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Ex vivo investigations of laser auricular cartilage reshaping with carbon dioxide spray cooling in a rabbit model

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Abstract

Laser cartilage reshaping (LCR) with cryogen spray cooling is a promising modality for producing cartilage shape change while reducing cutaneous thermal injury. However, LCR in thicker tissues, such as auricular cartilage, requires higher laser power, thus increasing cooling requirements. To eliminate the risks of freeze injury characteristic of high cryogen spray pulse rates, a carbon dioxide (CO_2) spray, which evaporates rapidly from the skin, has been proposed as the cooling medium. This study aims to identify parameter sets which produce clinically significant reshaping while producing minimal skin thermal injury in LCR with CO_2 spray cooling in ex vivo rabbit auricular cartilage. Excised whole rabbit ears were mechanically deformed around a cylindrical jig and irradiated with a 1.45-μm wavelength diode laser (fluence 12–14 J/cm^2 per pulse, four to six pulse cycles per irradiation site, five to six irradiation sites per row for four rows on each sample) with concomitant application of CO_2 spray (pulse duration 33–85 ms) to the skin surface. Bend angle measurements were performed before and after irradiation, and the change quantified. Surface temperature distributions were measured during irradiation/cooling. Maximum skin surface temperature ranged between 49.0 to 97.6 °C following four heating/cooling cycles. Significant reshaping was achieved with all laser dosimetry values with a 50–70 °C difference noted between controls (no cooling) and irradiated ears. Increasing cooling pulse duration yielded progressively improved gross skin protection during irradiation. CO_2 spray cooling may potentially serve as an alternative to traditional cryogen spray cooling in LCR and may be the preferred cooling medium for thicker tissues. Future studies evaluating preclinical efficacy in an in vivo rabbit model are in progress.

Keywords

Facial plastic surgery; Macrotia; Carbon dioxide spray; Laser cartilage reshaping

Introduction

Cartilage is the principal structural element of the nasal, auricular, and laryngotracheal regions. Congenital, iatrogenic, inflammatory, and traumatic defects often damage head and neck cartilages, leaving significant functional and aesthetic consequences. An important example of these facial malformations is congenital deformities of the human ear.

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Approximately one in 5,000 children are born with auricular malformations. The most common deformity, the protuberant ear (macrotia), is associated with ridicule and teasing when children reach school age, and this abuse often leads to significant psychological distress and emotional trauma [1, 2]. Surgical otoplasty is the standard of care to correct this deformity. Classic open surgery, however, requires incisions to expose cartilage tissue and alter its shape. It is a technically challenging operation that produces variable results depending on the preferences, experience, and skills of the surgeon.

Alternative methods have been developed to provide non-surgical approaches to reshaping cartilage. Specifically, cartilage biomechanics is temperature-dependent, with temperatures between 50–70 °C producing stress relaxation which leads to gross shape change [3, 4]. Thus, thermal-based mechanisms, such as radiofrequency [5], electrical current [6–9], or lasers, have been investigated to produce shape change in cartilage in place of classic “cut and suture” methods. The main advantage afforded by these optical technologies is that cartilage reshaping may be achieved through an incision-free procedure with limited cutaneous injury and no associated risks and economic costs of surgery and anesthesia which translates to a shorter recovery time, reduced costs, and lower overall discomfort for the patient.

Laser-assisted cartilage reshaping (LCR) was first demonstrated by Helidonis et al. in 1993 [10]. In LCR, cartilage is mechanically deformed into a clinically desired shape using a simple jig or moulage and then irradiated with the laser in the regions of internal stress concentration, effecting permanent, quantifiable, and consistent shape change. Since its inception, LCR has been extensively studied in animal models [11–16] as well as in clinical studies involving human subjects [16–18]. Compared to reshaping the nasal septum and laryngotracheal region, LCR to reshape the auricle can be directly performed without the need for an endoscope or specialized surgical hardware and is thus more tractable as a starting step for development towards real clinical use as it repurposes hardware built and designed for laser dermatologic therapies. The main limitation of LCR in ears is controlling the evolution of heat which evolves radially and axially (in the direction of light propagation) in tissue.

