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STRESS RELAXATION OF PORCINE SEPTAL CARTILAGE DURING Nd:YAG ($\lambda = 1.32 \mu\text{m}$) LASER IRRADIATION: MECHANICAL, OPTICAL, AND THERMAL RESPONSES

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ABSTRACT

Laser-assisted cartilage reshaping is mediated by thermally induced stress relaxation, and may be used to alter cartilage morphology for reconstructive surgical procedures in the upper airway and face without carving, morselizing, or suturing. Internal stress $\sigma(\mathbf{t})$, integrated backscattered light intensity $\mathbf{I}(\mathbf{t})$ from a He–Ne probe laser ($\lambda = 632.8 \text{ nm}$), and radiometric surface temperature $S_c(\mathbf{t})$ were measured during the reshaping of porcine nasal septal cartilage using a pulsed Nd:YAG laser ($\lambda = 1.32 \mu\text{m}$). Internal stress and integrated backscattered light intensity were observed to increase, plateau, and then decrease in similar ways during laser irradiation. The plateau region occurred when the cartilage front surface temperature approached 65°C . $\mathbf{I}(\mathbf{t})$ was utilized in a feedback control procedure to reshape cartilage specimens from a flat to a curved geometry. Immediately following laser irradiation, the tissues were rehydrated in normal saline for 15 min while wrapped around a small dowel. A stable shape change was retained for 21 days while the specimens were stored in normal saline at 5°C . The backscattered light intensity signal mirrors underlying changes in internal stress, and further rate of change or slope of $\mathbf{I}(\mathbf{t})$ is nearly zero when the surface temperature reaches about 65°C . Measurements of $\mathbf{I}(\mathbf{t})$ (or, equivalently, the fractional change in integrated backscattered light intensity $\Delta\mathbf{I}(\mathbf{t})/I_0$) may be used to control the process of laser-assisted cartilage reshaping and minimize nonspecific thermal injury due to uncontrolled heating. © 1998 Society of Photo-Optical Instrumentation Engineers. [S1083-3668(98)00504-8]

Keywords cartilage; phase transformations; laser mediated cartilage reshaping; plastic surgery; Nd:YAG laser; feedback control.

1 INTRODUCTION

Autologous cartilage is used in a wide variety of reconstructive and aesthetic procedures in the head and neck. Cartilage may be harvested from the pinna of the ear, nasal septum, or rib and used to reconstruct the upper airway and facial anatomy. Inasmuch as the shape and morphology of cartilage tissue harvested from these donor regions often do not accommodate the constraints of the recipient site where reconstructive surgery is being performed, reshaping of cartilage by carving, suturing,

or morselizing is necessary. Unfortunately, these traditional techniques can result in damage to the transplanted tissue and decrease viability.

Laser radiation may be used to alter the shape of native cartilage and create mechanically stable new morphologies, via thermal-mediated stress relaxation.^{1–4} The temperature distribution within the cartilage approximates the light fluence distribution established by the laser. The procedure allows creation of complex curvilinear cartilage shapes which would otherwise require surgical (destructive) manipulation of the tissue. Although the

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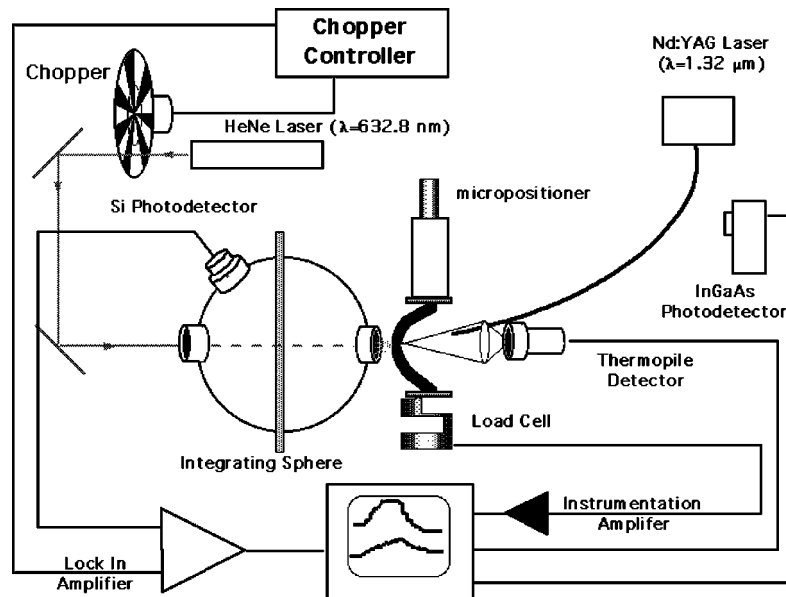


