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Evaluation of mechanical behavior of soft tissue by means of random laser emission

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We demonstrate the use of random laser emission for mechanical testing of bovine pericardium. An apparatus designed for tensile tests of soft and thin materials, incorporating optical and mechanical devices, allows for obtaining the mechanical behavior of the tissue samples. Using both, digital image correlation (DIC) and random laser emission analysis, the apparatus provides information regarding the response of the bovine pericardium under different stress levels. Our results show that changes in the spectral features of the random laser correlate well to the mechanical response obtained with conventional uniaxial tensile analysis coupled with DIC. Furthermore, parameters such as the shear and Young moduli are consistent with values reported previously and obtained with other techniques. Changes at the microstructural level of the tissue may thus be evaluated through spectral analysis of the random laser emission from biological samples. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4823783]

I. INTRODUCTION

Aortic and mitral valve disorders are among the top causes of heart related diseases; in most cases, these pathologies require valve replacement using bioprosthetic or mechanical heart valves.[1] Bioprosthetic valves based on bovine or porcine pericardium have been widely used as replacements for human cardiac valves, mainly because patients do not need a lifelong anticoagulation treatment.[2] The long-term durability of theses valves is mostly limited by calcification, which leads to progressive tissue degeneration and eventually to valve failure.[2,3] It has been shown that the mechanical behavior of the tissue used for the bioprosthetic valves determines important features such as leaflet flexibility, membrane stresses, and ultimately valve longevity.[4] Thus, methods for evaluating the mechanical properties of the biomaterials used for the valves are of great interest.

Several studies have been made to gain a better insight of the mechanical behavior of bovine pericardium.[4–6] Nevertheless, mechanical characterization of this kind of tissue is difficult because of its complex nature and its nonlinear mechanical response. Most reports are based on evaluating the macroscopic properties of the tissue via uniaxial loading tests, yielding the tensile strength, and the uniaxial extensibility of the material.[4,5] Recently, efforts have been made to obtain information of the micromechanical behavior of bovine pericardium using optical characterization methods.[7–9] Among other features, optical techniques allow for studying soft and thin materials and most importantly, they offer non-contact capabilities which can be simultaneously performed with mechanical tests. A promising approach to obtain information of microstructural changes in tissue is based on spectral analysis of random laser (RL) emission, obtained upon using the tissue itself as a scattering medium.[10,11] Since the microstructure of the tissue determines the scattering features of the random medium, changes at the microstructural level modify the emission spectrum of the RL.[10–12] In this work, we evaluate the RL emission from bovine pericardium during uniaxial loading tests using an apparatus specifically designed for soft materials. By means of digital image correlation (DIC) analysis, the micromechanical response of the tissue samples is obtained during the tests. Simultaneously, we register the RL emission from the samples and spectral features such as the full-width at half-maximum (FWHM) can be readily obtained. Our results show that changes in the FWHM of the RL spectrum correlate well with the DIC results, thereby suggesting that the spectral variations in the RL emission can be associated to microstructural changes in the tissue induced during the loading test.

II. MECHANICAL TESTER FOR SOFT AND THIN MATERIALS

The mechanical testing device used in our experiments is based on a load frame designed to apply uniaxial load on soft and thin samples of materials. Relevant parameters for this test, such as the force load and displacement, are monitored via electronic sensors. The apparatus further incorporates appropriate mountings for image acquisition via a long working distance microscope and a CCD camera. Additionally, an optical fiber coupled to a solid state spectrometer is used to perform spectral analysis of the RL emission from the samples. Data acquisition as well as synchronization of the electronic and optical devices are conveniently carried out with LabVIEW.

