ORIGINAL ARTICLE

Radiofrequency Cartilage Reshaping

Efficacy, Biophysical Measurements, and Tissue Viability

Michael W. Keefe, MD; Alexandre Rasouli, MD; Sergey A. Telenkov, PhD; Amir M. Karamzadeh, MD; Thomas E. Milner, PhD; Roger L. Crumley, MD, MBA; Brian J. F. Wong, MD, PhD

Objectives: To assess the feasibility of reshaping cartilage using radiofrequency (RF) heating, and to examine the effects of this process on tissue biophysical properties (optical and thermal) and cellular viability.

Methods: Mechanically deformed porcine septal cartilage was reshaped using 2 RF-generating devices. We performed dynamic measurements of tissue thermal and optical properties while heating cartilage with one of these devices. Cellular viability was assessed immediately and 7 days after treatment.

Results: A characteristic change in the diffuse transmittance of light through the cartilage occurred during heating. Change in transmittance has been shown to accompany the onset of stress relaxation in cartilage. Peak radiometric surface temperature during heating was 88.6°C. Specimens retained their user-specified curved shape for the observed period of 14 days. Chondrocyte viability in RF-heated tissue was 19% and 14% of that in untreated control specimens at days 0 and 7 after treatment, respectively.

Conclusions: Radiofrequency heating has been shown to effectively reshape cartilage while maintaining cellular viability, illustrating a novel application for a widely used technology.

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ALTERING THE shape of cartilage using classic surgical techniques requires carving, scoring, and/or suturing of the graft. The technique is limited by the availability of suitable donor tissue and complicated by unpredictable warping due to the release of interlocked stresses in the tissue matrix.[1-5] In 1993, Helidonis et al[6] introduced a technique to permanently reshape cartilage using photothermal stimulation from a laser to heat mechanically deformed cartilage grafts and thereby accelerate stress relaxation. Laser-mediated cartilage reshaping and the attendant biophysical changes in the tissue matrix have been described in detail.[7] A characteristic peak in the diffuse optical reflectance during laser-mediated cartilage heating has been observed to accompany the onset of stress relaxation that leads to permanent shape change. Photothermal heating provides precise control over the temporal and spatial distribution of temperature and allows the use of noncontact delivery methods. The control of tissue temperature is critical for maintenance of chondrocyte viability in the reshaping process. Recently, radiofrequency (RF) instruments have been developed that can generate temperature fields with the same spatial-temporal characteristics as selected laser wavelengths. Because RF technology can be manufactured and distributed at a significant cost advantage compared with presently available commercial medical lasers, development of cartilage reshaping instruments will require careful evaluation of approaches that use RF energy to heat the specimen.

Radiofrequency energy is commonly used in surgery to cauterize, coagulate, cut, fulgurate, and, more recently, ablate tissue.[8] Radiofrequency-based instruments allow innovative treatment of seizure foci within the brain, aberrant conduction pathways within the heart, hypertrophic prostate tissue, and hypertrophic, flaccid, or redundant soft tissue of the nasal conchae, palate, and tongue base.[9-13] Many modern clinical RF instruments are microprocessor controlled, allowing precise control of the generation of heat in the tissue surrounding the electrodes.[11,12]

In this study, we used 2 commercial RF generator systems in the novel surgi-

Author affiliations are listed at the end of this article.

cal application of reshaping porcine cartilage tissue grafts. The objectives of this study were (1) to successfully reshape cartilage specimens using RF-generated heat, and (2) to evaluate the biological response of RF-reshaped cartilage in terms of tissue viability. To assess shape change, 1 of the 2 generators was used to heat cartilage. Dynamic changes in tissue optical properties and radiometric surface temperature were measured, as the thermal and optical properties of cartilage change with heating.[14-16] After RF heating, short- and long-term tissue viability were determined using cell culture, chondrocyte isolation, and trypan blue exclusion combined with hemocytometry.[17]

METHODS

SPECIMEN PREPARATION

The intact crania of freshly killed 70-kg domestic pigs were obtained from a local abattoir (Clougherty Packing Company, Los Angeles, Calif), and the septal cartilages were harvested as previously described[18] within 3 to 4 hours after death. The septal mucosa was left attached to the cartilage specimens, which were maintained in chilled Hank solution (Gibco BRL, Grand Island, NY) until just before use. The mucosa and perichondrium were removed, and the specimens were cut into rectangular slabs ($10 \times 20 \times 2$ mm) with a custom guillotine microtome.[18] Only tissue from the most cephalic 40 mm of the septum was used to ensure uniform tissue properties.[17-19]