Fig. 1 Schematic of instrumentation used for measurement of integrated backscattered light intensity $I(t)$, internal stress $\sigma(t)$, and radiometric surface temperature $S_c(t)$. The cartilage specimen is held in compression between the load cell and single axis micropositioner so that the front surface is at the focal point of the IR thermopile detector. The Nd:YAG laser, thermopile detector, and He-Ne laser beams are focused onto the specimen at the point of maximum curvature. An intensity modulated (10 kHz) He-Ne laser beam is directed on the back surface of the cartilage specimen through an integrating sphere. A silicon photodetector and lock-in amplifier measure the integrated backscattered light signal $I(t)$. An InGaAs photodetector is used to detect the initial Nd:YAG laser pulse and trigger data acquisition.

mechanism of stress relaxation has not been conclusively established, the process is thought to involve collagen denaturation, alteration of weak van der Waals bonds between proteoglycan molecules and/or water flux.

Cartilage is a complex tissue composed of a three-dimensional collagen fibrillar framework containing a matrix of proteoglycan molecules that possess negatively charged ion groups (SO_3^- and COO^- moieties). The proteoglycans are compressed and their expansion is resisted by the surrounding collagen framework. For electrical neutrality (charge balance), cations (Ca^{++} and Na^+) also permeate the matrix. Mechanical deformation of the cartilage is resisted by the screened Coulomb potential between the negatively charged moieties residing on adjacent proteoglycan molecules.

We have previously reported on the thermal-optical response of cartilage to Nd:YAG ($\lambda = 1.32 \mu\text{m}$) irradiation during laser-assisted cartilage reshaping.¹ In this study, we report simultaneous measurement of internal stress $\sigma(t)$ during laser-assisted stress relaxation while monitoring $S_c(t)$ and $I(t)$, $I(t)$ is also utilized as a feedback signal to control the duration of laser irradiation during the reshaping of cartilage specimens. The alteration in internal stress during laser irradiation results in accelerated stress relaxation and represents a fundamental biophysical change that results in cartilage reshaping.

2 MATERIALS AND METHODS

Fresh porcine septal cartilage was obtained immediately following euthanasia from a local abattoir (Clougherty Packing Company, Vernon, CA). The cartilaginous nasal septum was removed from the skull via bilateral nasal bone osteotomies. Soft tissue including the perichondrium was dissected free from the specimen using a Freer elevator leaving only the cartilage material. The specimens were cut into rectangular shapes of thickness Δ_c , measured with a digital micrometer, and stored in physiological saline at ambient temperature for immediate use.

Cartilage specimens were held in compression between an aluminum plate attached to a calibrated one-dimensional micropositioner (model M-461, Newport Corp., Irvine, CA) and a thin beam load cell (0.25% full scale combined error, model LCL-113G, Omega Engineering Inc., Stamford, CT) coupled to an aluminum mounting plate (Figure 1). The internal stress [$\sigma(t)$] (a.u.) across the cartilage specimen was adjusted by translating the micropositioner. Voltage output from the load cell was amplified (Stanford Research Systems, SRS 650, Sunnyvale, CA), and recorded on a digital oscilloscope (Textronix DSA 601, Beaverton, OR) during laser irradiation. Cartilage specimens were irradiated with a Nd:YAG laser ($\lambda = 1.32 \mu\text{m}$, 50 Hz PRR (pulse repetition rate), NewStar Lasers, Auburn, CA) delivered by a $600 \mu\text{m}$ core diameter silica

multimode optical fiber. The laser spot size was estimated by measuring the burn diameter of irradiated thermal paper (Zap-It, Kentek, Pittsfield, NH). The laser power (6.94 W, 38.45 W/cm²) was measured using a pyroelectric radiometer (model 10A-P, Ophir, Jerusalem, Israel).

