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Pressure effects on soft tissues monitored by changes in tissue optical properties

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ABSTRACT

For pulsed laser tissue welding, an appropriate pressure needs to be applied to the tissues to achieve successful welds. In this study, we investigated the influences of pressure on *in vitro* optical properties of elastin biomaterial. The optical properties were measured as a function of pressure with a double integrating-sphere system. A He-Ne laser (633 nm) was used for all measurements. Each sample was sandwiched between microscope slides and then compressed with a spring-loaded apparatus. Transmittance and diffuse reflectance of each sample were measured under a pressure (0–1.5 kg/cm^2 and then released to 0). Absorption and reduced scattering coefficients were calculated using the inverse doubling method from the measured transmittance and reflectance values. Results from this study demonstrated: 1) The overall transmittance increased while the reflectance decreased as the tissue thicknesses were reduced up to 72% and the tissue weights were decreased about 40%, 2) The absorption and scattering coefficients increased with increasing the pressure, and 3) The pressure effects on the tissue optical properties were irreversible. Possible mechanisms responsible for the changes in the tissue optical properties were also investigated by changing tissue thicknesses or weights (through dehydration). This study implies that changes in tissue thickness and water content are important factors that affect tissue optical properties in different ways.

Keywords: Hydration, transmittance, diffuse reflectance, adding-doubling.

1. INTRODUCTION

Patch welding with a pulsed diode laser and indocyanine green (ICG) has been shown a promising method to successfully fuse two pieces of tissue with strong mechanical strength.[1] The advantages of the patch welding are: 1) The welding process can be simplified by welding patches to flat surfaces and 2) Collateral tissue damage is minimized by heating only the area stained with ICG. Firm contact between the two surfaces is necessary to create strong welds and consequently, during welding, the laser light must pass through the compressed tissue. However, previous studies have demonstrated that tissue optical properties are changed under compression.[2,3] The decrease in tissue thickness and the loss of water content might lead to the changes. Hence the deposition of the thermal energy at the site of welding is not only a function of laser irradiation parameters, but also the changes of optical properties in the surrounding tissue. An understanding of the pressure effects on tissue optical properties will enhance our ability to estimate the photothermal response of tissue to laser irradiation during patch welding procedure.

From preliminary work, it had been observed that dehydration could also cause both a decrease in tissue thickness and a loss of water content. A study by Çilesiz *et al.* demonstrated that the absorption coefficient increased by 20–50% in the visible range when 40% of total tissue weight was lost through dehydration.[4] Moreover, it was also noticed that the more dehydrated the biomaterial, the less pressure was needed to weld.[1] Unfortunately, there is not a comparative study to investigate the mechanisms possibly causing the changes in tissue optical properties due to the mechanical pressure or dehydration. It also remains unclear whether the changes are reversible or irreversible when the pressure is released. Thus, the objective of this study was to investigate the pressure effects on soft tissues, specifically elastin biomaterial, and possible mechanisms responsible for these changes.

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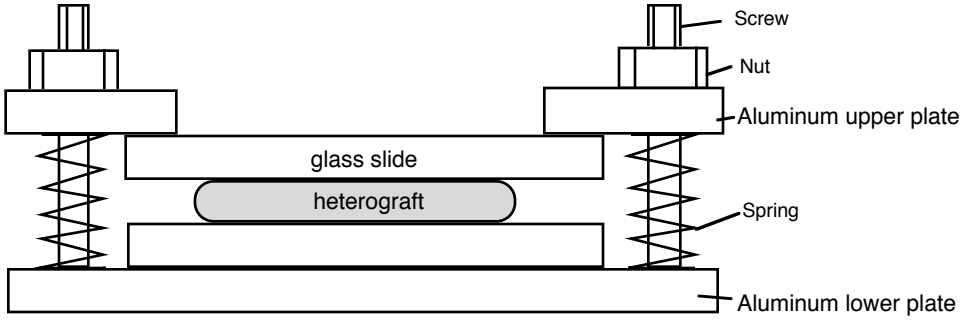

Figure 1. Cross section of the spring-loaded apparatus used for creating a constant pressure at the tissue sample. The apparatus consists of four posts with springs located at the corners of a 90×160 cm plate.

2. MATERIALS AND METHODS

2.1. Elastin Biomaterial

Porcine aorta were obtained at Carlton Packing Co., Carlton, OR. They were freshly cut and then were digested at 60°C for 1–1.5 hours in 0.5M sodium hydroxide (NaOH) to dissolve all tissue constituents but the elastin lamina. These modified vessels are termed elastin biomaterial. The biomaterial was placed in a room temperature deionized waterbath for 30 minutes, then boiled in deionized water for 30 minutes to remove the NaOH and to disinfect the vessels. The biomaterial was then kept in saline and autoclaved, and then stored at 4°C before use. Each sample was trimmed into about a 2×2 cm square using a sharp double-edged razor blade.