RF GENERATORS AND RESHAPING

Two RF generators were used to heat and reshape specimens. Preliminary reshaping investigations were performed using a microprocessor-controlled device (model 215 RF generator, 460 kHz; Somnus Medical Technologies, Inc, Sunnyvale, Calif [now part of Gyrus Medical, Inc, Maple Grove, Minn]) that was available only on a limited basis during this study. This unit was optimized to slowly heat (and subsequently denature) tissue, and is used for palatal, tongue base, and turbinate reduction operations. A series of thermocouples are incorporated into the needle-shaped electrode to provide temperature feedback control for the heating process. Cartilage slabs were manually bent into a curved shape while maintaining electrical continuity with a standard surgical grounding pad. The needle-shaped electrode (1000 single-needle coagulating electrode; Somnus Medical Technologies, Inc) was inserted at intervals along the length of the specimen. At each position, the RF energy was applied until the thermocouple readings reached 70°C (**Figure 1**). The deformed specimens were then rehydrated in isotonic sodium chloride solution for 15 minutes and photographed.

An open-loop–controlled RF device (Surgitron FFPF catalog S 2100, 2.8 MHz; Elmed Inc, Addison, Ill) was used for all remaining biophysical and viability experiments. This high-frequency instrument is optimized for use in intense thermal interactions such as cutting, fulgurating, coagulating, or cauterizing tissue. Using this device, we wrapped specimens around a wooden dowel rod (1 cm long × 0.95 cm in diameter) and secured them with plastic cable ties (Gardner Bender/APU Tools and Supplies, Milwaukee, Wis). Bipolar RF needle-point probes were independently inserted near one end of the slab, separated by a distance of 3 to 4 mm and perpendicular to the long axis of the specimen. The tissue was heated using the cut-coag mode of the instrument with an application time of 5 seconds. The power-setting knob on the generator is divided into 10 levels (arbitrary units), and a setting of 2 was used in this phase

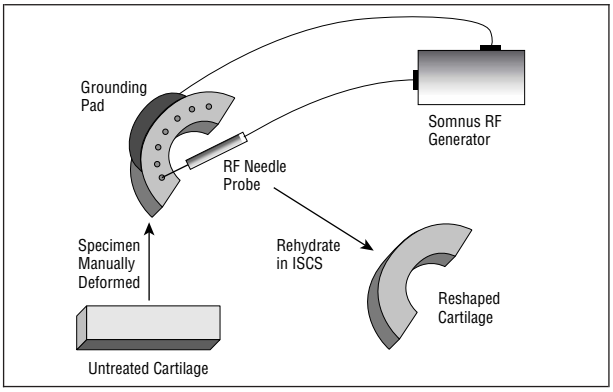

Figure 1. Cartilage reshaping with the model 215 radiofrequency (RF) generator (460 kHz; Somnus Medical Technologies, Inc, Sunnyvale, Calif). Manually deformed cartilage in contact with a grounding pad was heated at each electrode insertion point to a thermocouple reading of 70°C. The cartilage was then rehydrated in isotonic sodium chloride solution (ISCS).

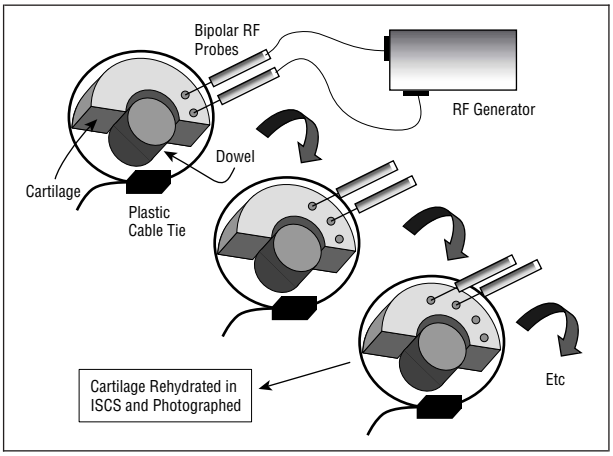

Figure 2. Cartilage reshaping with the Surgitron FFPF catalog S 2100 radiofrequency (RF) generator (2.8 MHz; Elmed Inc, Addison, Ill). The specimen was wrapped around a dowel and secured with a plastic cable tie. Bipolar RF electrodes were inserted and advanced in stepwise fashion, with heating of intervening cartilage at each insertion point. The cartilage was then rehydrated in isotonic sodium chloride solution (ISCS).

of the experiments. After heating at the first insertion point, the electrode closest to the end of the slab was removed and reinserted 3 to 4 mm beyond the second electrode. By advancing 1 electrode at a time in a stepwise fashion with heating of the intervening tissue with each insertion, the entire slab was incrementally heated (**Figure 2**). Specimens were then rehydrated in isotonic sodium chloride solution for 15 minutes while remaining secured to the dowel.[17,20,21] Each specimen was photographed before heating and after rehydration. The reshaped cartilage specimens were then stored in isotonic sodium chloride solution at 4°C and observed for 2 weeks. Additional photographs were taken at 7 and 14 days after RF reshaping to document shape change and memory effects.