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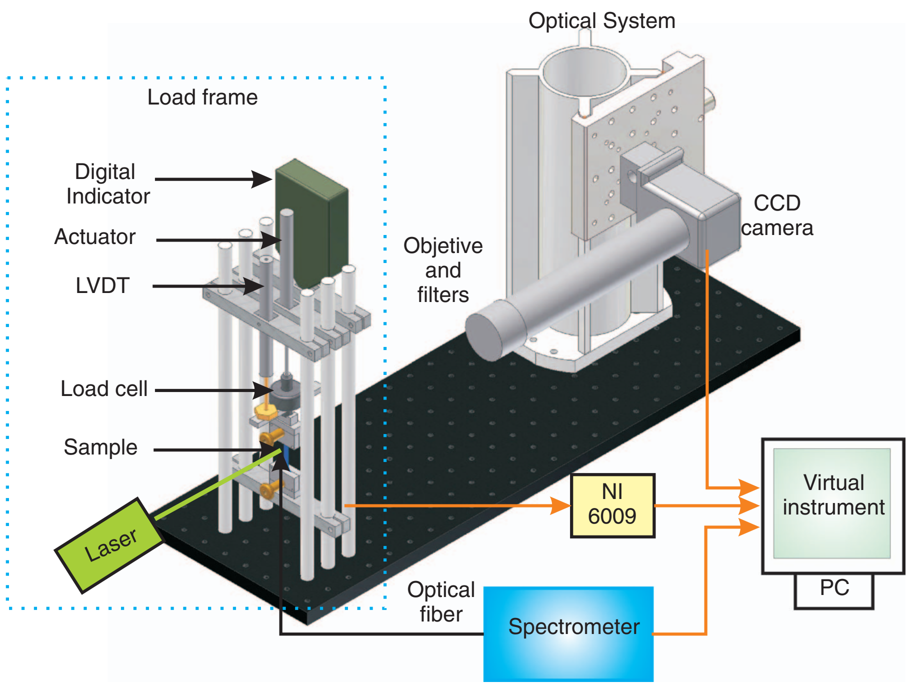


FIG. 1. Modular optomechanical apparatus for tensile tests of soft and thin materials. The mechanical load frame (dotted lines) and the optical systems used for image acquisition and laser emission analysis are controlled via virtual instruments.

A. Uniaxial loading control and data acquisition

The load frame of the apparatus was built using stainless steel bars supporting the axial load system composed by two rectangular cross-heads. A mechanical actuator is fixed to the upper cross-head of the frame, and a force sensor is coupled to the end of the actuator. As depicted in Fig. 1, the load frame is held together by a rectangular stainless steel beam, which also serves for housing the displacement sensor (a linear variable differential transformer, LVDT) and its corresponding reference device for calibration. The tissue samples are held using a special set of grips specifically designed to avoid damage and slip during the tensile test.

The mechanical actuator used in the load frame is a T-LA28A Zaber with a linear displacement range of 28 mm and providing up to 15 N of loading force. A stepper motor is used to control the actuator allowing to obtain linear displacements of 304.8 μm per revolution and 0.01 μm per microstep. With this arrangement, displacements can be controlled with an accuracy of ± 12 μm. A precision miniature load cell, model 31 from Honeywell Sensotec, is used to measure the axial load during the tensile tests. Tension and compression load forces (± 9.81 N range, 0.15%–0.25% accuracy) are registered with this device, which also compensates for off-axis loading effects thus minimizing the shear force components. This feature avoids shear stress contributions to the measurements which would modify the general state of stress of the sample during the test. Displacement of the linear actuator during the tests is measured by means of a LVDT, model MHR1000, from Lucas Schaevits. The output from the LVDT is processed with an ATA 2001 signal conditioner from the same manufacturer and a Mitutoyo digital position indicator (ID-F150HE) is used as displacement reference.

Data acquisition and control is performed with a National Instruments NI-USB 6009 data acquisition board, used to register time, displacement, and force data during the tensile tests. All the relevant data from the experiment, including digital image acquisition and random laser signals, were synchronized by means of a customized virtual instrument (VI) programmed in LabVIEW. The VI is composed by three modules: one for preliminary port initialization and configuration, and one for adjusting the initial settings of the experiment such as camera focusing and sample visualization. Finally, the remaining module corresponds to the uniaxial tensile test in which time, displacement, and force data are acquired while the images and the optical spectrum are simultaneously recorded. Subsequent data processing can be readily performed using the data files recorded with the VI.