One of the major achievements in making LCR a safer clinical treatment modality is the introduction of concomitant surface cooling which reduces skin temperature below injury threshold while allowing deeper tissue layers to be selectively heated. A number of cooling methods have been developed to modulate the spatial temperature distribution from laser irradiation. The best studied is the use of tetrafluoroethane (R134a) cryogen spray to cool the skin in spurts between laser pulses [19–23]. This method has been a gold standard in laser port-wine stain and tattoo removal as R134a has a low boiling point and absorbs a large amount of heat due to evaporation. However, due to extended evaporation times of residual cryogen, R134a has a tendency to cause frostbite when applied in large quantities (e.g., when thicker tissues such as ear, are irradiated at a high laser energy) [22]. R134a is also known to have a very high global warming potential [24]. In contrast, liquid carbondioxide (CO_2) is a promising alternative to address the problems associated with conventional cryogen spray cooling. Namely, liquid CO_2 does not leave residues on the skin which drastically reduces the risk of frostbite, has a very low global warming potential, and is less costly [24]. In this study, LCR with CO_2 spray cooling to reshape auricular tissue will be evaluated in an ex vivo rabbit model. The specific goal of this study is to identify candidate parameter sets that produce clinically significant reshaping while reducing skin thermal injury.

Materials and methods

Laser device and cooling setup

This study used ex vivo tissue and was performed in accord with the Institutional Animal Care and Use Committee at the University of California, Irvine. An investigative 1,450 nm wavelength diode laser with 6 mm spot diameter (Syneron/ Candela Corporation, Wyland, MA) was used to perform LCR. The laser was originally designed to use R134a cryogen as the cooling agent. To incorporate liquid CO_2, the existing cryogen lines could not be used since CO_2 spray requires delivery under greater pressure. A modified laser handpiece was used with a separate valve and nozzle connected to a 20 oz (approximately 591.5 ml) CO_2 aluminum paintball tank (Pursuit Marketing Inc, Santa Fe Springs, CA) through a high-pressure polyetheretherketone tubing line. This allowed for the CO_2 spray to be delivered under pressure based on similar user-determined R134a cooling parameters. Before each set of experiments, the CO_2 tank was filled to its full capacity, permitting roughly 200 sprays.

The CO_2 tank at room temperature does not deposit enough CO_2 to compare with the cooling effects of R134a. Therefore, to increase the deposition rate, the CO_2 tank was heated to approximately 29 °C, which increases the pressure to 1,022 PSI. A heating pad wrapped around the tank with a thermocouple and temperature controller (Omega Engineering, Stamford, CT) was used to maintain the temperature during experiments. This increased the pressure within the tank and thus in turn increased the deposition rate of CO_2 spray exiting the nozzle, generating an acceptable cooling effect. As the CO_2 contained within the canister is in a saturated state, the tank pressure remained steady at approximately 1,000 PSI as the tank emptied (i.e., until all liquid CO_2 is evaporated and no spray is expelled).

Tissue irradiation and cooling

The current experiments are based on a protocol previously developed by Holden et al. using a 1,450 nm wavelength diode laser [25]. Freshly sacrificed rabbit ears excised at the base of the auricle were obtained from a local abattoir. The ear cartilage tissue is comparable in quality to tissues used in previous ex vivo cartilage experiments [26, 27] and deemed to be in fair physiological condition. The ears were thoroughly shaved to remove the hair such that only hair follicles ends and the auricular skin were visible. Hair removal was necessary as excess hair could shield the skin from coming into contact with the CO_2 spray and diminishing the cooling effect.

The shaved ears were photographed exhaustively before treatment to record their native curvature from the longitudinal axis. Immediately after harvest and photography, the ears were then wrapped around a curved cylindrical, insulating polyvinyl chloride jig which contained 6 mm diameter perforations spaced 2 mm apart (roughly 9 mm between spot centers). A fiber optic light source inserted into the bore of the jig transilluminated the ear and aided in identifying the boundaries of the perforations which served to indicate the laser target sites on the ear surface (Fig. 1). Laser fluences of 12, 13, and 14 J/ cm^2 per cycle were delivered using a handpiece with a spacer approximating the laser spot. Irradiation and cooling were performed over the surface of the ear as it conformed to the shape of the cylindrical jig. Each laser cycle consisted of a pulse train of four laser pulses with duration of 52.5 ms. CO_2 spray was applied before the first laser pulse, between each subsequent laser pulse, and after the final laser pulse for a total of five cooling spurts [28]. The duration of the cooling spurts was varied as an independent variable, and these parameters were selected based on previous studies [28]. The laser system allows the user to define the combined duration of five cooling spurts within one cycle. Total cooling durations of 33, 35, 45, 55, 65, 75, and 85 ms were used (Fig. 2).