BIOPHYSICAL MEASUREMENTS: LIGHT SCATTERING AND SURFACE TEMPERATURE

To study the changes in tissue optical properties during RF heating, needle-point bipolar electrodes were inserted 2 mm apart into cartilage specimens that were secured in front of an integrating sphere (LPM-040-IG; Labsphere, Inc, North Sutton, NH) (**Figure 3**). Light from a diode laser ($\lambda = 650$ nm; 5 mW) (MWK Industries, Corona, Calif) was aimed at the region of tissue be-

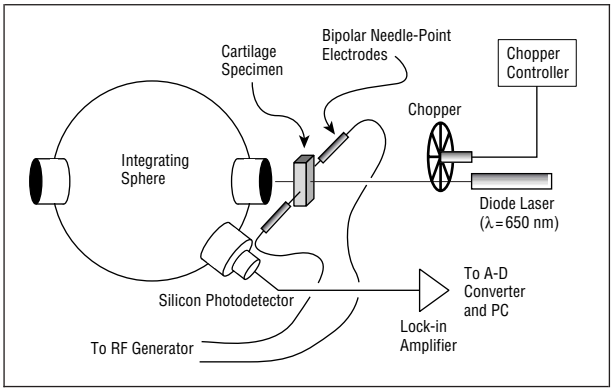

Figure 3. The apparatus used to measure the intensity of diffuse transmittance of laser light through cartilage during radiofrequency (RF) heating. Incident laser light is aimed at the area between the bipolar RF electrodes. A-D indicates analog-to-digital; PC, personal computer.

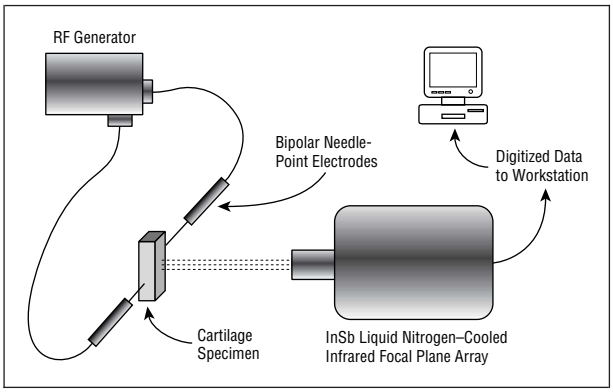

Figure 4. The apparatus used to measure the radiometric surface temperature of cartilage during radiofrequency (RF) heating. The indium antimonide (InSb) liquid nitrogen–cooled focal plane array was focused on the surface of the specimen between RF bipolar electrodes.

tween the 2 electrodes. Diffusely transmitted laser light was collected with the integrating sphere positioned at the opposite (nonirradiated) surface of the specimen. A mechanical chopper (SR540; Stanford Research Systems, Sunnyvale, Calif) modulated the intensity of the laser light, which was synchronously detected using a silicon photoreceiver (Model 2001; New Focus Inc, San Jose, Calif) and a lock-in amplifier (time constant, 300 milliseconds) (model SR 850 DSP; Stanford Research Systems). Signals were recorded using a 16-bit analog-to-digital converter (AT-MIO-16XE-50; National Instruments Corporation, Austin, Tex) and a personal computer running software written in LabView (National Instruments). The intensity of diffusely transmitted laser light was recorded during RF heating with the Elmed generator at a power setting of 1.25 for 30 seconds. The cartilage was allowed to cool for 5 minutes, and the process was repeated once. Reheating allowed correlation with previous laser studies, in which prolongation in the local extrema of light-scattering curves was observed during sequential irradiation.[19]

Radiometric surface temperature was measured using an indium antimonide, liquid nitrogen–cooled infrared focal plane array (Galileo model; Amber Engineering, Goleta, Calif [now part of Raytheon Company, Lexington, Mass])[22] (**Figure 4**). Infrared images were recorded at 0.5-millisecond intervals. Temperature $S_c(t,x,y)$ was determined by comparing the recorded signals with a calibration curve generated from measurements of a black-body radiation source (Omega BB701; OMEGA Engineering, Inc, Stamford, Conn). The RF generator was adjusted to a setting of 1.25 in the cut-coag waveform mode with a heating time of 5 seconds and electrode spacing of 2 mm. Temperature distributions were analyzed using a signal-processing software package (Advanced Visualization Software; Advanced Visual Systems, Waltham, Mass).