B. Image acquisition and random laser emission

A custom-designed long working distance optical microscope was coupled to the load frame for image acquisition. The optical system uses a monochromatic Sony XC-ST70 CCD camera (2/3 in CCD) with a C-mount. A Mitutoyo objective ($5\times$) is used to obtain appropriate magnification of the tissue surface; the image area obtained with our setup up is 3 mm^2 (2 mm $\times$ 1.5 mm images). An InfiniTube$^{\mathrm{TM}}$ assembly unit was also used to couple the CCD camera to the objective; this arrangement further allows for placing filter holders and a beam splitter within the optical axis of the microscope. The illumination system used in the setup is a Fiber-Lite DC950 coupled to an optical fiber bundle attached to the microscope via the beam splitter. Image acquisition from the camera is performed using a NI-PCI 1409 board from National Instruments.

Random laser emission from the tissue was obtained upon pumping the samples containing Rhodamine 6G with a Nd:YAG pulsed laser. The samples were prepared from bovine hearts (12–18 month calves) with intact pericardium collected fresh from a local slaughterhouse. The hearts were transported to the laboratory in cold saline solution and were processed within the following four hours. The pericardium sacs were removed from the hearts; subsequently, fat and excess tissues were removed from the bovine pericardium sacs. Sample sheets of selected sacs were cut and hung in custom-built frames; subsequently, they were cross-linked with glutaraldehyde (GA). The fixation process was carried out during 24 hours at 4 °C, using 0.5% GA in 0.1 M phosphate buffered saline solution with a pH of 7.4. Next, the sheets were washed in distilled water and subsequently cut with a special jig to obtain samples with a dog bone shape, a standard for tensile tests. Finally, the samples were immersed and stored in a solution of Rhodamine 6G (R6G) dissolved in glycerin with a concentration of 1 g/l.

The pump laser (New Wave Solo I) was operated at 532 nm wavelength providing nanosecond pulses (5–7 ns) and a spot of approximately 0.5 cm in diameter. The beam was oriented to illuminate the central region of the tissue and an optical fiber was used to capture the light emitted from the sample. The spectral features of the random laser emission were analyzed and recorded with a solid-state spectrometer (HR4000, Ocean Optics), with an optical resolution of 0.2 nm and an operating wavelength range of 200–1100 nm.

III. EXPERIMENTS

The tensile tests were conducted programming the VI to register data such as force, displacement, and spectral emission along with its corresponding image from the CCD camera. Random laser emission from the tissue samples was registered during the uniaxial load test using a fixed energy for the pump laser. The VI starts the uniaxial load test in steps of 50 μm and all data, mechanical and optical, are acquired in a synchronized manner. After the test is finished, the stored data are processed to determine relevant relations such as stress as a function of elongation ratio ($\lambda - \lambda^{-2}$). Analysis of the random laser spectra is based on evaluating changes in the full width at half maximum according to $\Delta\mathrm{FWHM} = \mathrm{FWHM}_i - \mathrm{FWHM}_0$. Thus, we compare the registered spectral width for a given elongation ratio (FWHM_i) with the initial width (FWHM_0) of the random laser emission obtained without elongation.

The mechanical behavior of the bovine pericardium samples during the tensile tests was obtained using spectral analysis of random laser emission and DIC. While the latter can provide information regarding the micromechanical features of the sample, random laser analysis might be used to obtain aspects related to the macromechanical response. As customary for tensile tests with elastomeric materials, λ in the elongation ratio is defined in terms of the strain (ϵ) as $\lambda = \epsilon + 1$.[13]

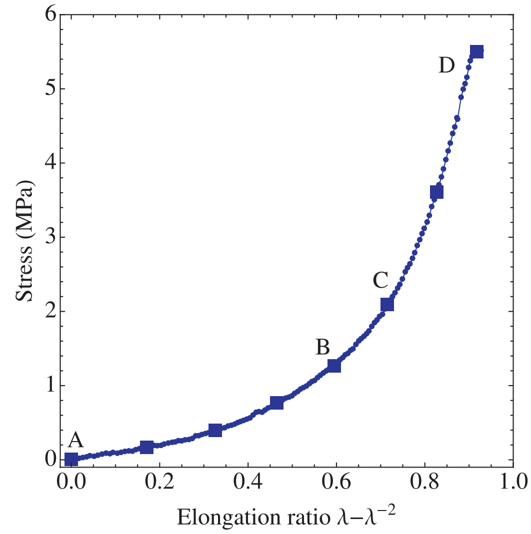


FIG. 2. Typical curve showing the stress as a function of elongation ratio for a bovine pericardium sample obtained during the uniaxial tension test.

