Long-term Viability and Mechanical Behavior Following Laser Cartilage Reshaping

Amir M. Karam, MD; Dmitriy E. Protsenko, PhD; Chao Li; Ryan Wright, BS; Lih-Huei L. Liaw, MS; Thomas E. Milner, PhD; Brian J. F. Wong, MD, PhD

Objective: To investigate the long-term in vivo effect of laser dosimetry on rabbit septal cartilage integrity, viability, and mechanical behavior.

Methods: Nasal septal cartilage specimens (control and irradiated pairs) were harvested from 18 rabbits. Specimens were mechanically deformed and irradiated with an Nd:YAG laser across a broad dosimetry range (4-8 W and 6-16 seconds). Treated specimens and controls were autologously implanted into a subperichondrial auricular pocket. Specimens were harvested an average±SD of 208±35 days later. Tissue integrity, histology, chondrocyte viability, and mechanical property evaluations were performed. Tissue damage results were compared with Monte Carlo simulation models.

Results: All laser-irradiated specimens demonstrated variable tissue resorption and calcification, which increased with increased dosimetry. Elastic moduli of the specimens were significantly either lower or higher than controls (all P<.05). Viability assays illustrated a total loss of viable chondrocytes within the laser-irradiated zones in all treated specimens. Histologic examination confirmed these findings. Experimental results were consistent with damage profiles determined using numerical simulations.

Conclusion: The loss of structural integrity and chondrocyte viability observed across a broad dosimetry range underscores the importance of spatially selective heating methods prior to initiating application in human subjects.

Arch Facial Plast Surg. 2006;8:105-116

LASER CARTILAGE RESHAPING was introduced in 1993 by Helidonis et al (as also reported in Sobol et al2). In laser cartilage reshaping, specimens are held in mechanical deformation and then heated using laser radiation. Heat generation accelerates the process of mechanical stress relaxation, which allows tissue to remain in stable new shapes and geometries. Although the mechanisms underlying laser cartilage reshaping have not been completely identified, numerous animal studies have investigated the various biophysical mechanisms underlying shape change.1-10 In contrast to conventional surgical techniques, which rely on sutures and scalpels to balance and relieve the forces that resist mechanical deformation,11-16 laser cartilage reshaping relies on heat generation to induce structural changes in the tissue matrix. Several studies have demonstrated the feasibility of laser-assisted cartilage reshaping in porcine and rabbit auricles, as well as in canine trachea.17-21

At the Russian Academy of Sciences in Troitsk, clinical evaluation of laser-assisted cartilage reshaping for correction of nasal septal deformities22 has now involved more than 200 patients (Emil Sobol, PhD, oral communication, 2004).

While several studies23,24 report the viability of cartilage tissue following laser irradiation in vivo, the laser dosimetry that results in effective laser reshaping remains unknown. The problem is challenging in that heat is needed to cause physical changes in the tissue to produce shape change, yet excess heat generation can result in significant cell death within the heated volume of tissue. Sobol et al2 theorized that a dosimetry parameter space may exist where both cell viability and shape change can be achieved. In contrast to this theory, early work by Karamzadeh et al5,23 and later by Mordon et al24 and Chiu et al,20,25 has shown that shape change in cartilage may be a consequence of spatially selective heating, a principle and technique that have been exploited successfully in dermatologic laser applications where heat is delivered selectively to specific regions of the tissue to cause intense thermal injury.5,21,25 In those applications, however, the surrounding tissues are largely spared of injury, allowing for the regeneration and regrowth of the tissue in the damaged regions.
The present study aimed to investigate the effect of laser dosimetry (ie, power density and irradiation time) on cartilage mechanical properties and viability and to compare results obtained for temperature elevation, thermal injury, and shape change with those predicted by numerical simulation.

METHODS

Eighteen Pasteurella-free New Zealand white rabbits (3.5-4.5 kg; 9-12 months old) were used in the study. All protocols and experimental design settings were reviewed and approved by the University of California, Irvine Institutional Animal Care and Use Committee.

ANIMAL SURGERY

Anesthesia, preoperative preparation, and the surgical technique have been described elsewhere and only an overview will be provided here. Animals were induced by administering ketamine hydrochloride (35 mg of 100-mg/mL ketamine hydrochloride per kilogram of rabbit body weight) and xylazine hydrochloride (5 mg/kg of 20-mg/mL xylazine hydrochloride) via an intramuscular injection, intubated, and then maintained on isoflurane gas (2%-4%), which was titrated to effect.