2.2. Experiments

Two experimental protocols were performed to investigate: 1) How does pressure affect tissue optical properties? and 2) What caused the changes? To address the first question, we measured tissue optical properties *in vitro* as a function of pressure with a double integrating-sphere system. A He-Ne laser (633 nm) was used for all measurements. Absorption and reduced scattering coefficients were calculated using the inverse doubling method[5] from the measured transmittance and reflectance values. The reflectance measurements were calibrated using a reflectance standard (Labsphere Inc.). Each sample was sandwiched between microscope slides. The glass slides were held together using a spring-loaded apparatus (Fig.1). This apparatus provided a constant force on the tissue sample. Four compression springs were calibrated using a universal material tester (V1000, LIVECO, Inc.), so that the applied pressure could be determined based on the tissue sample size (i.e., 2×2 cm square). The pressure was applied from 0, 0.5, 1, 1.5 kg/cm^2, and then released to 1, 0.5, 0 kg/cm^2 for each sample. For no pressure, each sample loosely adhered to the glass slides by tissue moisture. Tissue thickness was determined by averaging thicknesses measured at the center and near the edges of each sample using a micrometer. Five samples were used in this experiment.

To investigate the source of the changes, we measured tissue optical properties using the samples having either the same thickness or the same weight (through dehydration) as that of the compressed samples. To determine the weight loss, we weighed the samples before and after the dehydration. Each fresh sample was placed in a plastic container, and then slowly dehydrated at room temperature.

3. RESULTS

We observed that, in general, the overall transmittance increased and the diffuse reflectance decreased gradually as the pressure increased. The tissue thicknesses were reduced up to 72% and the tissue weights were decreased about 40%. Typical sample thicknesses and weights were 1.45 mm and 0.6 g respectively. A typical profile of the transmittance and the reflectance as a function of pressure is shown in Fig.2. Both the transmittance and the reflectance were irreversible; the transmittance increased up to 30% and the reflectance reduced by 12% from the initial values after the pressure was released. The corresponding absorption and scattering coefficients are shown in Fig.3. The absorption and scattering coefficients increased with increasing pressure, especially the absorption coefficient increased about twice as much as the initial value. The differences between the initial values and the final values (i.e., after compression) represent the irreversible optical properties due to the pressure effects.