IV. RESULTS AND DISCUSSION

A typical curve showing the stress as a function of the elongation ratio for a sample of bovine pericardium is shown in Fig. 2. The points in the curve marked as *A*, *B*, *C*, and *D* are used to identify different mechanical behaviors observed during the test. The zone delimited by points *AB* is obtained for small elongation ratios, when the collagen fibers of the sample are still in a compact arrangement. In contrast, the zone between points *CD* represents a stress condition under which the fibers are aligned along the same direction as that of the applied load. Points *BC* are the limits of a transition zone in which the fibers do not show a specific orientation. In general, the elongation ratios delimiting this transition zone will depend on the sample anisotropy. Changes in the orientation of the collagen fibers are apparent from the microscope images obtained during the tensile test shown in Fig. 3. Whereas images *i* and *ii* are typically obtained within the zone

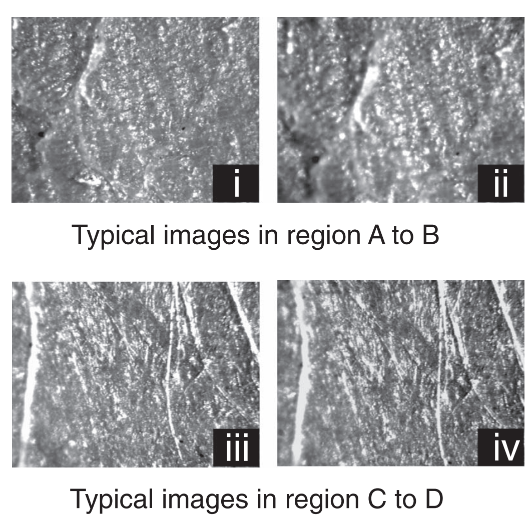
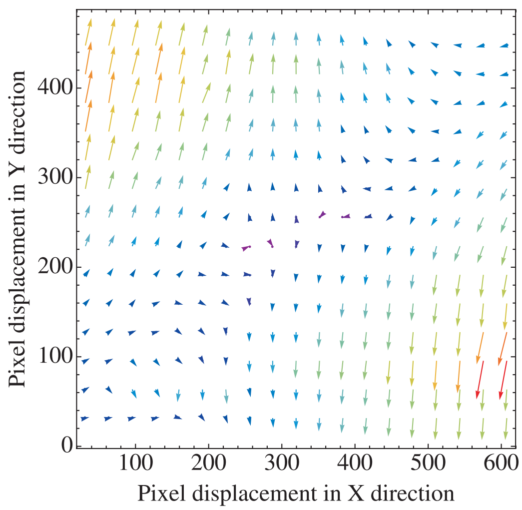
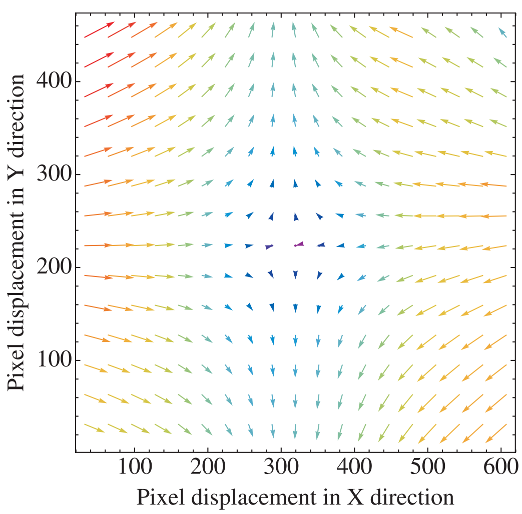


FIG. 3. Microscope images of the bovine pericardium sample for different loading conditions (see text for details).