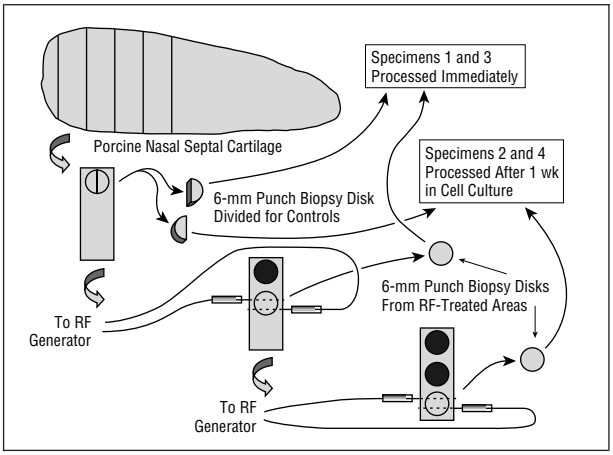

Figure 5. Schematic illustration of the experimental protocol used to treat cartilage specimens for chondrocyte viability determination. Two control and 2 radiofrequency-heated areas were excised by means of punch biopsy from each of 4 slabs from the cephalic end of porcine nasal septa.

CHONDROCYTE VIABILITY

The experimental protocol used to measure cell viability is illustrated schematically in **Figure 5**. Four cartilage specimens (labeled A through D, proceeding cephalad to caudad) were obtained from the most cephalic region of the septum in each of 5 pig crania. Each rectangular specimen was, in turn, divided into 4 regions labeled 1 through 4. Regions 1 and 2 were used as matched control specimens and were obtained by dividing a centrally placed 6-mm full-thickness punch biopsy specimen that was removed before RF heating of the specimen. Matched controls from each specimen were used, because previous investigations identified significant spatial variation in the cellular density and biosynthetic properties of this tissue model.[17,18] Region 1 was processed immediately and used as a control for region 3, whereas region 2 was placed in a cell culture and processed 1 week later as a control for region 4. The remaining tissue was heated in 2 separate areas by inserting bipolar electrodes (separated by 3-4 mm) and using a power setting of 2 on the Surgitron device (cut-coag waveform) for a total of 5 seconds per trial. Immediately after heating the first area (region 3), the modified tissue was removed with a full-thickness 6-mm punch. The remaining region of tissue (region 4) was heated, followed by punch biopsy excision. One of the 6-mm punch specimens was processed immediately (region 3), whereas the other was placed in a cell culture for processing 1 week after heating (region 4).

All tissue specimens were maintained in chilled Hank solution until placement in tissue culture or enzymatic digestion within 1 hour. Each specimen was weighed using an analytic balance, and then washed 3 times in phosphate-buffered saline (without calcium or magnesium) containing gentamicin (50 mg/L) and amphotericin B (5.6 mg/L) for 30, 15, and 15 minutes and transferred to sterile culture plates. Specimens 2 and 4 from each cartilage slab were placed in a cell culture medium (Dulbecco Modified Eagle Medium; Gibco BRL) with additives (gentamicin, 50 mg/L; penicillin, 100 000 IU/L; streptomycin, 100 000 IU/L; 2mM L-glutamine; and 10% fetal bovine serum [vol/vol]) and placed in a humidified 37°C incubator (5% carbon dioxide) for 1 week. The culture medium was changed every other day.

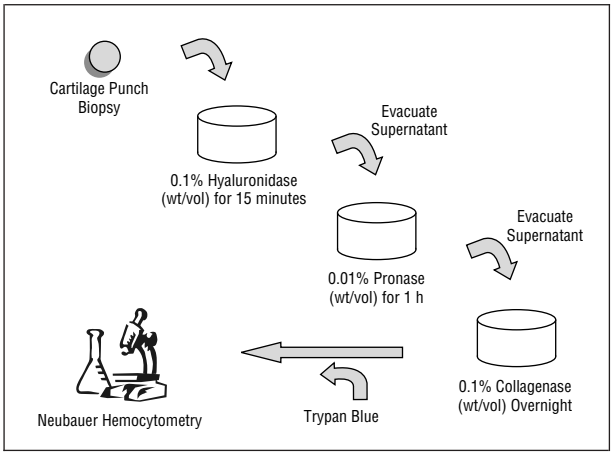

Figure 6. Schematic illustration of the experimental protocol used to isolate chondrocytes from the cartilage matrix via enzymatic digestion of the tissue.