The radiometric surface temperature [$S_c(t)(^{\circ}\text{C})$] of the laser irradiated cartilage was monitored using a thermopile sensor (Thermalert MI-40, response time of 120 ms (95%), spectral sensitivity of 7.6–18 μm , Raytek, Santa Cruz, CA). The detection system was calibrated by measuring the radiometric surface temperature of an aluminum block coated with highly emissive ($\epsilon=0.97$) black paint (TC-303 black, GIE Corp., Provo, UT) heated from 23 to 100 $^{\circ}\text{C}$ by a resistive element. The surface temperature of the aluminum block was measured with a precision thermistor (8681, Keithley Instruments, Cleveland, OH) in thermal contact with the block. The thermopile sensor was focused onto the center of the Nd:YAG laser spot on the specimen.

Backscattered HeNe laser light ($\lambda_0=632.8$ nm, 15 mW, Melles Griot, Irvine, CA) incident on the non-Nd:YAG laser irradiated surface of the cartilage specimen was collected in an integrating sphere (6 in., Labsphere, North Sutton, NH) and measured using a silicon photodetector (model 2001, New Focus, Mountain View, CA) to yield integrated backscattered light intensity $I(t)$.⁵ The HeNe beam was aimed at the center of the Nd:YAG laser spot on the specimen. The HeNe laser light intensity was amplitude modulated (10 kHz) with a mechanical chopper (Ithaco, Ithaca, NY) and synchronously detected by a lock-in amplifier (model SR 850, $\tau=5$ ms, Stanford Research Systems, Sunnyvale, CA). The fractional change in integrated scattered light intensity $\Delta I(t)/I_0$ was calculated by measuring the change in $I(t)$ relative to the baseline signal I_0 recorded prior to the onset of Nd:YAG laser irradiation. Both the scattered light and the radiometric temperature signals were displayed and stored on a digital oscilloscope.

The internal stress $\sigma(t)$, radiometric surface temperature $S_c(t)$, and integrated backscattered light intensity $I(t)$ were recorded during laser irradiation of the central region of the cartilage specimen under compressive deformation (Figure 1). Laser irradiation was terminated following observation of the peak value for $I(t)$ on the lock-in amplifier. Usually, irradiation continued for 2–4 s beyond observation of the peak (identification of the peak requires observation of a downward trend in $I(t)$ on the amplifier). Following the onset of laser irradiation, $I(t)$ undergoes a characteristic increase, and then a subsequent decrease, with a peak occurring when $S_c(t)$ reaches about 65 $^{\circ}\text{C}$.⁵ $I(t)$ was recorded along with either $\sigma(t)$ or $S_c(t)$, as the current apparatus could not be configured to measure three signals simultaneously.

