Near Infrared Raman Spectroscopy to detect the calcification of the annular mitral valve

R. Rocha[1], E. P.U. Otero[2], M. S. Costa[1], A. Balbin Villaverde[1], P. M. A. Pomerarantzeff[3] and M. T. T. Pacheco[1]

1-Universidade do Vale do Paraíba- UNIVAP
Instituto de Pesquisa e Desenvolvimento
Av. Shishima Hifumi 2911, Urbanova.
12244-000, São José dos Campos, SP, Brazil
2-Instituto de Física Gleb Wathaghin, Unicamp,
13083-970, Campinas, SP, Brazil
3-Universidade de São Paulo, USP
Instituto do Coração
Av. Dr Enéias de Carvalho Aguiar 44, Cerqueira César
01246-903, São Paulo, SP, Brazil

ABSTRACT

Cardiac valves are subjected to high repetitive mechanical stresses, particularly at the hinge points of the cusps and leaflets due to the over 40 millions cardiac cycles per year. These delicate structures can suffer cumulative lesions, complicated by the deposition of calcium phosphate mineral, which may lead to clinically important disease. Near Infrared Raman Spectroscopy gives important information about biological tissues composition and it is being used for diagnosis of some pathologies. The aim of this work was to detect trough the use of the Raman Spectroscopy technique the mitral annular calcification. A Ti:saphire laser operating at the near infrared wavelength of 785 nm was used for the excitation of the valve samples and the Raman radiation was detected by an optical spectrometer with a CCD liquid nitrogen cooled detector. In all, ten samples of normal and pathologic tissues were studied. They were approximately squared with the lateral size of 5 mm. It was observed that the Raman spectrum of the calcified mitral valve showed different behavior, when compared to normal tissues. Results indicate that this technique could be used to detect the deposition of the calcium phosphate mineral over the mitral valve.

Keywords: Raman Spectroscopy, Calcification, Annular Mitral Valve

1. INTRODUCTION

The aortic valves are submitted to intensive repetitive mechanic stresses, especially in the hinge points of the cusps and leaflets, owing to 40 million or more cardiac cycles per year[1,2]. These delicate structures can suffer cumulative damage complicated by the deposition of calcium phosphate mineral.

Optical spectroscopy techniques, such as reflectance, fluorescence, infrared absorption and Raman scattering can provide information about the tissue composition at the molecular level. Among these techniques, Raman spectroscopy has an excellent capability to provide valuable biochemical information for non-destructive diagnosis of cardiovascular disorders[3].

The Raman scattering is an inelastic process, that occurs when a sample is illuminated with a light source as a laser beam. In this process, energy from the incident photons is transfer to the sample molecules, exciting them to high vibrational modes. Scattered photons have a lower frequency than the incident ones due to the energy that is lost in the

5th Iberoamerican Meeting on Optics and 8th Latin American Meeting on Optics, Lasers, and Their Applications, edited by A. Marcano O., J. L. Paz, Proc. of SPIE Vol. 5622 (SPIE, Bellingham, WA, 2004) · 0277-786X/04/$15 · doi: 10.1117/12.589448

scattering process. The frequency shift of the excitation radiation corresponds to the different vibrational frequencies of the molecules of the sample material. The number of biomedical applications for the near infrared Raman scattering (NIRS) technique is growing very fast for the last ten years.

Raman spectra of tissues present narrow and well resolved bands with linewidth of the order of 10-20cm^{-1}, which reveal the presence of many biochemical molecules[3]. The relative contribution of these biochemical molecules for the Raman spectrum of the tissue are proportional to the relative abundance of molecules present in the tissue. This is the base for the information that the near infrared Raman spectroscopy can provide for diagnosis. The quantitative nature of the Raman spectra, combined with the ability to provide an unique identification of the biochemical molecules present in tissues, illustrates the potential of the Raman spectroscopy for disease diagnosis.

Among various biomedical investigations of Raman spectroscopy, some of the important diagnosis applications are in the atherosclerosis[4-6], breast cancer[3], colon cancer[7,8], skin cancer[3], analysis of blood metabolites[9-11] and cardiac valves[12].

The aim of this work was to detect trough the use of the Near Infrared Raman Spectroscopy (NIRS) technique the mitral annular calcification.

2. MATERIALS AND METHODS

2.1 Samples

The cardiac valves were obtained from patients that needed to have their annular mitral valve removed through a surgical procedure. The surgery was accomplished at the hospital "Incor-HC-FMUSP, São Paulo, Brazil". All patients signed a consent form. In all, five valves were studied. A total of ten squared samples with the lateral size of 5 mm were extracted, two for each valve. They were chosen by the visual observation of the valve's tissue, one was from the normal section of the tissue (non-calcified) and the other from the calcified region. Then, samples were snap-frozen and stored in liquid nitrogen (-160°C). Prior to the Raman measurements, the samples were warmed up to room temperature and added to them a few drops of saline solution (0.9%) during Raman data acquisition.