(a)



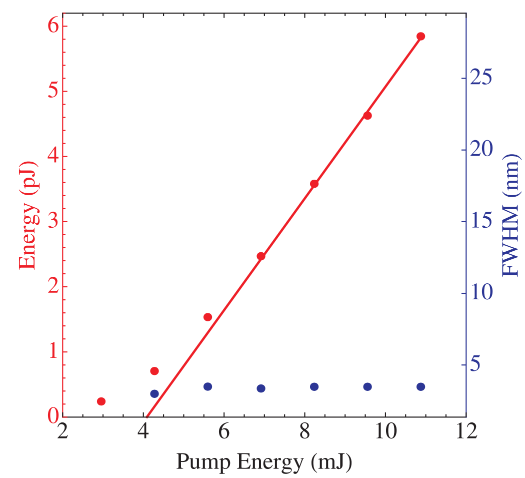
(b)

FIG. 4. Micromechanical response of the bovine pericardium samples: (a) displacement vector field obtained from images *i* and *ii* (region delimited by points *A* and *B* in the stress curve) and (b) displacement vector field obtained from images *iii* and *iv* (region delimited by points *C* and *D* in the stress curve).

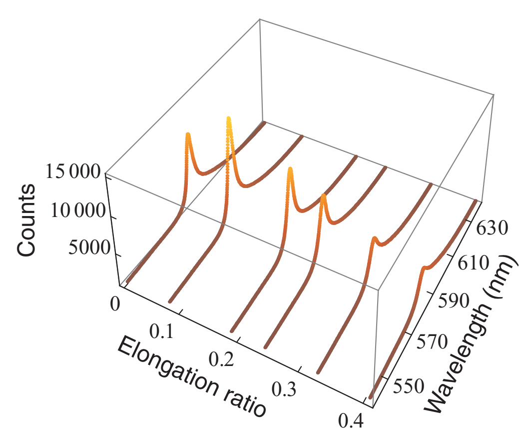
delimited by points *AB* in the stress curve, images *iii* and *iv* are representative of the *CD* zone.

DIC analysis was performed using pairs of images associated to the stress curve shown in Fig. 2. Upon applying an adaptive cross-correlation algorithm, the vector displacement field between images can be readily obtained.[8] Fig. 4(a) shows a typical vector field obtained from images *i* and *ii*; notice that the resulting vectors do not show a hyperbolic pattern as typically observed in tensile tests. This suggests that the collagen fibers are indeed arranged in a compact manner, as observed in the microscope images. As shown in Fig. 4(b), once the fibers are aligned along the direction of the load, the displacement vector field obtained from images *iii* and *iv* clearly shows a hyperbolic pattern. Thus, we can assume that the fibers are arranged in a less compact fashion and along a preferential direction. The displacement vector fields provide information about the micro-mechanical behavior of the sample; for the elongation ratios used in these experiments, the obtained vector fields clearly show different behaviors associated to two different linear regimes previously reported for bovine pericardium.[5,8]

RL emission from the tissue sample was verified using the spectrometer and upon analyzing the emitted intensity as a function of pump power. The curves obtained upon measuring the RL emission and the FWHM as the pump power was increased were used to identify the laser threshold for the tissue sample. In particular, we considered the laser threshold to be at the crossing of both curves; as shown in Fig. 5(a), above threshold we found a linear relationship between the RL emission and the pump power while the FWHM remained constant. Both features are customarily used to identify RL operation.[14,15] Random laser action in human tissue has been associated with the structural conformation of the tissue itself.[10] In the bovine pericardium samples, the light emitted by the R6G is scattered by the collagen fibers within the tissue, yielding random laser action for pump powers above threshold.[16] During the tensile test the pump power was kept constant (13 mJ) and well above the threshold value found for the sample (3 mJ).



(a)



(b)

FIG. 5. (a) Output energy and FWHM as function of pump energy for the bovine pericardium sample. (b) Evolution of the random laser spectrum from the bovine pericardium during the elongation test.