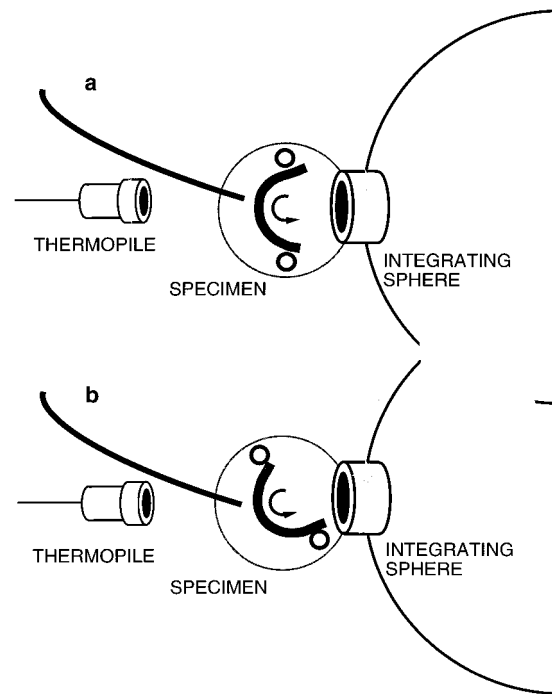


Fig. 2 Cartilage reshaping jig. (a) The cartilage specimen is held in compression between two aluminum rods 2.5 cm apart mounted on a rotational stage. In reshaping experiments only $I(t)$ and $S_c(t)$ are monitored. $I(t)$ is monitored on the lock-in amplifier display, and when $dI(t)/dt \approx 0$ laser irradiation is stopped. (b) Following laser irradiation, the stage is then rotated to a new position so that nonirradiated tissue can be brought into the laser irradiation zone.

The integrated backscattered light intensity signal $I(t)$ was used in a feedback control loop to optimize the process of cartilage reshaping using a shaping jig, schematically illustrated in Figure 2. Cartilage specimens (thicknesses=1.5–2.5 mm) were maintained in compression between two aluminum rods held approximately 2.5 cm apart [Figure 2(a)]. These in turn were attached to a calibrated rotational stage with vertical orientation. This arrangement permitted sequential irradiation of the entire cartilage specimen in a stepwise fashion, while simultaneously monitoring $I(t)$ and $S_c(t)$. When $I(t)$ peaked (i.e., $dI(t)/dt=0$), laser irradiation was stopped and the shaping jig was rotated so that a new region of the tissue could be irradiated [Figure 2(b)]. The process was repeated until the entire surface of the cartilage (opposed to the Nd:YAG laser) was irradiated. Reshaping can be accomplished with either the convexity or concavity of the specimen facing the beam for laser wavelengths with optical penetration depths larger or comparable to Δ_c . Next the cartilage specimens were removed from the shaping jig and wrapped around a plastic dowel of similar curvature, secured with a thin aluminum foil band, and allowed to rehydrate in normal saline for 15 min at ambient temperature. When the laser irradiated cartilage specimens were allowed to rehydrate in saline without being

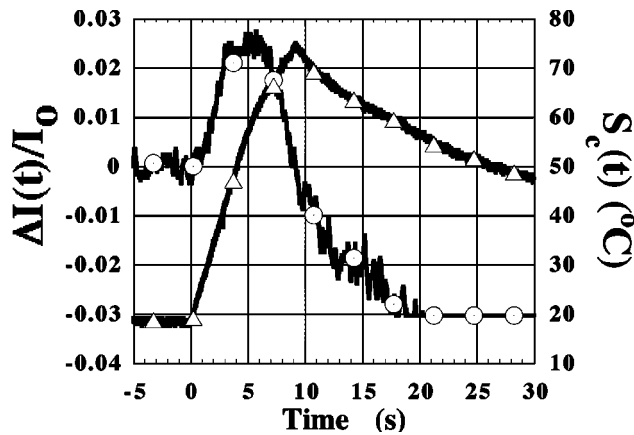


Fig. 3 Simultaneous measurements of radiometric surface temperature [$S_c(t)$ ($^{\circ}\text{C}$)] and fractional change in integrated backscattered light intensity [$\Delta I(t)/I_0$]. The laser irradiation time was 9.5 s. $\Delta I(t)/I_0$ initially increases, reaches a peak (between 4 and 6 s), and subsequently decreases despite continued laser irradiation and increasing surface temperature. In this time interval (4–6 s), $S_c(t)$ approaches 65 $^{\circ}\text{C}$ and a slope change in $S_c(t)$ is observed ($-\Delta-\Delta-\Delta-S_c(t)$, $-\circ-\circ-\circ-\Delta I(t)/I_0$).

wrapped around a dowel, the cartilage would return to the native configuration (flat) in less than 15 min. A minimum immersion time interval of approximately 15 min was empirically selected to allow maintenance of the new shape following laser irradiation. Longer rehydration periods (30 or 45 min) produced no additional change in the final cartilage shape. For clinical applications, reshaped cartilage is often surrounded by hydrated soft tissue and hence is in an aqueous environment. Reshaped cartilage specimens were maintained in a normal saline solution (at 5 $^{\circ}\text{C}$) for 21 days following laser irradiation and serially photographed at regular intervals.