A midline 3.5-cm-long nasal dorsal incision, from the level of the frontonasal suture to 1.0 cm above the nasal tip, was made to provide exposure for a laterally based osteoplastic flap (1.0 × 3.0 cm) centered over the septum. After the nasal cavity was exposed, bilateral submucoperichondrial flaps were elevated, and a 1.0 × 3.0-cm central segment of the septal cartilage was removed. Both sides of the septal mucosa were approximated, the osteoplastic flap was closed, and the skin incisions were sutured together.

LASER RESHAPING AND TEMPERATURE MEASUREMENT

Figure 1 illustrates the experimental protocol. The excised cartilage graft was immediately divided into 2 rectangular slabs (1 × 5 × 15 mm each) with a razor blade. One served as the control specimen and the other was irradiated with the laser. The cartilage for laser irradiation was placed in a custom jig assembly designed to maintain the specimen in a curved semicircular geometry (Figure 2). The jig was attached to a computer-controlled rotary translation stage, which positioned the specimen relative to the laser beam. Light from an Nd:YAG laser (λ = 1.32 µm, 5.4-mm-diameter spot size, 50-Hz pulse repetition rate; New Star Lasers Inc, Roseville, Calif) was directed perpendicularly at the specimen by using a multimode optical fiber (600 µm) and a collimating lens. This chassis was coupled to an infrared thermopile detector, which was used to estimate surface temperature. Thus, real-time measurements of surface temperature were recorded during the laser irradiation. Each specimen was irradiated using a different power–irradiation time pair (4-8 W, 6-16 seconds) as indicated in Table 1. The laser was directed at 3 predetermined positions in a nonoverlapping vertical arrangement along the surface of the specimen (Figure 2). Signals from the thermopile were recorded with the use of an analog-to-digital converter and a computer workstation. A 15-second delay was programmed between each irradiation spot to reduce overheating due to heat conduction. After irradiation, the cartilage specimen was immersed in an ambient-temperature isotonic sodium chloride solution bath for 15 minutes while maintaining the semicircular geometry. The distance between the ends of the slab was measured after rehydration and removal from the jig. Control specimens were secured in the same jig for 15 minutes (while immersed) but not irradiated. After removal from the jig and measurement, the control and laser-irradiated specimens were immersed 3 times (15 minutes each) in an antibiotic solution containing phosphate-buffered saline with gentamicin.
Figure 2. Laser irradiation pattern for rabbit nasal septal cartilage. Positions 1, 2, and 3 illustrate the sequence of laser irradiation along the surface of the cartilage during reshaping.

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Laser Power, W</th>
<th>Time, s</th>
<th>Calcification</th>
<th>Peak Surface Temperature, °C</th>
<th>Acute Bend Angle, Degrees*</th>
<th>Viable Chondrocytes</th>
<th>Fibroblast Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>16</td>
<td>Partial</td>
<td>75</td>
<td>55</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>12</td>
<td>Partial</td>
<td>71</td>
<td>38</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>10</td>
<td>Partial</td>
<td>69</td>
<td>35</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8</td>
<td>Partial</td>
<td>70</td>
<td>25</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>6</td>
<td>Partial</td>
<td>68</td>
<td>16</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>10</td>
<td>Partial</td>
<td>60</td>
<td>45</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>16</td>
<td>Partial</td>
<td>93</td>
<td>65</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>12</td>
<td>Partial</td>
<td>76</td>
<td>54</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>10</td>
<td>Total</td>
<td>74</td>
<td>45</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>8</td>
<td>Total</td>
<td>68</td>
<td>33</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>6</td>
<td>Partial</td>
<td>64</td>
<td>26</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>6</td>
<td>Partial</td>
<td>77</td>
<td>62</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>8</td>
<td>Partial</td>
<td>81</td>
<td>59</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>10</td>
<td>Partial</td>
<td>85</td>
<td>61</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>12</td>
<td>Partial</td>
<td>74</td>
<td>77</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>6</td>
<td>Partial</td>
<td>94</td>
<td>62</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>8</td>
<td>Partial</td>
<td>116</td>
<td>67</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>10</td>
<td>Partial</td>
<td>120</td>
<td>71</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Measured according to the method of Wright et al.
sulfate (200 mg/L) and amphotericin B (22.4 mg/L) under sterile conditions.