2.2. Raman spectroscopy

The excitation laser source consisted of a Ti: Sapphire laser (Spectra Physics, 3900S model) pumped by a 6W multiline Argon laser (Spectra Physics, model stabilite 2017), tuned at 830 nm. The Raman radiation from the sample was collected and coupled into the spectrometer entrance slit by a series of lenses. The optical detection system was composed by a high coupling spectrometer (Kaiser Optical Systems, Inc. f/1.8i) and a liquid nitrogen cooled CCD, "Deep Depletion" detector (Princeton Instruments EEv with 1024x256 pixels). The spectrometer resolution was estimated to be around 10 cm^{-1}. The detection system was controlled by a personal computer that saved and processed the Raman spectra. The intensity of the excitation laser was strongly reduced in the collecting system by using a set of holographic notch filters (Kaiser Optical Systems, model HLBF 830, USA). The exposure time for collecting Raman signal was 100s in all experiments.

The optical detection system, spectrometer and CCD detector, was calibrated using as a reference an organic compound, the indene C_9H_8, with a well established Raman spectrum and that presents intense bands covering the spectral range from 700 to 1700 cm^{-1}. The contribution of the sample fluorescence to the Raman spectra was eliminated by subtracting a third order polynomium fitting from the gross spectrum.

A schematic diagram of the experimental setup for Raman spectroscopy is displayed in Figure 1, showing the excitation laser source, the spectrograph, the CCD detector, the controller, the personal computer and the corresponding optics.

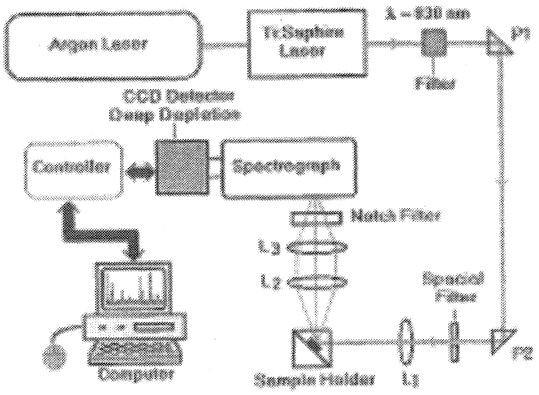

Figure 1. Schematic diagram of the experimental setup.

3. RESULTS AND DISCUSSION

A characteristic Raman spectrum from a sample of normal annular mitral valve is shown in Figure 2. The spectrum is displayed for the Raman shift range of 800 to 1900 cm^{-1}. The contribution to the spectrum of the fluorescence of the sample was already subtracted. Light from the Raleigh scattered radiation is so strong below 800 cm^{-1} that any sample's Raman peak could be identified. It can be seen three main bands appearing in the spectrum of a normal mitral annular mitral valve. The strong bands at 1670, 1448 and 1270cm^{-1} can be ascribed to the amide I (C=O stretching), C-H bending (H-C-H union) and amide III, vibrational modes of structural proteins, respectively[13].

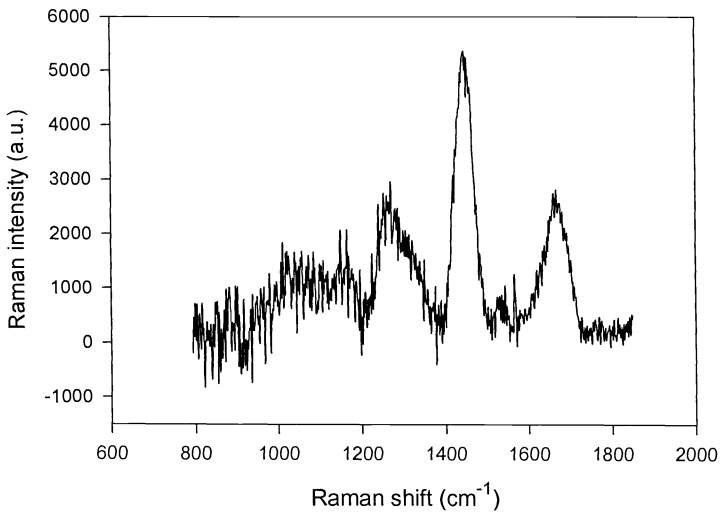

Figure 2: Characteristic Raman spectrum from a tissue of normal annular mitral valve

Likewise, a Raman spectrum of a calcified annular mitral valve is displayed in Figure 3. It can be observed six Raman bands in this case, corresponding to the Raman shifts of 967, 1051, 1275, 1448, 1680 and 1750 cm^{-1}. The strongest peak is located at 967 cm^{-1}. The observed bands at 967 and 1051 cm^{-1} are ascribed to phosphate and carbonate symmetric stretching vibrations of calcium hydroxyapatite and of carbonate apatites, respectively; materials that were deposited on the tissue.

