

BRIEF COMMUNICATION

Raman and Surface Enhanced Raman Scattering Applications in Shock Wave Therapy Related Research

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The biological applications of Raman scattering (RS) in its different forms continues to grow exponentially, and the literature is so extensive that in a short communication references will not even attempt to do justice to the field. The fingerprint for molecular structure provided by vibrational spectroscopy, and their relation to functionality in biochemical systems can be used for the development of a quantitative technique for biomarkers. These vibrational fingerprints in the spectra are used to track and characterize species such as small low-molecular-weight metabolites and also follow molecular species in large living organisms. Today, researchers are making great progress applying RS to unravel the structure/function issues in proteins, nucleic acids, and lipids. Recently, the efforts are devoted to bioanalytical and medical diagnostic applications. In our group, for biomedical applications, we integrate a full range of Raman experimental methodologies, including Raman microscopy, resonance Raman scattering microscopy, near-infrared Raman, and the ultrasensitive analytical technique surface-enhanced Raman scattering.¹ This molecular approach is then integrated with the biomedical research in an attempt to understand the biological processes.² Here, we present the first steps towards the molecular understanding of the important improvements of rotator cuff supraspinatus tendons diseases that have seen after shockwave treatment. Neo-angiogenesis stimulation and hypercellularization are the result of short time periods of treatment. The beginning of this work, necessarily, requires an extensive background research dedicated to the creation of the appropriate database for fingerprint characterization of the biomolecules present in the tissue.

This is an enormous task that involves a large group of multidisciplinary researchers with a top-down approach of the medical team (the real samples) and a bottom-up approach of the spectroscopist, all helped by the statistical analysis and modelling of the physics group.

The preliminary results have been selected from our Raman scattering and plasmonic driven technique of Surface-enhanced Raman scattering.³ The background information included the studies of the basic amino acids forming collagen, two different types of collagen and 52 biopsies of tendon tissues. Briefly, the inelastic Raman scattering was collected using a micro-Raman system with a spatial resolution of 1 micron squared and the sample is illuminated with laser lines at 442 nm, 514.5 nm, 632.8 nm, or 785 nm, depending on the optimization of the experimental conditions. SERS was attained using overlayers of silver and also colloidal silver nanoparticles. Typical Raman spectra of the amino acids most commonly found in collagen are shown in **Figure 1**.

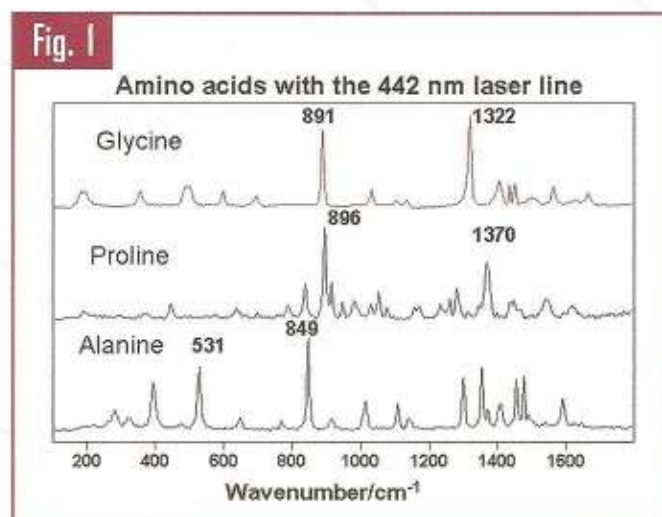
It can be seen, that each molecule of the amino acid has its own characteristic spectral pattern, and characteristic wavenumbers can be

identified for each one of them. The extensive and complete analysis and computational work for each molecule will be published separately.

The collagen detection and characterization was demonstrated using to commercially available collagens; the rabbit skin (TR in the spectra), and ox bone (CB in the spectra). The experimental SERS was obtained by depositing 6 nm mass thickness of silver by vacuum evaporation onto the collagen sample. Micro-Raman was recorded using point-by-point-mapping. The mapping shows that a typical pattern repeat itself on the silver coated collagen for both collagens. These typical spectral patterns are shown in **Figure 2**.