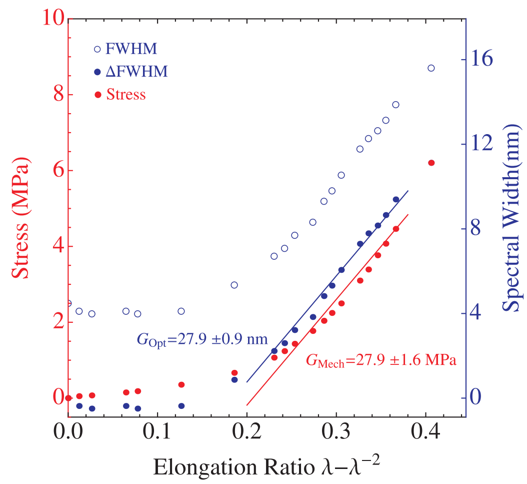


FIG. 6. Changes in the spectral width of the laser emission during the tensile test and mechanical response of bovine pericardium.

Fig. 5(b) shows a sequence of spectra acquired for different elongation ratios. The ΔFWHM for each spectrum acquired during the test was evaluated and plotted as a function of the elongation ratio, this is shown in Fig. 6. Notice that the ΔFWHM tends to increase as the elongation ratio increases, thereby suggesting that the scattering effects within the tissue have been changed. This in turn affects the laser threshold and leads to extinction of laser emission, in coincidence with previous studies of random lasers involving variations of the volume fraction of scattering centers.[15]

Changes in the spectral features of the RL are expected if the structure of the tissue is altered.[10,11] As verified through DIC analysis, elongation of the bovine pericardium sample leads to changes in the arrangement of collagen fibers. Since these fibers contribute to the scattering effects within the tissue, fiber reorientation may be used to explain the spectral variations registered in the RL emission. Inspection of Figs. 5(b) and 6 reveals that at the beginning of the tensile test, the spectrum becomes narrower than its initial value. This might be associated to a different disposition of scatterers associated to the rearrangement of collagen fibers thereby modifying the RL spectrum. For larger values of the elongation ratio, the spectral width increases significantly and the RL emission decreases noticeably. Furthermore, the FWHM registered for these elongation ratios (see data with open circles in Fig. 6) suggests that the optical signal corresponds to amplified spontaneous emission (ASE) and the laser action has therefore ceased. Under this condition, the collagen fibers seem to be aligned along the direction of the load; thus, changes in the RL spectrum provide evidence of the modified scattering effects achieved during the tensile test. It is worthwhile noticing that collagen fiber alignment during tensile tests of bovine pericardium has been observed in previous studies.[5] These results have been adequately fitted by a nonlinear model representing a phase transition in an elastic material leading to different mechanical responses. Our results are therefore consistent with previous reports obtained with different characterization techniques.

Upon comparing the stress curve and ΔFWHM of the RL emission we also find a clear correlation between both sets of measurements. As shown in Fig. 6, the experimental curves for stress and ΔFWHM as function of elongation ratio show a similar trend. For large values of the elongation ratio both plots exhibit a linear tendency associated to collagen fiber alignment.[5] The slope of the stress curve in this region can be used to evaluate the shear modulus (G) of the sample;[13] notice further that the mechanical behavior of the sample is very similar to that of an elastomer and the Young modulus may thus be estimated as $E = 3 \times G$.[17] Hence, the slope of the stress curve leads to a shear modulus of $G_{mech} = 27.9 \pm 1.6$ MPa and the slope of the ΔFWHM curve yields $G_{opt} = 27.9 \pm 0.9$ nm. This suggests that changes in the FWHM are closely related to the mechanical response of the tissue. The resulting Young modulus for the bovine pericardium is thus $E = 83.7$ MPa, which is also consistent with previous reports.[8,18]

V. CONCLUSIONS

We have demonstrated that RL emission analysis can be incorporated into tensile tests of tissue such as bovine pericardium. It is evident from our results that the spectral features of the laser will vary as a function of the elongation ratio. This suggests that structural information of the tissue may be obtained from spectral analysis of the RL emission. Although further investigation is required to fully understand the relationship between structural changes and RL emission, a simple description of the observed phenomena may be based on changes in the scattering features within the tissue induced through mechanical deformation. Indeed, as the bovine pericardium samples are elongated, the scattering is expected to change due to collagen fiber alignment. This in turn changes the RL emission because the density of scattering centers per unit volume decreases accordingly. A reduction in the density of scatterers leads to shorter residence times within the tissue suppressing laser emission and increasing the FWHM of the spectrum. Therefore, changes at the microstructural level of the tissue can be associated with the spectral features of the random laser emission.

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