REIMPLANTATION

A small vertical incision was made along the base of the right auricle. The reshaped specimen and paired control were then autologously reimplanted into a subperichondrial pocket overlying the auricular cartilage in a transverse orientation (Figure 3). This location was selected because of the intrinsic favorable curvature of the auricle and an intact perichondrium—factors we hypothesized would optimize graft survival and shape retention. The degree of curvature of the auricle is significantly less than that of the acutely reshaped cartilage specimens. Placement of the curved specimens within the subperichondrial pocket resulted in an overall reduction of the curvature. A single 0-0 blue polypropylene suture was placed through the center of the irradiated specimen before implantation to facilitate identification at harvest. The perichondrium and overlying skin were closed in separate layers. Following surgery, the animals were observed daily and examined for signs of pain, wound infection, and other operative complications.

SPECIMEN RETRIEVAL

Specimens were removed following euthanasia an average ± SD of 208 ± 35 days after implantation. Samples were dissected free from the subperichondrial pockets with the use of a dissection microscope. Specimens were evaluated for gross shape change, presence of calcification, and overall mechanical integrity (Table 1). Specimens were photographed and prepared for mechanical analysis, confocal imaging, viability assessment, and histologic examination.

MECHANICAL PROPERTIES

Specimens were maintained in isotonic sodium chloride solution until just before mechanical testing (no longer than 15 minutes). The width, thickness, and length of each specimen were measured with a digital caliper. The specimens were visually examined and separated on the basis of the presence and pattern of calcification. Specimens that showed evidence of partial calcification (nonhomogeneous) were grouped together, and the calcified segments were excised and analyzed separately from the noncalcified regions. Likewise, specimens that showed homogeneous patterns of calcification or noncalcification were grouped and evaluated together.

Figure 4 shows the experimental setup for measurement of the elastic modulus of cartilage specimens. The cartilage specimen was secured between plastic clamps and attached to a motorized stage. The free end of the specimen was brought in contact with a blunt ball-tipped pin, mounted on a calibrated load cell (Model LCL 227 g; Omega Engineering, Inc, Stamford, Conn). The pin was blunted to prevent penetration into the specimen and gliding across the surface. The cantilevered cartilage specimen was deflected by moving the encoded motorized stage (Model M-126 PD; Physiks Instruments, Karlsruhe/Palmbach, Germany), controlled by a personal computer.
computer, back and forth a distance of 1 mm at a velocity of 5 mm/s. To reduce measurement errors and variation due to specimen nonuniformity, a micropositioner (Newport Corporation, Irvine) moved the pin-and-load cell assembly along the specimen’s surface to several different positions separated by 0.3 to 1 mm. The experiments were performed at each location with the application of 5 cycles of deflection.

The elastic modulus ($E$), of the cartilage specimen was estimated using the following equation for elastic flexure of a cantilevered bar:

$$E = \frac{\Delta F L^3}{\Delta X I}$$

where $\Delta F$ is the change in force; $\Delta X$, the change in displacement; $I$, the distance between the pin and the edge of the clamp; and $L$, the moment of inertia. For a cross section of a rectangular bar with a width $w$ and thickness $b$, $I = wb^3/12$. The calculated elastic modulus was averaged across all pin locations and deflection cycles.

**VIABILITY ANALYSIS**

After mechanical analysis, an approximately 500-µm-thick cross-sectional slice was cut lengthwise from the dissected tissue sample. The tissue slice was incubated in a live/dead assay system for visualization with a confocal microscope. The 2-color fluorescent viability dye solution was prepared using green fluorescent cyanine dye and red fluorescent ethidium homodimer-2 (SYTO 10 and DEAD Red, respectively; Molecular Probes Inc, Eugene, Ore). Live/dead assay has previously been used to identify live and dead cells in laser-irradiated cartilage with flow cytometry and with confocal microscopy. The assay determines cell viability on the basis of cell membrane integrity, which is considered an accurate indicator of cell viability. The assay uses a 2-fluorescent nucleic acid stains that weakly bind to DNA and RNA. SYTO 10 permeates all cells in the sample and weakly binds to the cell’s nucleotides with low affinity. SYTO 10 is excited at 488 nm and fluoresces green at 520 nm. The second stain, DEAD Red, can only permeate cells with compromised membranes, weakly binds to DNA and RNA with high affinity, and thereby displaces any bound SYTO 10. DEAD Red is excited at 488 nm and fluoresces red at 615 nm. The sectioned specimens were placed in a live/dead assay staining solution consisting of 4 µL of SYTO 10 and 2 µL of DEAD Red in 1 mL of Hank balanced salt solution. This ratio of dye concentration was found to be optimal for our experiments because SYTO 10 tends to photobleach at a much higher rate than DEAD Red does.