3 RESULTS

Figure 3 depicts cartilage radiometric surface temperature $S_c(t)$ and the fractional change in integrated backscattered light intensity $\Delta I(t)/I_0$ as a function of time during and following laser irradiation. We note that there is a pronounced change in the slope of $S_c(t)$ at approximately 65 $^{\circ}\text{C}$ and after 4–6 s. During the same time interval, $\Delta I(t)/I_0$ reaches a maximum and then decreases monotonically (the stationary region). Laser irradiation lasted 9.5 s and the peak for $\Delta I(t)/I_0$ occurred prior to the cessation of laser radiation. These findings are consistent with previous observations in porcine auricular cartilage.⁵ Figure 4 depicts the simultaneous measurement of fractional change in the integrated backscattered light intensity signal $\Delta I(t)/I_0$ and internal stress $\sigma(t)$. Laser irradiation was for 10 s and $\sigma(t)$ is depicted using normalized arbitrary units. Inasmuch as the maxima of both light scattering

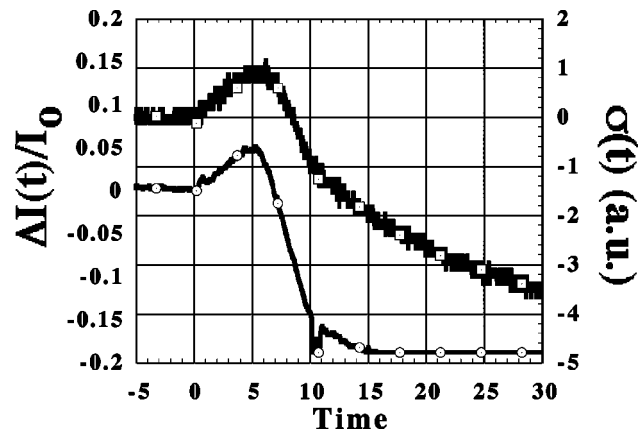


Fig. 4 Simultaneous measurements of internal stress $\sigma(t)$ (normalized, arbitrary units) and fractional change in the integrated backscattered light intensity $\Delta I(t)/I_0$. The laser irradiation time was 10 s. The peaks for $\Delta I(t)/I_0$ and $S_c(t)$ occur simultaneously, despite continued laser irradiation. $\sigma(t)$ continues to decrease after laser irradiation. Prior to the onset of laser radiation (–10–0 s), the base line stress relaxation rate for native cartilage is recorded ($-\square-\square-\square-\sigma(t)$, $-\circ-\circ-\circ-\Delta I(t)/I_0$).

and internal stress are nearly coincident, $\Delta I(t)/I_0$ may be used as a control signal to optimize the process of laser-assisted reshaping.

Figure 5 is a serial photographic montage of a cartilage specimen that has undergone laser-assisted reshaping across its entire length: (a) the cartilage specimen before reshaping, (b) immediately after laser radiation and rehydration in normal saline solution (while wrapped around a plastic dowel), (c) the same specimen in normal saline solution after 7 days, and (d) after 21 days, respec-

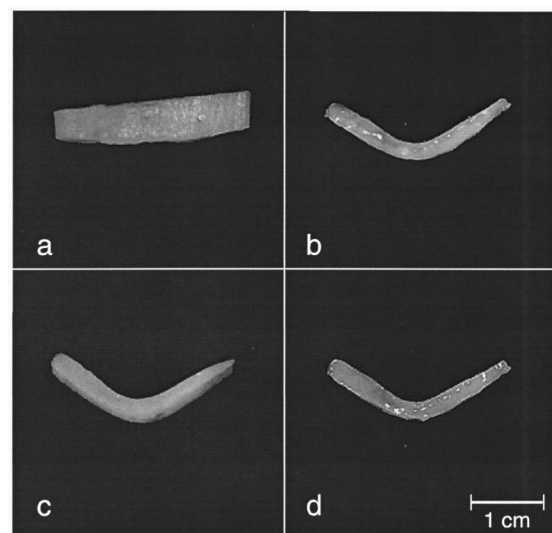


Fig. 5 Serial montage of a cartilage specimen that has undergone reshaping across the entire length: (a) the cartilage specimen before reshaping, (b) immediately after laser radiation and 15 min rehydration in normal saline solution (while wrapped around a plastic dowel), and (c) the same specimen in normal saline solution (c) after 7 days and (d) after 21 days.