It can be said that there are four characteristic wavenumbers in both collagen samples. Characteristic here means that these Raman bands have similar relative intensities and are observed at approximately the same wavenumber maxima; 799, 1003, 1353 and 1637 cm^{-1} . The band at 736 cm^{-1} , clearly marks the difference between the two forms of collagen. Notably, the vibrational spectra of collagen has also been studied using a pulsed source neutron spectrometer.⁴

The final and more challenging part of the work is prove of concept that good SERS spectra can be obtained from the tissue (biopsies) provided by the medical team. The SERS spectra obtained for several of these samples are shown in **Figure 3**. The technique is the same applied to obtain the spectra of collagen. It can be seen that the spectra are of excellent



quality and we have found the experimental conditions that avoid sample burning and sample degradation. We are in the process of recording now a substantial amount of new data on these and other samples (thousands of spectra) that should give us the statistical

validation for characterization of the molecular species.

We are also beginning to work on the identification of other molecular components in the tissue.

The preliminary data are encouraging. It can be seen that there are characteristic wavenumbers that can be assigned to collagen, marked in Figure 3 in all five samples. The experimental work will continue as to enhance our database, and then we will use multicomponent analysis, specially adapted to our needs, to extract the information from the spectral maps obtained by SERS of tissue samples. The aim of the work is to provide a spectral characterization of the tissues before and after shockwave treatment.

Fig. 2

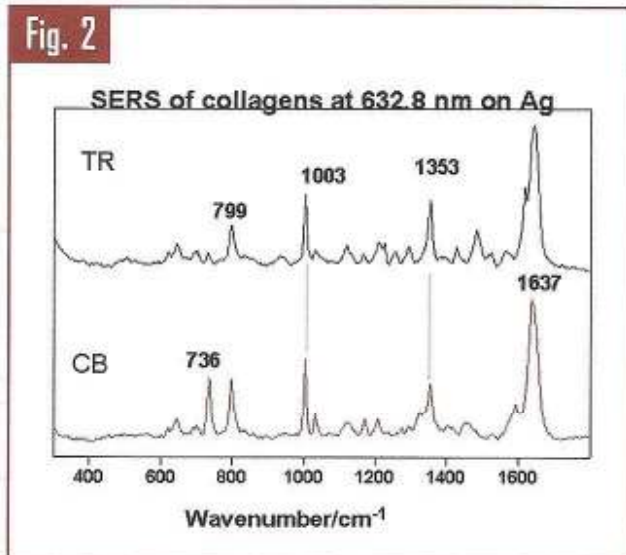
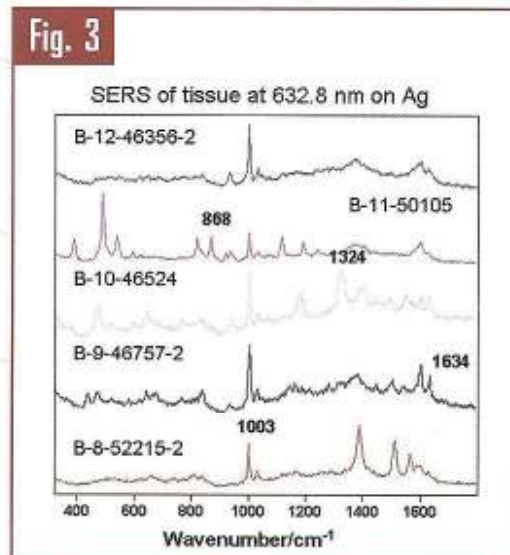


Fig. 3



References

1. Aroca, R. *Surface-enhanced Vibrational Spectroscopy*; John Wiley & Sons: Chichester, 2006.
2. Kneipp, K.; Aroca, R.; Kneipp, H.; Wentrup-Byrne, E.; Editors *New Approaches in Biomedical Spectroscopy. (Symposium held in Honolulu, Hawaii December 2005.)* [In: *ACS Symp. Ser.*, 2007; 963], 2007.
3. Baker, G. A.; Moore, D. S. *Analytical and Bioanalytical Chemistry* 2005, 382, 1751-1770.
4. Middendorf, H. D.; Hayward, R. L.; Parker, S. F.; Bradshaw, J.; Miller, A. *Biophysical Journal* 1995, 69, 660-673.