**HISTOLOGIC EXAMINATION**

The remaining portions of the specimens were fixed in formalin, serially dehydrated using graded ethanol solutions, and embedded in paraffin. The 6-µm sections were stained with hematoxylin-eosin and examined microscopically to correlate with confocal microscopic findings.

**NUMERICAL MODEL OF CARTILAGE IRRADIATION**

Laser irradiation (Nd:YAG, $\lambda = 1.32$ µm) of rectangular slabs of cartilage was numerically modeled. A previously described thermo-optical model was modified to include calculations of thermal damage based on a rate process damage mechanism, as follows:

$$\Omega(\tau) = A \int_0^\tau \exp \left(-\frac{E_a}{RT}dt\right)$$

where $\tau$ is heating time, $\Omega$ is the thermal damage parameter and $\Omega \geq 1$ corresponds to 1; $R$, the universal gas constant; $T$, the absolute temperature; $A$, a rate constant or frequency factor; and $E_a$, the activation energy per mole of molecules. The values of $A$ and $E_a$ were previously estimated on the basis of experiments that quantified the short-time decrease in viable rabbit septal chondrocytes as a function of exposure time in temperature-controlled isotonic sodium chloride baths. These variables allow estimation of cell viability given knowledge of the heating profile as a function of time.

The optical and thermal properties of cartilage, thermal damage settings, and geometry of the simulated cartilage specimen used in the numerical model are listed in Table 2. The temperature profiles as a function of time and space were calculated for a single laser exposure using each of the dosimetry setting pairs listed in Table 1. These data were used to estimate the peak temperature for each dosimetry pair as previously described. The model was used to estimate the spatial distribution of thermal injury by numerically integrating equation 2 and permits identification of regions of intense thermal injury, $\Omega(\tau)$, is greater than 1. Because a circular laser beam was used in the experiments and simulations, the spatial extent of severe thermal injury (ie, cell death) could be estimated. Results are presented as mean±SD unless specified otherwise.

**MACROSCOPIC TISSUE PROPERTIES**

Table 1 gives measured bend angles immediately following laser irradiation for each dosimetry pair. A direct relationship between laser dosimetry and the degree of bend...
angle was observed up to a power density of 20.4 W/cm². At greater doses, the degree of curvature flattened (Table 1). Bend angles were derived from recent ex vivo experiments designed to determine the relationship between laser dosimetry and the resultant bend angle. Details of these studies are provided elsewhere.29

All laser-irradiated specimens demonstrated some loss of tissue integrity and calcification, increasing with increased dose (Figure 5). Nearly all irradiated specimens contained calcified zones associated with regions where laser energy was directed. The extent of tissue calcification increased with laser dosimetry and generated a radial pattern of calcification. Nonirradiated controls showed relatively intact preservation of tissue bulk with no evidence of calcification.

After tissue recovery at 208±35 days, irradiated specimens (both stiff [calcified] and soft [noncalcified]) did not maintain curvature and conformed to the general shape of the auricular base. Specimens that were very stiff or very soft lacked any of the original curvature.

SURFACE TEMPERATURE MEASUREMENTS

Peak surface temperature measurements increased with increasing dosimetry. The range of surface temperatures was between 60°C and 120°C (Table 1).

MECHANICAL MEASUREMENTS

We found no evidence of calcification in any of the 18 control specimens; however, specimen flexibility did vary

![Figure 5](https://archfaci.jamanetwork.com/)

**Figure 5.** Range of tissue resorption and calcification following laser irradiation. A, A control specimen shows no evidence of calcification or resorption. B, A sample irradiated at 4 W for 12 seconds shows loss of tissue integrity and heterogeneous patches of calcification. C, A specimen irradiated at 4 W for 16 seconds shows extensive calcification extending beyond the borders of the native cartilage.