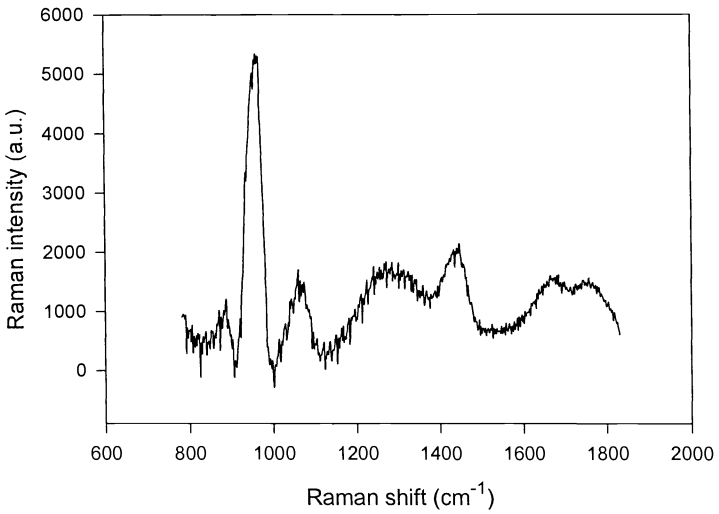

Figure 3: Characteristic Raman spectrum from a tissue of a calcified annular mitral valve

It can be observed by comparison of the two spectra that the three peaks exhibit in the normal tissue are also present in the calcified; but, now we have two new and strong peaks for the calcified tissue, that correspond to the calcium accumulated on the valve's tissue. It appears also a new weak peak at 1750 cm^{-1}, that it is almost superposed to the 1680 cm^{-1} peak. Small differences between the peak positions of different spectra are not significant. Those may be due to calibration variations and spectral resolution.

4. CONCLUSION

These preliminary results indicate that the NIRS could be an efficient technique to detect the deposition of the calcium phosphate mineral over the mitral valve. However, further studies are required to validate this method and to evaluate its sensitivity and specificity. Multivariate statistics such as principal components analysis could be used to improved this technique.

REFERENCES

1. Christensen G. "Cardiovascular and renal effects of atrial natriuretic factor". Scand J. Clin Lab Invest, **53**:203-7, 1993.

2. Peters NS. et al. "Cardiac arrhythmogenesis and the gap junction". J Mol Cell Cardiol., **27**:37-40, 1995.

3. Hanlon EB. et al. "Prospect for "in vivo" Raman spectroscopy". Phys. Med. Biol. **45:**R1-59, 2000.

4. Lucas A. et al. "Development of an avian model for restenosis". Atherosclerosis **119:**17-41, 1996.

5) Sathaiah S, Silveira L Jr., Pasqualucci CA, Zangaro RA, Chavantes MC. and Pacheco MTT. "Correlated diagnosis of atherosclerosis with non-invasive Raman spectroscopy and pathological techniques". International Laser Congress,Greece,**72** ,1996

6) Sathaiah S, Pasqualucci CA and Pacheco MTT. "Application of near Infrared Raman spectroscopy for less invasive diagnosis of atherosclerosis". Proc. International Conference on Lasers 97, U.S.A., 1997.

7) Manoharan R et al. "UV resonance Raman spectroscopy for detection of colon cancer" Lasers in Life Sci. **6**: 217-49, 1995.

8) Boustany NN et al. "Analysis of normal and diseased colon mucosa using ultraviolet resonance" Raman spectroscopy. Proceedings of SPIE, Advances in Lasers and Light Spectroscopy to Diagnose Cancer and other Diseases III: Optical Biopsy, San Jose CA vol. **2679:** 66-70, 1996.

9) Pilotto S, Pacheco MTT, Silveira L Jr, Balbin Villaverde A and Zangaro RA "Analysis of near-infrared Raman Spectroscopy as a new technique for a transcutaneous non-invasive diagnosis of blood components". Laser Med. Sci ,**16**:2-9, 2001.

10) Jianan Y , Wilson B C and Suria D . " Concentration measurements of multiple analytes in human sera by near-infrared laser Raman spectroscopy". Appl Opt **38(25)**:5491-7, 1999.

11) Berger A, Koo TW, Itzhan I, Horowitz G and Feld MS. "Multicomponent blood analysis by near-infrared Raman spectroscopy". Appl Opt **38(13)**:2916-6,1999.

12) Otero E.U., Sathaiah S., Silveira L. Jr., Pomerantzeff PMA and Pasqualucci CAG. "Raman spectroscopy for diagnosis of calcification in human heart valves". Spectroscopy: An International Journal **18(1)**:75-84, 2004.

13) Stone N et al. "Near-infrared Raman Spectroscopy for classification of epithelial pre-cancers and cancers". J. Raman Spectrosc, **33**:564-73, 2002.