Determining mechanical properties of rabbit skin with light reflection technique verified with latex

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Abstract. An experimental technique to evaluate the elastic limit for rabbit skin is developed and described. The experimental technique is designed to measure small changes in the normalized reflectivity as a function of applied stretch, with a laser wavelength of 632.8 nm and power of 1 mW. When the samples of rabbit skin are stretched, the reflectivity increases until a critical point (elastic limit) is reached. After this point, the curve drops off very quickly. The elastic limit for the male dorsal samples is less than that for the female samples, with an average strain of 14.4 and 34.6% in males and females, respectively. Moreover, our results show that the average slope of the regression lines is greater for the male dorsal samples than the dorsal female samples. This indicates that the male dorsal skin is rougher than that of the female.
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Keywords: laser reflection; polarization imaging; rabbit dorsal skin; rabbit ventral skin; skin elasticity; skin roughness.

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1 Introduction

The study of optical properties of tissues is very important to many medical and biomedical applications of lasers and other light sources. Reflectance of light can determine subsequent biological effects in therapeutic uses such as laser surgery[1] and photodynamic therapy.[2] For diagnostic purposes, a noninvasive optical spectroscopy study was considered for the brain,[3] breast,[4] muscles,[5] and skin.[6] Furthermore, measurement of the stretching of skin and estimation of stress in skin is an important problem in plastic surgery. Excessive tensile stresses delay wound healing and cause scar tissue.[7] Techniques of light reflection have been used to study the intrinsic properties of skin, and mathematical models have been developed to take these into account.[8]

Measurements of the mechanical properties of skin have numerous implications in surgical repair, dermal disorders, and the diagnosis and treatment of skin trauma. Research in the area of skin aging and cosmetic product assessment can also benefit from new methodologies for the measurement of mechanical properties. The mechanical behavior of skin is strongly influenced by the concentration and structural arrangement of constituents such as collagen and elastin, the hydrated matrix of proteoglycans, and the topographical site and their significant role in the organism.[9] A measurement of skin stretch was based on an optically based technique, where the reflected light from the skin is considered to be diffused due to multiple scattering properties for the skin and reflection from different layers of skin.[10] This optical technique is based on changes in the reflectivity of polarized light as the skin is stretched. As the skin is stretched, the roughness of the tissue is reduced, resulting in a smoother surface and a consequence increase in the polarized reflected light.

In this work, a noninvasive technique (light reflection technique) has been successfully introduced to measure the deformation obtained from the stretching device. Mechanical properties of dorsal and ventral skin of female and male rabbit skin, such as elastic limit, can be calculated. The difference in roughness and elasticity between female and male skin is also illustrated.

2 Experimental Technique

2.1 *Optical Components*

Our experimental setup is similar to that described in the literature.[10] The experimental setup is designed to measure small changes that take place in the reflection characteristics of the skin as a result of applied stretch. The optical arrangement consists of a 1-mW He–Ne laser of wavelength 632.8 nm (used as a light source), mechanical chopper, polarizer P, skin sample stretched on skin holder, analyzer A, converging lens, and a photodetector connected to a lock-in amplifier (see Fig. 1). The intensity of the laser light was kept low enough to protect the skin from damage. The angle of

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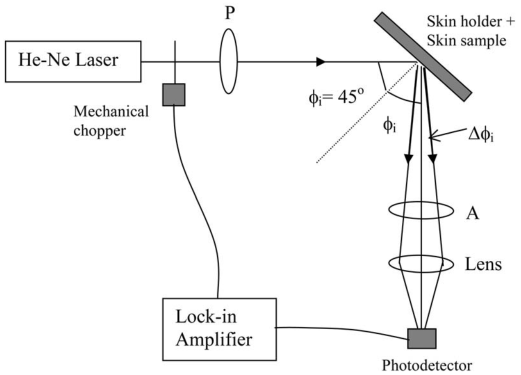

Fig. 1 Experimental setup: laser light is reflected from the skin sample stretched on the skin holder. P is the polarizer for linearly polarizing the incident light, and N is the analyzer for linearly polarizing the reflected light.

