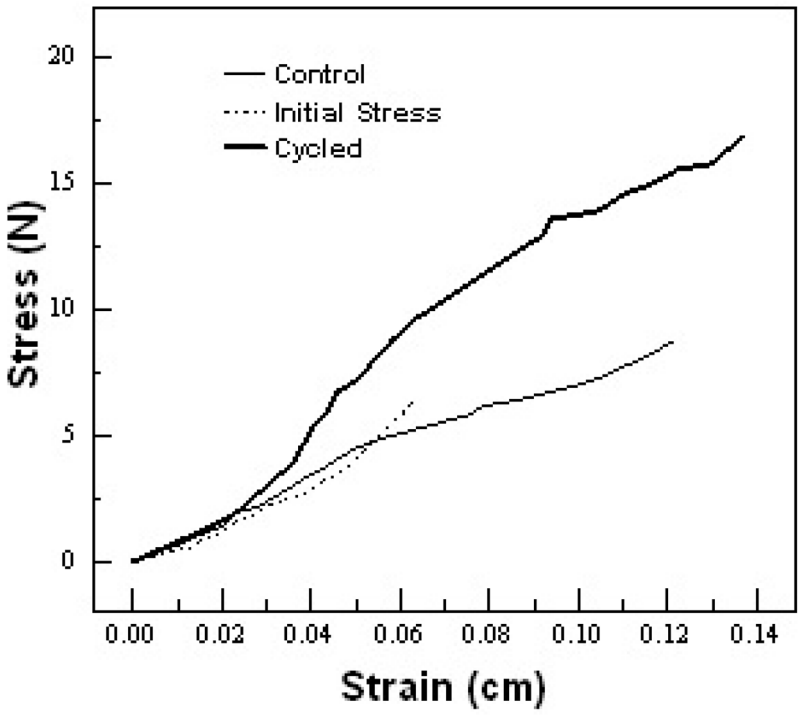

Fig. 2.

The control ligament was stretched until breaking (14 N). The second ligament was stretched to a tension of 7N and held at constant tension for 1 hr. The tension was then released and the ligament stretched until it ruptured.

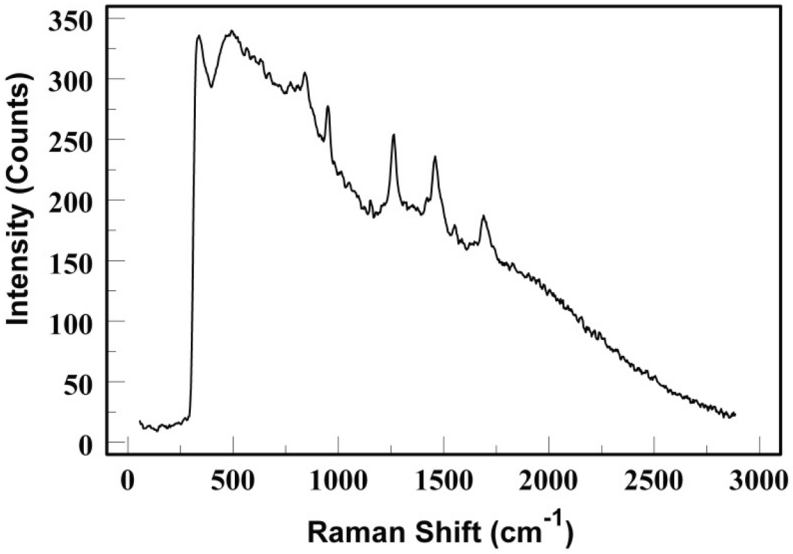

Fig. 3.
Raman spectrum of rabbit ligament under tension of 5 N. The Raman lines are visible on top of fluorescence.

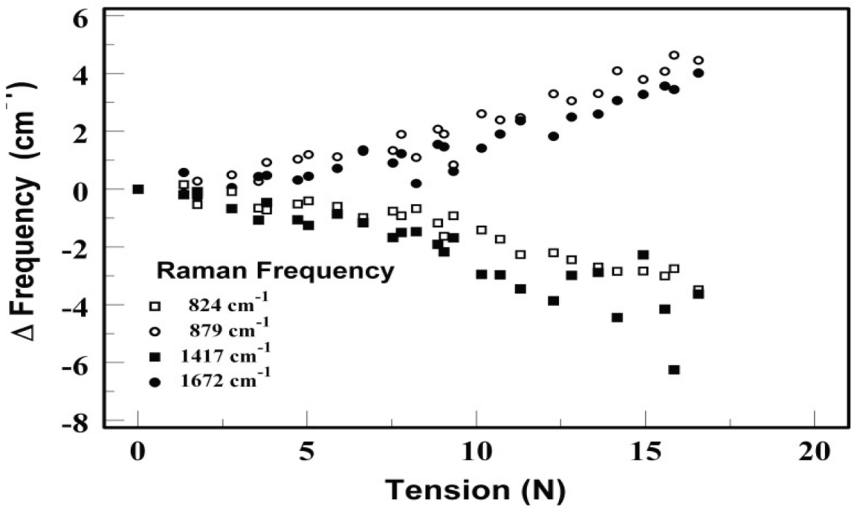

Fig. 4.
Comparison of Raman frequencies as a function of applied stress.