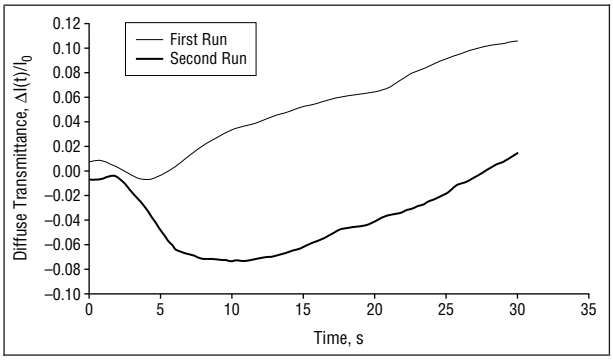

Figure 7. Plot depicting the diffuse transmittance (I[t]) of laser light during radiofrequency heating. Two heating cycles are depicted. The slope of $\Delta I(t)/I_0$ vs time reaches 0 at 4 seconds with the first cycle and 10 seconds with the second. I_0 represents the signal intensity of diffused transmitted light before radiofrequency energy is applied.

Chondrocytes in regions 1 and 3 from each cartilage slab were isolated from the extracellular matrix using a 3-step enzymatic digestion[23] (0.1% hyaluronidase [wt/vol] for 15 minutes; 0.01% pronase [wt/vol] for 1 hour; and 0.1% collagenase [wt/vol] overnight), all at 37°C in humidified 5% carbon dioxide) (**Figure 6**). After overnight digestion in collagenase, we used Neubauer hemocytometry combined with trypan blue exclusion to access viability.[24] The intact cell membranes of live chondrocytes exclude the dye, whereas dead cells stain blue owing to incompetent cell membranes. We calculated the number of live cells per gram of tissue in each punch biopsy region. Similarly, after 1 week in cell culture, enzymatic digestion and hemocytometry were performed on the specimens from regions 2 and 4 of each slab to evaluate latent thermal effects on cell viability.

RESULTS

Diffuse transmittance, recorded during 2 sequential RF heating cycles, is depicted in **Figure 7**. A 5-minute cooling interval elapsed between successive applications of RF energy. Absolute minima for intensity of diffusely transmitted laser light are observed at approximately 4 seconds with the first heating and 10 seconds with the second heating.

Figure 8 is a thermal image recorded using the indium antimonide infrared focal plane array and represents a 4.5-mm^2 region of interest, 5 seconds after the onset of RF heating with the Elmed RF generator. Isothermal lines at 3°C intervals from 58°C to 88°C are shown. The maximum temperature on the image is 88.6°C. The average radiometric temperature in a 1-mm^2 region centered in the area of maximum heating is illustrated as a function of time in **Figure 9**.

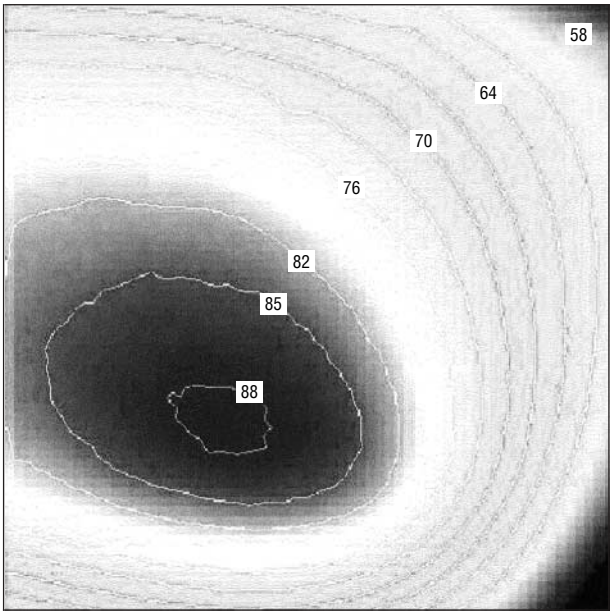

Figure 8. A thermal image produced using the indium antimonide liquid nitrogen–cooled infrared focal plane array. This image represents the radiometric surface temperature (measured in degrees Celsius) of a 4.5-mm^2 area of a cartilage specimen 5 seconds after the commencement of radiofrequency heating with the Surgitron FFPF catalog S 2100 radiofrequency generator (2.8 MHz; Elmed Inc, Addison, Ill).