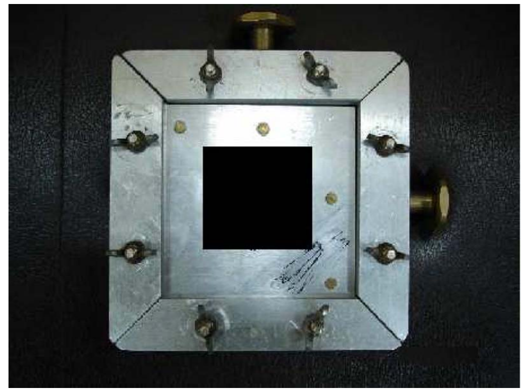

Fig. 2 Skin stretch device. This device allows the skin to be stretched in two dimensions.

incidence in this study was $\phi_i = 45$ deg. The incident light was linearly polarized using a polarizer (P). The reflected light is collected onto a photodetector using a lens after it passes through an analyzer and a converging lens. The incident light polarization was set perpendicular to the plain of incidence. The analyzer was set in one of two perpendicular orientations: either parallel or perpendicular to the plain of polarization. The laser power is detected using a photodetector by mechanically chopping the incident laser light and utilizing a lock-in amplifier and standard phase-sensitive detection techniques. The reflectivity was calculated by taking the difference between the two components of the reflected light, using the following simple equation:

$$R = R_1 - R_p, \tag{1}$$

where R_1 is the reflected light intensity as the analyzer is set parallel to the polarizer, and R_p is the reflected light intensity as the analyzer is set perpendicular to the polarizer.

2.2 *Methods and Materials*

A simple device was built using facilities available in the physics department at the University of Bahrain to apply stretch to skin pieces, as shown in Fig. 2. The four sides of rectangular skin samples are clamped onto a sample-holder stretching device. Two adjacent clamps can move with respect to the sample-holder plate by the use of screws. The skin is held on the sample holder by two adjacent pieces that are orthogonal to each other. The skin is placed on an area 10 $\times 10$ cm.

The experiments were preformed on rabbit skin samples. The rabbits with known breed were bought from a local butcher who raises them at a particular farm for use as food. The animals were chosen in a similar size range and the age varied from 10 to 12 months. The rabbits were killed using concentrated chloroform and used immediately. They were placed in a dissecting tray and fixed on their ventral side by special dissecting pins. The skin was shaved using a sharp blade and it was then marked to nearly 12 cm width $\times 14$ cm length. To protect the skin from any damage, the marked sides were gently raised by forceps to make a shallow mid-dorsal incision. The same procedure was repeated for the mid-ventral surface side. The skin samples from the dorsal and ventral surfaces were individually placed in a standard buffered saline solution to be used immediately for light reflection technique. The temperature in the laboratory was controlled to be 20 ± 2 °C. During the experiment, the backside of the skin (the side facing the sample holder) is kept wet with a saline solution to minimize friction between the two materials.

Skin samples were stretched by equal displacements using two screws. This allows the center of the sample to be stationary with respect to the stretch device. Various amounts of stretch, up to 50 mm, are applied to the $(10 \times 10\text{-cm})$ skin samples.

3 Results

3.1 *Reflectivity of latex*

To test our system and to demonstrate the performance of our proposed system, measurements were made on commercially obtained latex. The use of latex is well known as a test material to study the mechanical properties of soft tissues. For example, it was used as test material in a study of the mechanical properties of normotensive and hypertensive pulmonary arteries from rats,[11] and in a study of the mechanical properties of guinea pig skin using a light reflection technique.[6] For our study, we used square sheets of latex, from high quality commercially available examining gloves. All of the samples were taken from the same box, and cut from the middle of the glove to ensure uniformity. The measurements were made on all the samples in the same day while the temperature in the laboratory was controlled to be $20° \pm 2$ C. While the measurements and analysis of the homogeneous latex samples differ from subsequent measurements that are made on skin samples, the parameters measured are similar, which means that the uncertainty applies to both. The reflectivity versus strain was measured, where the strain (elongation) is the change of length (stretch) of the sample under tensile stress. The tensile strain is defined as the ratio of the elongation Δl to the original length l_o, such as tensile strain$=\Delta l / l_o$. The reflectivity was calculated by taking the difference between the two components of the reflected light, using Eq. (1). Figure 3 shows the measured data of the normalized reflectivity versus strain for three samples of latex gloves. The intensities are normalized with respect to the un-