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Five rabbit ears were used for each laser dosimetry and cooling parameter set except for one group that consisted of four ears (13 J/cm^2, 35 ms). The irradiated area was centered on the midsection of the pinna and dosimetry was based on thickness. For thin areas (lateral, measuring 0.10–0.50 mm) of the ear, four pulse cycles per irradiation site was applied; for thick areas (medial, measuring 0.50–1.5 mm) six pulse cycles per irradiation site was applied. Four rows in the middle third section of each ear were irradiated, with each row consisting of five to six irradiation sites. Care was taken to not irradiate areas in proximity to large vessels. Photographs of both profile views of each ear were taken immediately after irradiation to record bend angle and skin surface injury. Graphical analysis software (NIH ImageJ, Bethesda, MD) was used to measure bend angle (from the longitudinal axis of the native ear when viewed laterally) before (i.e., natural curvature) and after irradiation. Signal factor analysis of variances (ANOVA) with a significance level of 0.05 was performed on bend angle data using Excel (Microsoft Corporation, Redmond, WA). Clinically significant reshaping is defined as any shape change that is statistically different from the control, native state.

Cutaneous thermal injury

Cutaneous thermal injury was assessed using a qualitative, visual rating scale. Both the skin surfaces directly irradiated as well as the side opposite irradiation was evaluated using this scale. Tissue samples showing no signs of thermal injury were rated as “no injury.” *Burns* were defined as areas of gross tissue damage, progressing from local skin discoloration to hair follicle destruction (in live animals, likely corresponding to erythema and alopecia, respectively) to significant charring. “Minimal injury” was defined as having occasional burns (some, but not all, irradiated sites) on some, but not all, treated ears. “Moderate injury” was defined as having occasional burns over all laser target sites on all treated ears. Finally, “severe injury” was defined as having consistent burns (all irradiated sites) on all treated ears.

Surface temperature

To record surface temperature distributions over each laser target site, tissue specimens were pinned against a flat cardboard backing with the laser handpiece’s height gauge, maintaining the trajectory of both laser and CO_2 spray perpendicular to the skin surface (Fig. 3). An infrared camera (InSb 3–5 μm Phoenix® DAS, FLIR Systems, Boston, MA) was calibrated with a black body (BB701, Omega Engineering, Stamford, CT) to detect temperatures between −18 and 149 °C and then used to measure radiometric temperature on the surface of the rabbit ear. The camera recorded videos at 2,000 frames per second on a rectangular area 4 by 8 mm centered on the spot of laser irradiation. The above laser fluences and cooling durations were tested. Graphical analysis software (NIH ImageJ, Bethesda, MD) was used to measure the average intensity of the irradiated spot in each frame of the video, and Excel was used to create a graph of intensity over time for each parameter. Using the calibration curve obtained from the black body, pixel intensity could be interpolated to a surface temperature estimate. Linear regression analysis with a least squares approach was performed in Excel to analyze the relationship between cooling spurt duration and surface temperature within similar laser fluences. In total, nine, seven, and 12 samples were assessed for laser fluences of 12, 13, and 14 J/cm^2, respectively.

Results

Cartilage reshaping

In accordance with prior findings, irradiation using the laser device produced clinically significant reshaping of the rabbit auricular cartilage with shape retention at 12, 13, and 14 J/cm^2 (Fig. 4). Furthermore, a reshaping effect, which increased with laser dosimetry, was identified. The average pre-treatment rabbit ear native bend angle was 22.5±5.8 ° from the longitudinal axis. The *change* in bend angle (the difference between bend angle following irradiation/cooling and native bend angle) achieved with 12, 13, and 14 J/cm^2 was 49.6±2.8 °, 55.4 ±2.3 °, and 65.6±2.5 °, respectively. The maximum change in bend angle was 71.0±5.5 ° and was achieved using 14 J/cm^2 and 45 ms cooling spurt duration. Single-factor ANOVA comparing different cooling durations with similar laser fluences revealed no statistically significant differences in bend angle for 12 J/cm^2 (p=0.42) and 14 J/cm^2 (p=0.46) but differences among those treated with 13 J/cm^2 (p=0.006).