![Figure 6](https://archfaci.jamanetwork.com/)

**Figure 6.** Average (and standard deviation) elastic modulus of the control specimens. The average modulus of the control specimens (C) is shown at the far left for comparison.

<table>
<thead>
<tr>
<th>Table 2. Cartilage Slab Geometry and Optical, Thermal, and Thermal Damage Settings Used in the Numerical Model for Cartilage Irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
</tr>
<tr>
<td><strong>Width</strong></td>
</tr>
<tr>
<td><strong>Thickness</strong></td>
</tr>
<tr>
<td><strong>Initial temperature</strong></td>
</tr>
<tr>
<td><strong>Thermal conductivity</strong></td>
</tr>
<tr>
<td><strong>Density</strong></td>
</tr>
<tr>
<td><strong>Heat capacity</strong></td>
</tr>
<tr>
<td><strong>Convection coefficient</strong></td>
</tr>
<tr>
<td><strong>Absorption coefficient</strong></td>
</tr>
<tr>
<td><strong>Scattering coefficient</strong></td>
</tr>
<tr>
<td><strong>Scattering angle</strong></td>
</tr>
<tr>
<td><strong>Tissue refraction index</strong></td>
</tr>
<tr>
<td><strong>Laser beam radius</strong></td>
</tr>
<tr>
<td><strong>Laser beam profile</strong></td>
</tr>
<tr>
<td><strong>Frequency factor</strong></td>
</tr>
<tr>
<td><strong>Activation energy</strong></td>
</tr>
</tbody>
</table>

![Figure 7](https://archfaci.jamanetwork.com/)

**Figure 7.** Elastic moduli of the laser-irradiated specimens compared with the average modulus of the control group. Data from the nonhomogeneous group (irradiated specimens 3, 5, 9, 10, and 11) are arranged in pairs, showing the elastic modulus of non-calculated and calculated portions separately. The lowest elastic modulus of 0.17±0.03 MPa was measured in the soft portion of the specimen from animal 3. Specimens from animals 6, 7, 8, 14, and 16 demonstrated a high degree of calcification. Mechanical flexing of these specimens in an experimental apparatus resulted in random fracturing of calcified material, which prevented accurate estimation of elastic modulus. However, from data obtained before the fracturing occurred, it was possible to estimate that the elastic modulus of these specimens exceeded 43 MPa.

**Figure 8** shows elastic modulus as a function of the laser energy delivered per a single irradiation spot for the noncalcified and calcified portions of the nonhomogeneous and homogeneous specimens, respectively. The elastic modulus of the calcified portions of the nonhomogeneous samples was enhanced with increasing energy level, from 1.4±0.1 MPa at 24 J to 21±1.7 MPa for the least and most stiff specimens, respectively.
calcified portions remained significantly lower (from 0.17±0.03 MPa to 0.61±0.07 MPa) than the control value (9±6 MPa) (P=.048).

The nonhomogeneous specimens—specimens with spatially separated calcified and noncalcified portions—were present only at energy levels of 50 J or below. Homogeneously calcified specimens with elastic moduli greater than 10 MPa appeared at energy levels of 50 J and above. However, soft homogeneous specimens with elastic moduli varying from 0.25±0.10 to 0.97±0.06 MPa were present throughout the investigated energy ranges.

**CHONDROCYTE VIABILITY**

Confocal microscopy using the live/dead assay revealed no evidence of live, healthy chondrocytes within the laser-irradiated region of each specimen for any laser dosimetry pairs used in this experiment (Figure 9). Instead, the results consistently showed an extracellular matrix devoid of chondrocytes that was in the process of being invaded by fibroblasts (Figure 9). The distinction between live and dead cells was ultimately of minimal value because the assay tested the viability of the only cell type present—invading fibroblasts. The pervading red signal found in the confocal images represented dead fibroblast tissue, which was vulnerable to desiccation and readily perished during the staining procedure. The importance of the live/dead assay is its utility in distinguishing fibroblasts from chondrocytes by way of their respective cellular morphology. As the confocal images of the null controls showed, live chondrocytes are round and were evenly distributed throughout the matrix, in contrast to elongated fibroblasts, which permeated the dead matrix.