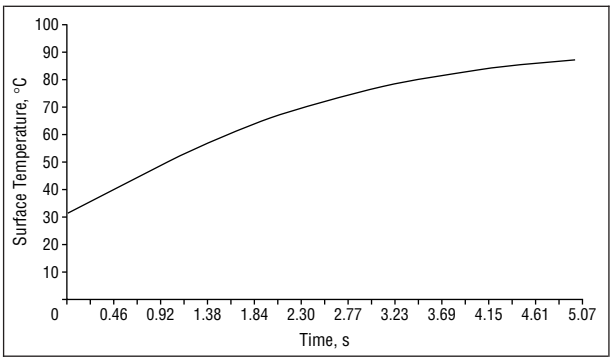

Figure 9. Plot depicting the average radiometric surface temperature vs time of a 1-mm-diameter area of cartilage centered in the area of maximum radiofrequency (RF) heating with the Surgitron FFPF catalog S 2100 RF generator (2.8 MHz; Elmed Inc, Addison, Ill) and bipolar needle-point electrodes.

Figure 10 is a photographic montage of a cartilage specimen before (Figure 10A) and immediately after RF reshaping (Figure 10B) and on days 7 (Figure 10C) and 14 (Figure 10D). No memory effects or reversion to prebending shape were observed during this interval.

Tissue viability was evaluated in each of the 20 RF-heated specimens subjected to immediate enzymatic digestion and each of the 20 specimens that underwent delayed digestion after a week in cell culture. The average viability or cell survival (cells per gram of cartilage) of

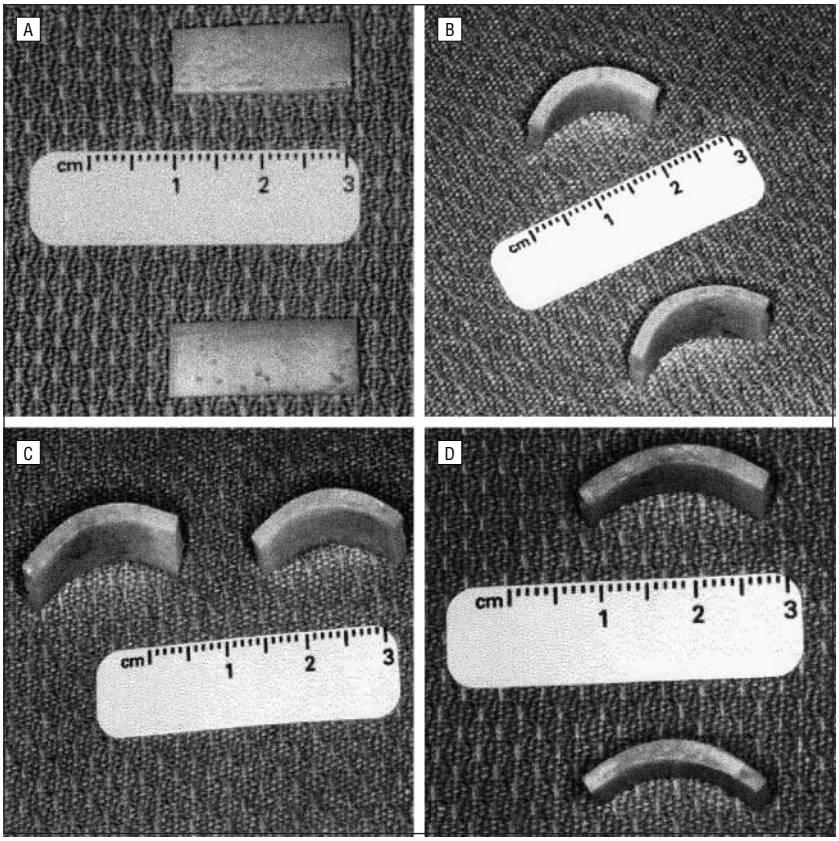

Figure 10. Photographs of cartilage specimens before (A), immediately after (B), 7 days after (C), and 14 days after (D) radiofrequency-mediated reshaping.

region 3 specimens (immediate digestion), when normalized to their matched controls, was 19%. The average viability for region 4 specimens (delayed digestion) normalized to controls was 14%. These finding are presented graphically in **Figure 11** and **Figure 12**. Rasouli et al[25] previously demonstrated that the cell viability in controls after this digestion process is 99% to 100%.