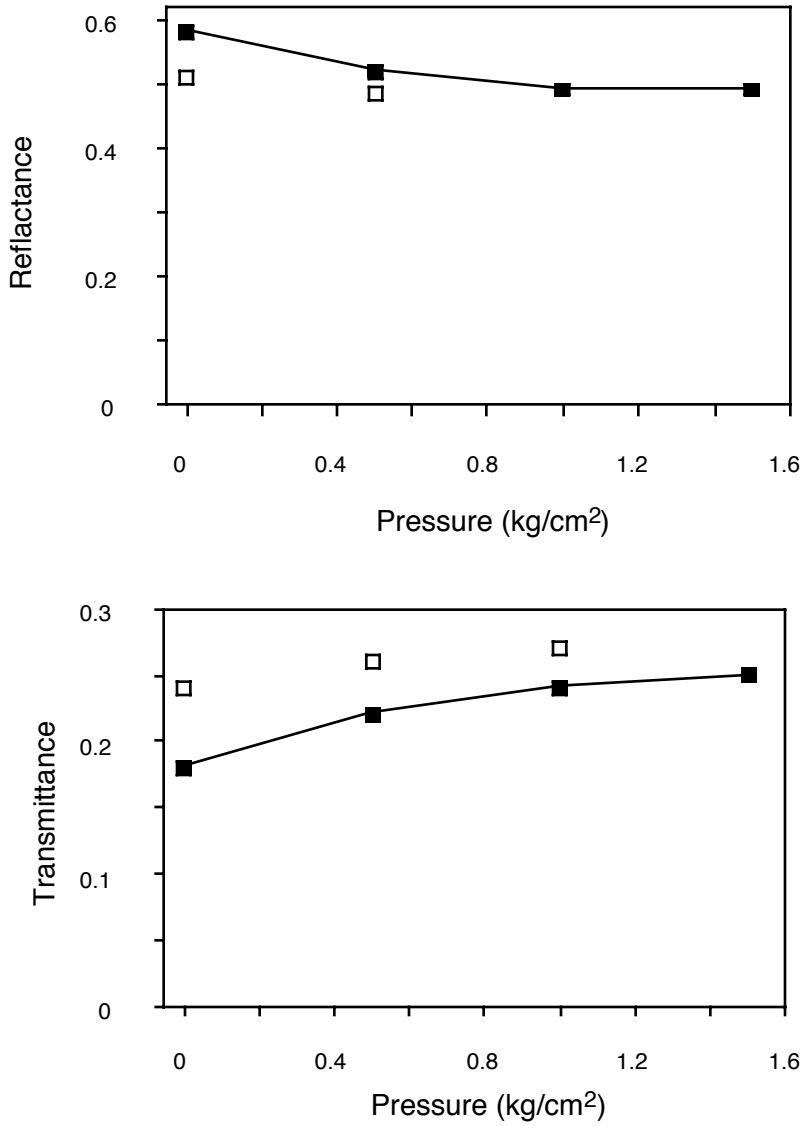

Figure 2. Elastin heterograft diffuse reflectance (top) and transmittance (bottom) as function of pressure. The filled marks represent the data measured with increasing pressure, while the open marks are the data measured as the pressure is released.

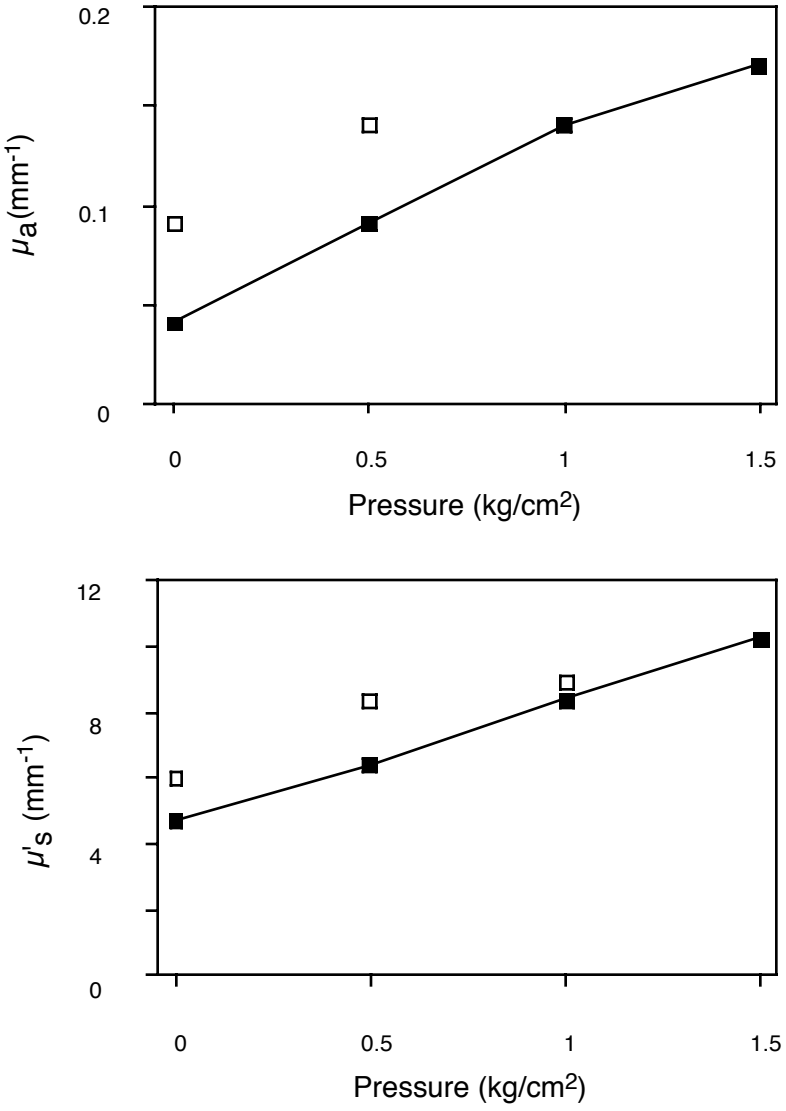

Figure 3. Elastin heterograft absorption coefficient (top) and reduced scattering coefficient (bottom) as function of pressure. The filled marks represent the data measured with increasing pressure, while the open marks are the data measured as the pressure is released.

Table 1. Optical properties of the samples with similar thickness. The tabulated values are the average of three samples. The errors are the standard deviation.

	Thickness (mm) ±0.02	Trans. (%) ±0.02	Refl. (%) ±0.02	μ_a (mm^{-1}) ±0.02	μ_s' (mm^{-1}) ±0.80
No pressure	0.54	0.35	0.45	0.09	5.37
Compressed ($1\,kg/cm^2$)	0.53	0.24	0.49	0.14	8.31

Table 2. Optical properties of the samples with similar weight. The tabulated values are the average of three samples. The errors are the standard deviation.