**HISTOLOGIC FINDINGS**

Review of the histologic images reinforced the confocal microscopy observations. Images revealed empty lacunae with fibroblasts tunneling through a deteriorated matrix devoid of chondrocytes in all laser-irradiated specimens (Figure 10). The matrix was replaced by scar tissue and calcification in the irradiated spots. The calcification density was the greatest at the center of the laser spot. However, regions outside the laser-irradiated zone and controls showed normal cellular and extracellular architecture. The lack of viable chondrocytes in the irradiated regions was consistent with numerical simulation results.

**NUMERICAL SIMULATION OF CARTILAGE IRRADIATION**

Figure 11 compares measured peak radiometric temperature on the irradiated cartilage surface during laser irradiation (Texp) with values predicted by numerical simulation (Tsim). The experimental values are an average of measurements recorded during the 3 consecutive irradiations on the specimen for a given set of laser settings. The simulated and experimental values correlated well at temperatures below 90°C (correlation coefficient, 0.95). When the radiometric temperature was above 90°C, the simulated values were consistently lower. Figure 12 correlates the radius of the region of thermal damage (ie, Ω>1) estimated from the numerical simulation with the bend angle of cartilage specimens measured immediately after laser irradiation. The relationship between bend angle and laser dosimetry using the apparatus described in this study has been described previously. A thermal damage radius exceeding 2.5 mm corresponds to the diameter of a damage zone that exceeds the width of cartilage sample, although the simulation
calculates damage longitudinally along the specimen and places an upper limit of 7.5 mm on the radius. In experiments, damage zones from adjacent irradiation spots overlap because of heat diffusion; thus, numerical simulations underestimate damage. Figure 12 demonstrates that, across the range of laser power and irradiation times, the radius of the thermal damage varied linearly with bend angle (correlation coefficient, 0.91).

In laser-assisted cartilage reshaping, heat creates physical changes within the tissue matrix, which leads to the acceleration of stress relaxation and to the establishment of a new equilibrium shape. Heat also may injure chondrocytes, the constitutive cells of cartilage tissue responsible for the generation and maintenance of a healthy tissue matrix. An important question under debate is whether a laser dosimetry parameter space exists where competing objectives of shape change and cell viability are achieved. Although the results of this study do not provide any evidence of such a parameter space, the question may not be relevant to the clinical utility of laser-assisted cartilage reshaping. Alternatively, the success of laser-assisted cartilage reshaping in both animals and humans may rely on the spatially selective heating of cartilage tissue,5,23,24 where heat significantly alters the mechanical properties of discrete regions of tissue without regard to cell viability. Adjacent tissue does not undergo significant thermal modification, and the net shape change is produced as a consequence of focal tissue denaturation (and cell damage) combined with wound healing and tissue regeneration owing to the presence of normal surrounding tissue. To verify what occurs in clinical laser-assisted cartilage reshaping, it is imperative that the relationship between dosimetry and tissue viability be determined in a long-term in vivo model, because wound healing, tissue regeneration, and tissue repair cannot be adequately evaluated using ex vivo tissue specimens in culture. Hence, this study focused on evaluating long-term results with an emphasis on obtaining both physical and biological measurements of tissue behavior. In previous investigations,3,5,7 the acute effect of laser dosimetry on bend angle in cartilage was studied exhaustively in rabbit and porcine nasal septal cartilage and has provided some information on what might be effective laser settings to reshape tissue when using Nd:YAG laser irradiation and volumetric heating. Recent work by our group29 indicates that the maximum bend angle increases with laser dose immediately following laser irradiation, until the power density exceeds 20.4 W/cm². Above this threshold, the bend angle does not significantly increase with power density. The dosimetry settings used in this study ranged from levels too low to create shape change to those high enough to do so, based on our ex vivo studies.

Cartilage is an avascular tissue and undergoes a wound healing and tissue repair process, which is different from most other soft tissues. Cartilage injury can result in depopulation of chondrocytes and subsequent changes in the matrix, including depletion of proteoglycans, calcification, ossification, scar/fibrosis, and even repopulation by chondrocytes provided a stem cell source such as perichondrium is close to the injured region.24 The laser wavelength used in this study (1.32 µm) produces relatively uniform longitudinal heating of the specimen. Similarly, large laser spots were used to reduce lateral temperature gradients.