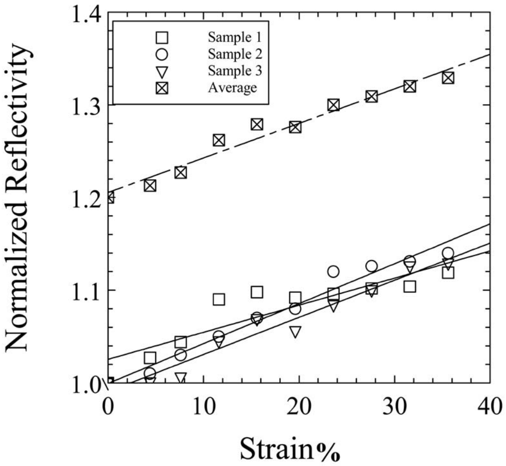

Fig. 3 Normalized reflectivity (reflected light intensity polarized parallel to the incident light polarization minus the reflected light intensity polarized perpendicular to the incident light polarization) versus strain for commercial latex examination gloves. The three curves represent three different samples, and the offset curve is the average of the samples.

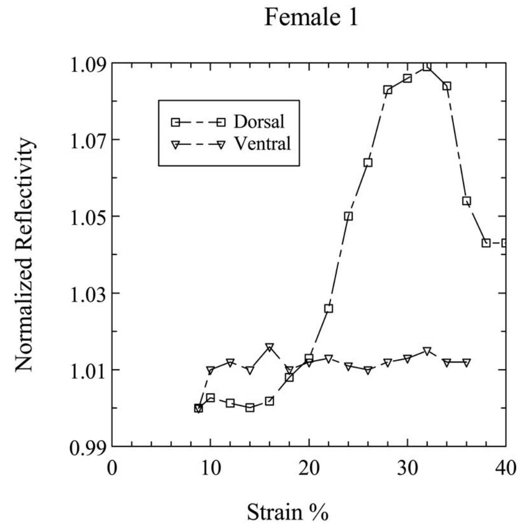

Fig. 4 Normalized reflectivity versus strain for dorsal and ventral skin taken from a female rabbit.

stretched intensity. In all tests, an almost linear change in reflectivity versus strain is observed. The slopes of the linear regression lines were determined by a least-squares fitting, and the correlation coefficients R were calculated using the Rt-Plot program. A correlation greater than 0.8 is generally described as strong, whereas a correlation less than 0.5 is generally described as weak, where perfect correlation of +1 occurs only when the data points all lie exactly on a straight line. The correlation coefficient is calculated with the assumption that the data are stochastic (i.e., bivariate Gaussian). The slopes and the correlation coefficient R for samples 1, 2, and 3 are: (0.401 ± 0.030, 0.978), (0.431 ± 0.026, 0.986), and (0.292 ± 0.053, 0.890), respectively. The average of the three samples is shown of the same graph with slope and correlation coefficient R (0.314 ± 0.018, 0.987). The uncertainty of the slope for the average is about 5.7%, which indicates that the system provides highly accurate measurements, and hence the results of the latex test demonstrate the validity of the test.

3.2 *Reflectivity of Rabbit Skin*

The experiments were performed on six different rabbits (three animals from each sex) with a He–Ne laser wavelength of 632.8 nm. Samples of skin were freshly removed from dorsal and ventral areas of the body. We noticed that the dorsal skin for both sexes is thicker that the ventral skin, and this was also determined by other authors studying frog skin.[12] As stretch is applied to the samples, the reflected light intensity is measured as the difference between the two perpendicular components of the reflected light (component parallel to the polarization of the incident light minus the perpendicular component). The intensities are normalized with respect to the unstretched intensity. Figures 4 and 5 show the results obtained for the female samples F1 and F2. Figures 6 and 7 show the results for the samples M1 and M2 taken from male rabbits. As the stretch is applied to the samples, the reflectivity increases until a critical point is reached. After this point, the curve drops off quickly. From the figures it can be noted that the dorsal skin for all samples has higher variation of reflectivity with strain. The elastic limit for the male dorsal skin samples is reached earlier than the female samples, indicating that the female skin is more elastic and more stretchable than the male skin. Typical data are shown in the figures. The initial parts of the curves are considered to be linear, and the data are fitted to a least-squares linear fit to determine the initial slope. The quality of the fit is indicated by the linear correlation coefficient (R) with a value of 1, indicating a perfectly linear set of data. Table 1 summarizes the data for the six dorsal samples.