	Weight (g) ±0.02	Thickness (mm) ±0.02	Trans. (%) ±0.02	Refl. (%) ±0.02	μ_a (mm^{-1}) ±0.02	μ_s' (mm^{-1}) ±0.80
Dehydrated	0.32	0.72	0.29	0.57	0.04	6.46
Compressed ($1.5\,kg/cm^2$)	0.36	0.42	0.25	0.49	0.17	10.20

The measurements for the samples with similar thickness are listed in Table 1 and those with similar weight are listed in Table 2. The samples with similar thicknesses did not have similar optical properties (see Table 1). More light was transmitted through the compressed sample than the normal one, although the absorption and scattering coefficients for the compressed sample were greater than those for the uncompressed sample. The dehydrated samples were thicker than the compressed samples with similar weight (see Table 2). The transmittance and reflectance for the dehydrated samples were slightly greater than those of the compressed sample, while differences in the absorption and reduced scattering coefficients between them were significantly different.

4. DISCUSSION

In this study the pressure effects on tissue optical properties and the possible mechanisms for these changes were investigated. We observed that, in general, under compression the transmittance, absorption, and reduced scattering increased while the diffuse reflectance decreased. Compression caused the reduction in sample thickness and leakage of fluids from the sample. The overall transmittance increased gradually with decreasing the sample thickness. These observations may be explained with the following two equations[6]:

$$\mu_a = \rho\sigma_a \tag{1}$$

$$\mu_s = \rho\sigma_s \tag{2}$$

where μ_a and μ_s are absorption and scattering coefficients of the biomaterial respectively, ρ is the density of the absorbing or scattering centers. σ_a and σ_s are the absorption cross section and the scattering cross section. If variation in tissue density and refractive index cause light scattering in soft tissue, then under compression ρ increased as a result of reduction in the spacing among the cellular components, while σ_a and σ_s remained roughly constant or were slowly decreased because a more index-matched environment might be created as the proteins refractive index became closer to that of the elastin fibrils. Thus, the absorption and scattering coefficients were increased. Chan *et al.* suggested that the increase of the volumetric water concentration because of the reduction in tissue thickness may also be an explanation for the increase of absorption coefficient with compression.[3]

Previous investigations have shown that the optical properties of aorta at 633 nm for different species (e.g., bovine and human) were different. For example, values of the optical properties of bovine aorta were $0.04\,mm^{-1}$ for the absorption coefficient and $2.19\,mm^{-1}$ for the reduced scattering coefficient at 633 nm,[7] while values from normal

human aorta were $0.05\,\mathrm{mm}^{-1}$ and $4.1\,\mathrm{mm}^{-1}$ for the absorption coefficient and for the reduced scattering coefficient respectively.[8] The results of this study showed that the optical properties of elastin biomaterial were similar to those of normal human aorta (see Fig. 3 and Table 1), although there are several tissue constituents (e.g., collagen, fiber, elastin) in the normal human aorta, while only elastin in the elastin biomaterial used in this study.

The absorption coefficient for the compressed tissue sample was greater than that for the uncompressed one, although they had the similar thicknesses (see Table 1). However, the compressed samples were very dense and compact, while the physical structures of the uncompressed samples were loose under light microscopy. The changes in the physical structure under compression may give rise to the increase in absorption and scattering due to the reasons mentioned above. Furthermore, the results of this study showed that the pressure effects on the tissue optical properties were greater than the effects of dehydration for the samples with similar weights. Although both compression and dehydration cause a reduction in tissue thickness and a loss of water content, the processes were different. Under compression, tissue thickness was reduced because spacing of elastin layers was decreased after the water was squeezed out, and even the elastin layers were squeezed as sufficient pressure was applied. On the other hand, the process for dehydration was slow (e.g., it took at least 3 hours to obtain the sample with the similar weight through dehydration.). The loss of water content was due to the evaporation rather than using extra pressure. The elastin layers may remain loose even after dehydration, so that the sample did not become very dense. Moreover, the terms, σ_a and σ_s, in Eqs. 1 and 2 may also remain roughly constant, since the samples were not deeply dehydrated in this study. Thus, the absorption and scattering coefficients for the dehydrated samples were less than those for the compressed samples.

The changes in the physical structure of soft tissue are most likely responsible for the changes in soft tissue optical properties under compression. The changes will be irreversible if the structure cannot recover after compression. We observed that the tissue thickness did not return to its initial value after releasing the pressure. This may be an explanation for the irreversible changes in the optical properties after releasing the pressure.

In conclusion, this study demonstrated: 1) The overall transmittance increased while the reflectance decreased as the tissue thicknesses were reduced up to 72% and the tissue weights were decreased about 40%, 2) The absorption and scattering coefficients increased with increasing the pressure, and 3) The pressure effects on the tissue optical properties were irreversible. Possible mechanisms responsible for the changes in the tissue optical properties were also investigated by changing tissue thicknesses or weights (through dehydration). This study implies that changes in tissue thickness and water content are important factors alerting tissue optical properties, but the contributions they make are different.

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