Of note, systematic evaluation of each dosimetry pair (Table 1) demonstrated that no viable populations of chondrocytes were identified in the region of laser energy deposition in any specimen studied. This included dosimetry pairs that produced negligible shape change (e.g., 4 W and 6 seconds). Given that no dosimetry settings and corresponding temperature elevations maintain cell viability, even under circumstances where minimal shape change occurs, we believe that laser cartilage reshaping may not involve balancing shape change and stress relaxation with the preservation of cell viability within the same space. Effective shape and
the preservation of cell viability are mutually exclusive phenomena.

Our results underscore the importance of spatially selective heating in cartilage reshaping, which was the technique elegantly demonstrated by Mordon et al\textsuperscript{24} and Chiu et al\textsuperscript{20,25} for reshaping rabbit ears. The concept of spatial selectivity in laser therapy has become the cornerstone of dermatologic laser surgery\textsuperscript{20,23-25} and was advocated for use in laser cartilage reshaping as early as 1998.\textsuperscript{3}

In a pioneering in vivo study, Mordon et al\textsuperscript{24} used near-infrared laser irradiation in combination with contact cooling to reshape rabbit auricular cartilages. Biopsy specimens of the laser-treated region obtained at 1, 3, and 6 weeks demonstrated the presence of a layer of complete cartilage matrix, as shown in Figure 10. The asterisk represents a region on the periphery of the laser spot showing the presence of normal cartilage matrix and lacunae with chondrocytes. The dashed line indicates the transition zone between nonirradiated (viable) and irradiated (nonviable) cartilage. The laser-irradiated zone demonstrates the loss of chondrocytes and the empty lacunae. A cross section through a specimen irradiated at 4 W for 6 seconds illustrates an acellular matrix and empty lacunae (original magnification ×20). C and D, Infiltration of fibrous tissue into the irradiated cartilage matrix (C, specimen irradiated at 4 W for 10 seconds; D, specimen irradiated at 4 W for 6 seconds; original magnification for both ×20). E, The presence of dense calcification within the cartilage matrix of a specimen irradiated at 8 W for 8 seconds (original magnification, ×20). F, A control specimen illustrates normal cartilage microstructure and the presence of chondrocytes within the lacunae (original magnification, ×40).
chondrocyte death adjacent to a layer of viable chondrocytes. Similar findings were reported by Chiu et al.20 The fact that this was accomplished without incisions or graft removal means that an intact perichondrium was preserved, either in appositional or adjacent regions. Perichondrium is rich in stem cells and highly vascularized.

In contrast, the present study used extracorporeal irradiation and reshaping of graft tissue with near-infrared laser wavelengths that minimized axial temperature gradients (and hence any axial spatial selectivity) and facilitated the study of the isolated effect of dosimetry (or temperature) on tissue biophysical behavior alone. The lack of any viable chondrocytes in the laser irradiation zones suggests that the success of cartilage reshaping in humans is the result of spatial selectivity of heating. Hence, the task of reshaping cartilage focuses on delivering adequate laser energy to specific regions of the specimen where heat can relieve internal stresses produced by mechanical deformation.

The results of our mechanical analysis further support this approach. A significant difference (P = .048) between the elastic moduli of control and irradiated samples was observed. The presence of tissue calcification was observed in irradiated specimens only. The elastic moduli of irradiated specimens were either significantly higher or lower than those of control specimens. The presence of focal zones of calcification tended to increase with power density. Experimental specimens could be separated into the following 3 distinct subgroups: (1) very soft without calcification, (2) very stiff with complete calcification, and (3) inhomogeneous with intermingled regions of soft (noncalcified) and stiff (calcified) tissue. Soft, low-modulus noncalcified tissue was found in specimens irradiated at every power level; however, stiff, high-modulus calcified tissue was only present in samples exposed to a total energy of 50 J or greater. These findings suggest that the calcification process may evolve in a stepwise pattern following laser irradiation. The initial laser interaction may result in loss of viable chondrocytes followed by deterioration of matrix architecture, infiltration of fibroblasts, and subsequent collagen deposition. The rate and degree of calcification follows a dose-dependent relationship, although the mechanism of laser-induced mineralization is unclear. The effect likely results from loss of tissue viability. This observed phenomenon would have a deleterious impact on clinical outcome, especially in structures such as the external nose and ear.