4 Discussion

The data obtained from the latex samples show a linear behavior between the normalized reflectivity and strain, which is in agreement with a theoretical and experimental study performed by others.[10] The theoretical results showed that the

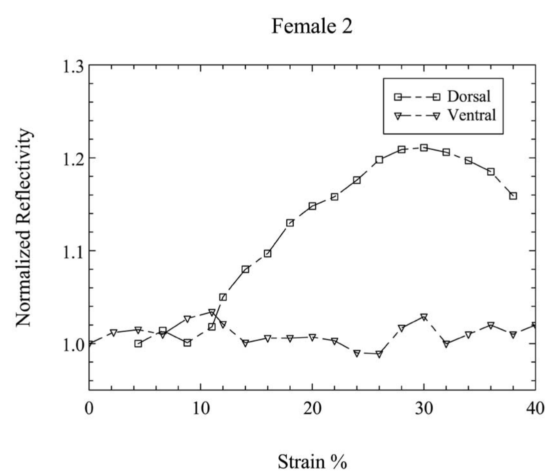

Fig. 5 Normalized reflectivity versus strain for dorsal and ventral skin taken from a female rabbit.

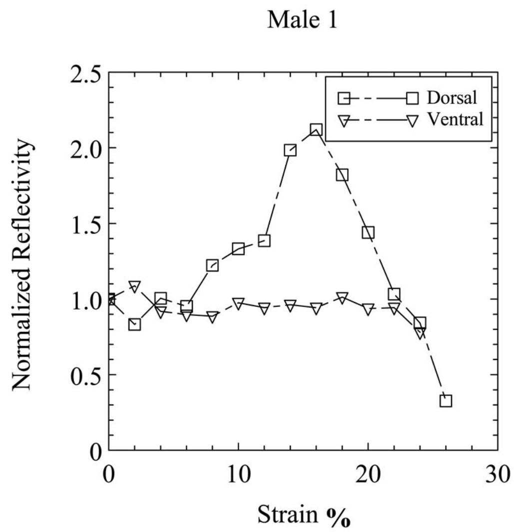

Fig. 6 Normalized reflectivity versus strain for dorsal and ventral skin taken from a male rabbit.

reflected power $P(\phi_i)$ is linearly proportional to the linear extend Δx of the surface, which was in agreement with their experimental results done on latex examination gloves and guinea pig dorsal skin. The theoretical model was based on the assumption that the intensity of the light reflected or transmitted from a rough interface depends on the local angle of incidence and polarization. The second assumption was that light is diffusely scattered due to rough air-skin interface. The roughness z of the surface was considered as a sine wave and given as

$$Z = A \sin(gx), \tag{2}$$

where A is the height of the roughness with a wavelength of $2\pi/g$. If the incident light power is P_o, then the reflected power of a rough surface is given as

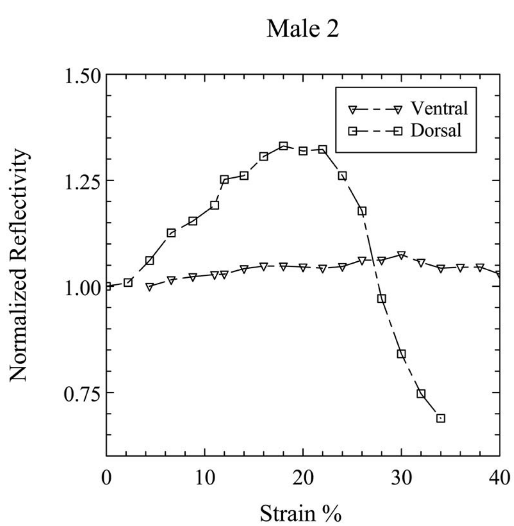

Fig. 7 Normalized reflectivity versus strain for dorsal and ventral skin taken from a male rabbit.