tively. $I(t)$ was used to determine when laser irradiation should be terminated [after observing the peak value for $I(t)$]. The need to evaluate the permanency of the shape following laser irradiation has been addressed previously.^{3,6}

4 DISCUSSION

Laser-assisted cartilage reshaping has the potential to change reconstructive surgery in the upper airway, head, and neck by allowing construction of complex shapes without the loss of tissue bulk due to surgical manipulation. The stress relaxation rate of native (nonirradiated) porcine septal cartilage held in compression between the load cell and the micropositioner is illustrated from -10 to 0 s in Figure 4. Mechanical relaxation in the absence of laser irradiation takes a prohibitively long period of time which is impractical in the operating room. Laser irradiation accelerates this process via a thermally mediated mechanism. At the onset of laser irradiation ($0-4$ s), internal stress $\sigma(t)$ actually increases and approaches a plateau that is coincident with the plateau observed for $I(t)$ (and it also occurs when $S_c(t)$ is approximately 65°C). During the plateau period ($4-8$ s), both $\sigma(t)$ and $\Delta I(t)/I_0$ peak, and then they subsequently decrease well after irradiation has ceased. Although the origin of the transient increase in $\sigma(t)$ during irradiation ($0-4$ s) is not clear, the process may represent the focal expansion of water in the irradiated tissue volume during laser heating. Collectively, the observed behaviors of $\sigma(t)$, $I(t)$, and $S_c(t)$ suggest that the cartilage is undergoing a phase transformation.⁷

Changes in internal stress $\sigma(t)$ and the stress relaxation rate $d\sigma(t)/dt$ appear to be thermally mediated, but the molecular basis of these findings is not known. Light scattering experiments provide insight into several possible mechanisms. When the surface temperature reaches about 65°C , a stationary region in the fractional change in the integrated backscattered light intensity signal ($d\Delta I(t)/I_0/dt = 0$) is observed. Sobol et al. have suggested that the change in the light scattering properties of cartilage may be due to the formation of isolated regions of water movement with anomalous refractive index values, leading to an increase in backscattered light intensity.² During heating, additional regions of the tissue undergo this change in refractive index and eventually coalesce, resulting in a decrease in the overall scattered light signal. At the molecular level, Sobol et al. have suggested that water is undergoing a transition from its bound state (to proteoglycans or collagen) to a free or mobile state. This bound to free transition is temperature dependent. Hence, during laser heating, water moves through the matrix, and charged moieties on the proteoglycans are no longer shielded by water molecules. In a cartilage specimen under mechanical stress, cooling results in the reformation of weak bonds (hydrogen, polar bonds) between proteogly-

can groups and water flux and new stable shapes are formed. Alternatively, the process of stress relaxation may be partially due to the denaturation of collagen. A change in the slope of $S_c(t)$ occurs at about 65°C and this is synchronous with changes in $I(t)$ where $d\Delta I(t)/I_0/dt = 0$. Temperature above 65°C may have deleterious effects on tissue viability if heating is sustained for long time intervals; in this temperature range, protein systems begin to undergo denaturation. This is the approximate denaturation temperature for collagen and suggests that the helixes may unwind and then recoil as the tissue is allowed to cool.⁸ Inasmuch as protein denaturation (and subsequent cell death) are time and temperature dependent, rapid laser heating may be used to reshape cartilage while at the same time minimize nonspecific thermal injury and chondrocyte death.