The reasonable agreement between experimental and simulated peak temperatures (Figures 11 and 12) further validates our numerical model as a tool to calculate temperature distributions during laser heating of cartilage. Thermo-optical models have been used extensively in dermatologic laser applications and have been invaluable in the optimization of dosimetry settings for several clinical procedures, such as the treatment of vascular lesions. In the present study, the principal value of the thermo-optical simulation is in its use in combination with equation 2, such that tissue damage can be estimated. Although the Arrhenius rate-process models assume a 1-step interaction, they have been surprisingly accurate in estimating thermal damage. These results link thermal injury with acute shape change and demonstrate that shape change and tissue injury are highly correlated. Given the strong correlation between thermal damage and bend angle, the prospect of being able to achieve significant shape change without thermal injury appears remote for the laser devices used in this study. Hence, the clinical effectiveness of shape change is likely a consequence of spatially selective heating, in which damaged tissue regions are small and surrounded by healthy tissue or stem cell–rich perichondrium. Regardless, the behavior of cartilage following any physical modification process is unpredictable, and even measures such as morselization and carving can produce chondrocyte death.7,37 Hence, even standard surgical approaches may significantly traumatize the tissue and compromise cell viability.

Figure 11. Correlation of simulated maximal temperature at the cartilage surface (T_max) and experimentally measured values (T_exp). Each data point corresponds to a particular combination of laser power and irradiation time.

Figure 12. Correlation of simulated values of thermal damage radius and experimentally measured final bend angles of reshaped cartilage. Shown are the levels of damage radius corresponding to the radius of the laser spot (horizontal line 1) and also half the width of the cartilage sample corresponding to half the distance between irradiation spots (horizontal line 2).
We evaluated a broad range of laser dosimetry pairs (and hence temperature profiles) that produced varying degrees of acute shape change in rabbit septal cartilage tissue. The bulk heating model used in this study resulted in compromise of chondrocyte viability, loss of structural integrity, and drastic changes in elastic modulus after 7 months. These findings were observed even when dosimetry that did not produce significant acute shape change. Because no appreciable axial temperature gradient was produced during Nd:YAG laser irradiation of the grafts, the results of our study provide information on the temperature-time criteria that lead to cell death in septal cartilage and are in accord with estimates produced by our numerical models. Our study underscores the importance of spatially selective heating, which is likely fundamental to the successful clinical application of this therapy in human subjects.

Accepted for Publication: November 15, 2005.

Correspondence: Brian J. F. Wong, MD, PhD, Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Sciences Rd E, Irvine, CA 92612 (bjwong@uci.edu).

Funding/Support: This study was supported in part by an Undergraduate Research Opportunities Program Grant from the University of California, Irvine; grants D00170, DC005572, and RR-01192 from the National Institutes of Health, Bethesda, Md; the American Society for Lasers in Surgery and Medicine, Inc, Wausau, Wis; and grant FA9550-04-1-0101 from the Air Force Office of Scientific Research, Arlington, Va.

Previous Presentation: This study was presented in part at the Combined Otolaryngological Spring Meetings, American Academy of Facial Plastic and Reconstructive Surgery; May 13, 2005; Boca Raton, Fla.

Acknowledgment: We thank George Peavey, DVM, and Laurie Newman for their insights and assistance with the surgical protocol and care of the animals. Tatiana Krasieva, PhD, provided technical support for confocal imaging.

CONCLUSIONS

In clinical studies, Ovchinnikov et al reported the successful use of holmium:YAG laser (λ = 2.12 µm) wavelengths to alter the shape of nasal septal cartilage in humans. The thickness of human nasal septal cartilage has been estimated to vary from 2 to 4 mm; the optical penetration depth of the laser wavelengths used by Ovchinnikov and colleagues (they later switched to erbium-doped fiber lasers, which emit light at 1.54 µm) was significantly less than these dimensions, and their irradiation was performed in a transmucosal fashion. In all likelihood, the shallow absorption and short radiation times resulted in the heating of only the superficial layers of the septum, producing physical changes only in the most superficial regions of the cartilaginous septum. The deeper regions of the cartilaginous septum and the contralateral septal mucosa were largely spared thermal injury, and the relatively small spot size ensured that reasonable wound healing would occur owing to the presence of viable tissue surrounding the laser irradiation site.

REFERENCES