Table 1 Measured elastic limits, slopes, and corresponding linear correlation coefficient (R) for measurements on six different rabbits.

Sample	Elastic limit Average strain %	Initial slope	R (correlation coefficient)
Female 1	29.86	0.01003±0.0005	0.98
Female 2	32.08	0.0044±0.00059	0.92
Female 3	42	0.0008±0.00010	0.82
Average	34.6	0.0051±0.00045	
Male 1	19.58	0.0199±0.0009	0.99
Male 2	15.83	0.0747±0.0157	0.89
Male 3	13.75	0.0402±0.0075	0.94
Average	16.4	0.0449±0.010	

$$P(\phi_i) = P_o r(\phi_i) \cos \phi_i \Delta x, \tag{3}$$

where $r(\phi_i)$ is the Fresnel reflection power coefficient and Δx represent the linear extent of the surface that contributes to the reflectivity. The previous equation predicts that the reflected power is linearly proportional to the stretch Δx, which is in agreement with our experimental results for the latex and also for the rabbit skin, before reaching the elastic limit. Equation (3) was approximated for very rough skin ($\mathrm{Ag} \gg 1$), and can be written as

$$P(\phi_i) = P_o r(\phi_i) \frac{\Delta \phi_{\mathrm{ref}}}{\pi \mathrm{Ag}}, \tag{4}$$

where $\Delta \phi_{\mathrm{ref}}$ is the difference between the angle of reflection of the angle of incidence ϕ_i due to reflection of a rough surface (see Fig. 1 for definitions). For the proposed experiment, P_o, ϕ_i, and $\Delta \phi_{\mathrm{ref}}$ are kept constant, hence as the soft tissue is stretched, both amplitude A and the wave number g decrease, leading to increase in the detected power. The change of roughness of the epidermis-dermis interface may be primarily responsible for the observed change in light reflection owing to skin stretch.

All the examined skin samples showed the same behavior, where a nearly linear relationship between the amount of stretch and the reflected light intensity is measured, which is in agreement with the previous theoretical model, until a critical point is reached (elastic limit). After this point, the relationship is not linear and the slope of the curve drops off very quickly. This means that the skin starts to feel soft and the elasticity is lost. Table 1 summarizes the results for the elastic limits for the dorsal skin for the six samples studied. The diversity of the results within each group of male or female could be attributed to the slight age differences between the rabbits used. The mean value for the elastic limit for the male dorsal samples is less than that for the female samples, where for the male samples an average strain is 16.4%, while for the female it is 34.6%. This result designates that the female samples of skin can withstand greater stress without losing elasticity. Our findings are consistent with an earlier study[12] of mechanical properties of aquatic pipid frog skin, which showed that female skin is more elastic than male skin, where female skin has a lower modulus of elasticity than that for the male. Furthermore, these authors found that the maximum strain at break for the female frog skin is higher than that for the male frog skin. Our results show also that for the ventral skin, the elastic limit cannot be reached with the 40% examined range of strain. The linear behavior between the normalized reflectivity and strain was also found for *in-vivo* human skin.[6]

Our results also show that the average slope of the regression lines is greater for the male dorsal samples than that of the female. As the interface within the skin (the epidermis-dermis interface), and other biological elements in the skin are stretched, the interface and the extracellular matrix flatten out. Hence, any changes in roughness of the epidermis-dermis interface could be primary responsible for the observed alteration in the light reflection due to skin stretch. The mechanical behavior and accordingly the optical behavior of the skin could be strongly influenced by the concentration and structural arrangement of the extracellular proteins such as collagen and elastin. It was found that the mean diameter of collagen fibrils is significantly smaller in female than in male rabbit skin,[13] and the same results were found for mice.[14] The difference in mean diameter of collagen fibrils may contribute to the roughness of the skin, and because the male skin contains bigger collagen fibrils, then it is rougher than the female skin. Hence, we can conclude that higher value for the slope contributes to a rougher skin.

Moreover, our results indicate a higher reflectivity for the dorsal skin than the ventral skin for both female and male rabbits. According to our analysis in the previous paragraph, we may conclude that dorsal skin for both female and male rabbits is rougher that ventral skin.

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