COMMENT

In this study, we demonstrated the feasibility of reshaping porcine nasal-septal cartilage using RF-generated heating. After heating and rehydration, the newly achieved shape is stable during the observed period of 14 days and cellular viability is maintained. The results of our study suggest that RF-based cartilage reshaping represents the use of a promising biomedical technology in a surgical application with potentially broad clinical utility. The optimization and refinement of RF technology for cartilage reshaping may ultimately achieve similar results as laser reshaping, only at a fraction of the present-day cost of medical lasers. The numerous potential applications of this procedure in plastic and reconstructive surgery represent an expansion of the armamentarium of techniques available to the surgeon. As observed in laser cartilage-reshaping studies, RF heating of cartilage results in changes in bulk properties of the material, causing stress relaxation and shape change. As heat alters matrix protein conformation, the biophysical properties of the tissue (eg, modulus and thermal conductivity) change. Changes in the tissue optical properties, in particular, are easily monitored using standard, diffuse light-scattering measurement techniques. In this study, characteristic minima in the intensity of transmitted light were observed with each RF energy application. These minima parallel the onset of accelerated stress relaxation and represent a correlate to previous laser-based cartilage reshaping studies that have shown a maximum in the backscattered laser light with the onset of stress relaxation.[15,20]

Sobol et al[7] have proposed that the matrix changes accompanying laser reshaping may be a result of several processes, including (1) a bound–to–free water transition in the matrix, (2) local mineralization of tissue caused by the interactions of free sodium or calcium ions with charged carboxyl or sulfate groups on proteoglycans, (3) local depolymerization of proteoglycan aggregates, (4) breaking of bonds between collagen and/or proteoglycan subunits, and (5) denaturation of the collagen framework.[7] The bound–to–free water transition may be responsible for the change in the light-scattering properties, as multiple isolated areas of water movement with anomalous refractive index values coalesce to contribute to an overall increase in backscattered light, or in our case, a decrease in diffusely transmitted light. As water bound to proteoglycans or collagen in the unheated cartilage is mobilized and migrates with heating, a concomitant exposure of charged moieties on the proteoglycans occurs. When the deformed cartilage cools and is rehydrated, formation of new weak bonds occurs, stabilizing the new shape of the cartilage.[7]

The molecular changes that result in change of cartilage shape likely occur without frank denaturation of the collagen or proteoglycan macromolecules within the matrix. Gaon and Wong[26] measured changes in the elas-

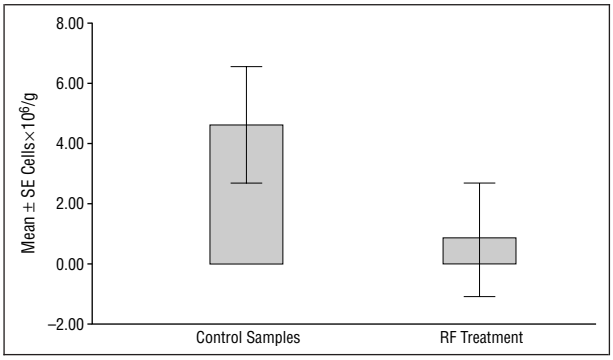

Figure 11. Graph depicting chondrocyte survival (live cells per gram) in 20 samples digested immediately after radiofrequency (RF) heating and normalized to matched control samples. Bars depict SE. Average survival was 19%.

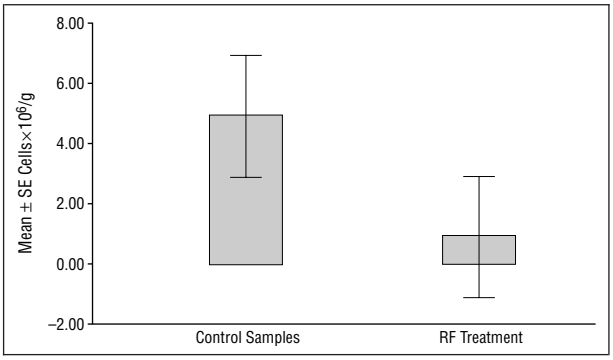

Figure 12. Graph depicting chondrocyte survival (live cells per gram) in the 20 samples digested after 7 days in cell culture following radiofrequency (RF) heating and normalized to matched control samples. Bars depict SE. Average survival was 14%.

tic modulus of cartilage before and after laser irradiation and noted a significant reduction in the modulus after heating. However, if specimens were allowed to rehydrate in isotonic sodium chloride solution for 15 minutes, the original mechanical properties of the specimen would return. In contrast, the modulus of specimens boiled for 60 minutes (total thermal denaturation) did not return to baseline values after rehydration. Similar findings were identified by Youn et al,[27] who observed reversible changes in cartilage birefringence after laser heating, using polarization-sensitive optical coherence tomography.