While the molecular basis for laser-assisted cartilage reshaping is unknown, this technique represents a potentially useful approach for head and neck reconstruction and aesthetic surgery. A more practical issue is identification of appropriate laser dosimetry parameters to allow controlled tissue heating. Radiometric measurements of the surface temperature during heating have several limitations: (1) detectors collect infrared (IR) radiation over $6-20\ \mu\text{m}$ in tissue depth; (2) surface evaporation during laser irradiation may result in peak temperatures within the specimen; (3) heat flow in the tissue may be quite variable; and (4) the measured radiometric surface temperature weakly correlates with the mechanical change in the tissue (Figure 4).

During laser irradiation, cartilage undergoes mechanical relaxation ($d\sigma(t)/dt = 0$) that is accompanied by synchronous changes in $I(t)$ ($d\Delta I(t)/I_0/dt = 0$) (Figure 4); worth noting is the fact that peaks in $I(t)$ (Figure 3) and $\sigma(t)$ (not illustrated) are seen to occur when $S_c(t)$ reaches approximately 65°C . As a consequence, $\Delta I(t)/I_0$ may be a more useful control signal during laser-assisted cartilage reshaping because of the strong correlation with changes in $\sigma(t)$ as noted in Figure 4. $I(t)$ is dependent on the absorption and scattering coefficients of the irradiated cartilage at the probe beam wavelength, and with visible or near-IR (NIR) wavelengths a penetration depth of several hundred microns is possible. In contrast to $S_c(t)$, $I(t)$ represents the integrated backscattered light intensity signal that originates from changes in tissue optical properties through a large volume of the irradiated specimen (for visible and NIR wavelengths). A principal advantage of an optically based feedback control system over surface temperature techniques is that the entire tissue volume is probed. The backscattered light intensity signal (or, alternatively, a transmitted light signal) may be used as a feedback variable to modulate laser intensity or to

translate the cartilage in a semi-automated reshaping device.

In Figure 5, $I(t)$ was used as a feedback signal to limit laser irradiation during the reshaping of a cartilage specimen. When $d(\Delta I(t)/I_0)/dt=0$, laser irradiation was terminated, the specimen rotated, and an adjacent region brought into the target zone and irradiated; during irradiation $I(t)$ was closely observed on the lock-in amplifier. Following sequential irradiation across the surface of the specimen exposed to the Nd:YAG laser, the shape became stable. If the tissue were rehydrated without securing the cartilage to a shaping jig, the specimen would return to its native shape (flat) within minutes. We hypothesize that this process is secondary to rapid rehydration of water depleted regions of tissue in the irradiated cartilage. Immediate rehydration leads to an influx of free water which rapidly expands the collagen-proteoglycan matrix. In contrast, when the tissue is allowed to undergo rehydration when it is secured around a dowel of similar diameter to that used for reshaping, rehydration occurs in a constrained environment. Tissue rehydration and expansion of the collagen-proteoglycan framework are limited and new bonds/conformations are formed within the imposed structure. Fifteen minutes was the minimum rehydration time we measured for a change to a stable shape to occur. The reshaped cartilage specimens using the backscattered light intensity signal ($\Delta I(t)/I_0$) were stored for 3 weeks in physiologic saline at 5 °C (without being wrapped around a dowel). No change in specimen shape was observed in this time period (see Figure 5). Beyond 21 days, native and irradiated cartilage (*ex vivo*) undergo noticeable biologic degradation (even at 5 °C). In contrast, during surgery cartilage autografts are placed in a vascularized tissue bed and may remain viable for decades.

5 CONCLUSIONS

Laser irradiation can be used to reshape cartilage into complex geometries, and has the potential to radically change head and neck reconstructive surgery. The molecular basis for this complex phenomenon is not completely understood, and experiments are underway in our laboratory to clarify the mechanism(s) involved. Clinically, the most impor-

tant issue is preservation of chondrocyte viability. If cartilage is heated to temperatures much greater than 65 °C, the tissue becomes plastic, but chondrocyte destruction may occur if the duration of the heating is prolonged. With moderate heating, reshaping is possible without chondrocyte destruction, but the exact laser parameters are unknown.⁹ Feedback control of this process using a light scattering signal may be a practical method by which to limit nonspecific tissue injury due to uncontrolled heating yet still achieve adequate shape change.

Acknowledgments

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