Radiofrequency cartilage reshaping is clearly a temperature-dependent process. The nonuniform temperature field generated during RF heating using the generator available in our laboratory precluded the use of simple thermopiles or thermocouples to estimate tissue temperatures. Hence, an infrared focal plane array was used to image radiometric temperature and generate images of the spatial distribution of temperature during heating. As expected, RF reshaping demonstrated features similar to those observed in laser reshaping. The surface temperature of the tissue reached a maximum of 88.6°C in less than 5 seconds, which is quite similar to measurements obtained during laser heating.[21] The maximum temperature in the recorded radiometric temperature image was in an oval area with its long axis parallel to and approximately midway between the 2 bipolar RF needles. A gradual diminution of temperature is seen on the image in proportion to distance from this maximum (Figure 10).

Although 2 commercially available RF generators used clinically for a wide range of procedures were used to heat cartilage specimens in this study, neither device is optimized for the generation of rapid uniform temperature profiles in tissue. The Somnus instrument was designed to produce volumetric heating in soft tissue for a prolonged interval, with thermocouples providing temperature estimates for microprocessor control. The Elmed device is used primarily for cutting soft tissue and generates a highly localized electric field. Neither instrument produces a temperature field that emulates that generated by a laser, and no attempt was made to optimize electrode design (given the power limitations of these instruments). Although the optimal temperature, time, and space parameters are not precisely known for laser or RF reshaping, several investigations have attempted to identify parameters that result in tissue shape change with reduced cell injury.[19,25] Despite the limitations of the present RF systems, results of measurements using the quantitative viability methods demonstrate that chondrocytes survive after RF cartilage reshaping.

The use of heat to reshape cartilage for aesthetic or reconstructive surgery may provide a less invasive method to alter tissue structure with reduced morbidity relative to classic open procedures. A laser-based approach is being used in Russia at present to correct nasal septal deviations. Dr Sobol's group has performed more than 150 minimally invasive laser septoplasties as office-based procedures using only local or topical anesthesia.[28] Laser energy is delivered transmucosally to mechanically deformed cartilage in situ to heat and reshape the tissue. In contrast to laser reshaping, in which heat generation depends primarily on the distribution of light in tissue, the initial thermal field produced by RF sources is determined by the spatial variation of the electric field and current density. To generate similar temperature fields using RF sources, electrode design and generator frequencies will need optimization.

Our measurements of chondrocyte viability after heating must be interpreted in light of the RF generator used in this study. The instrument parameters were selected on the basis of preliminary measurements of albumin (egg white) denaturation using bipolar RF electrodes.[29] In retrospect, these parameters may have led to overheating of the cartilage tissue, as the dielectric properties of albumin and cartilage differ substantially. Real-time optical monitoring during the heating could possibly have reduced thermal injury, but the logistics of doing both simultaneously were intractable with the present apparatus. Despite this potential of excessive heat generation, chondrocyte viability was maintained using the same instrument parameters that reshaped cartilage. Optimization of electrode geometry, generator frequency, power, and heating time would likely yield improved cell viability.

Although rapid spatially uniform heating of cartilage is thought to produce optimal results with respect to stress relaxation or reshaping and to viability, this was not accomplished using 2 needle-point electrodes as evidenced by the infrared focal plane array image (Figure

10). A solution to this problem may be to design a pair of electrodes with a predetermined shape that could deform the tissue like a mandrel or waffle iron and also deliver the RF energy. With this design and optimization of the instrument variables, more uniform and rapid heating of the cartilage specimen may be possible.

CONCLUSIONS

The results of our studies indicate that RF-mediated cartilage reshaping is a potentially useful procedure in plastic and reconstructive surgery. Full use of available donor site tissue with the ability to alter its shape will diminish the need for harvesting auricular and rib cartilage, along with the morbidities that accompany these procedures.

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From the Beckman Laser Institute (Drs Keefe, Rasouli, Telenkov, Karamzadeh, and Wong) and the Department of Biomedical Engineering, Henry Samuel School of Engineering (Dr Wong), University of California–Irvine; the Division of Facial Plastic Surgery, Department of Otolaryngology–Head and Neck Surgery, University of California–Irvine Medical Center, Orange (Drs Keefe, Karamzadeh, Crumley, and Wong); the Biomedical Engineering Program, Department of Electrical and Computer Engineering, The University of Texas at Austin (Drs Telenkov and Milner); and the Department of Surgery, Long Beach Veterans Administration Medical Center, Long Beach, Calif (Drs Keefe and Wong). The authors have no commercial or proprietary interest in the devices or equipment mentioned in this article, have no financial interest, and have received no payment as a consultant, reviewer, or evaluator of the same.

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Corresponding author and reprints: Brian J. F. Wong, MD, PhD, Beckman Laser Institute, University of California–Irvine, 1002 Health Sciences Rd E, Irvine, CA 92612 (e-mail: bjwong@uci.edu).

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