Cutaneous thermal injury

Observations of tissue surface injury are summarized in Table 1. Overall thermal injury increases with a decrease in cooling spurt duration and an increase in laser fluence. No gross injury was observed at 12 J/cm^2, 45 ms; 12 J/cm^2, 55 ms; 13 J/cm^2, 55 ms; and 14 J/cm^2, 55 ms, suggesting that these dosimetry parameters are potentially promising for use in in vivo preclinical studies. Cooling pulses with duration above 55 ms appear to deposit a visible layer of frost, leaving the skin surface persistently cold; these cooling parameters may represent the threshold above which frostbite may occur and thus may not be clinically appropriate.

Surface temperature

The infrared camera recorded surface temperature distributions in real-time while pulses of laser light and cooling spray were delivered; a sample temperature history is shown in Fig. 5. The maximum surface temperature (T_m) consistently coincided with the surface temperature measured immediately following delivery of the final laser pulse. T_m ranged from 49.0 (12 J/cm^2, 85 ms) to 97.6 °C (14 J/cm^2, 25 ms) and correlated well with gross skin injury as determined by the injury scale. As expected, T_m decreased with increasing cooling pulse duration (R^2=0.80, 0.73, 0.71 with p=0.001, 0.01, and 0.0005 for 12, 13, and 14 J/cm^2, respectively) and decreasing laser fluence (Fig. 6).

Discussion

Currently, cryogen spray cooling is the gold standard in dermatology for achieving efficacious subdermal laser heating with minimal thermal injury to the superficial layers of the skin and can be readily adapted to LCR in ears. Up to this point, LCR studies have largely concentrated on applications of cartilage reshaping in the nasal septum and ear [16–18, 29], drawn from preclinical studies based on rabbit models [12, 14, 25, 26, 30, 31]. As composite structure of the human ear is considerably thicker than rabbit ear, LCR of human auricular cartilage has increased laser heat and cooling requirements. However, high cooling intensities using the R134a spray frequently results in cutaneous freeze injury similar to frostbite, possibly due to slow evaporation of residual cryogen on the skin surface [22]. A promising new alternative to surface cooling using R134a is CO_2 cooling [24]. In CO_2 cooling, liquid CO_2 is released from its storage canister through a solenoid valve and a nozzle. Since CO_2 cannot maintain a liquid state at atmosphere pressure, part of the liquid CO_2 changes to gas through flash boiling and part of the liquid CO_2 freezes to dry ice. A high-velocity mixture of dry ice and CO_2 gas is the final media for CO_2 cooling. The spray duration can be precisely controlled to milliseconds which enables selection of optimal cooling duration of the skin layer without affecting the temperature of the subsurface cartilage. There is no residual dry ice deposition on skin once the CO_2 spray is terminated because dry ice particles are immediately carried away by the gas phase, in contrast to cryogen residue forming on the skin surface during prolonged cooling and, oftentimes, resulting in freezing injury associated with conventional R134a cooling.

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Due to its volatile behavior, the CO_2 spray, which rapidly exits the nozzle in its evaporated gaseous phase, tends to cover a much larger area compared to conventional R134a cooling, which takes on a more globular, liquid phase. As a result, the CO_2 spray is expected to require higher deposition rates and volumes compared to the R134a spray although at a lower cost and producing much less environmental harm (e.g., global warming). For instance, previous work demonstrated that conventional R134a cooling can prevent skin injury at 33 ms cooling duration [25] while the present study showed a similar effect above 45 ms cooling duration with the CO_2 spray. Future challenges in optimization of the cooling apparatus will likely focus on improvement of the CO_2 spray design to produce consistent spray areas and maintenance of high flow and pressure.

In this study, measurement of surface temperature during simultaneous laser heating and cooling served as a rough proxy for the extent of skin protection afforded by the CO_2 spray. At heating rates typically achieved using a surgical laser (10–20 °C/s), the elastic modulus and shape of cartilage undergo significant changes when heating exceeds 60–70 °C [3, 32–34]. When applied directly to the skin, the same temperature can produce necrosis of the entire epidermal layer within seconds [35]. Thus, the goal of cooling is to minimize effects of skin heating due to absorption of laser energy in skin and heat diffusion from beneath the skin surface. This study found no gross skin injury (burn) or frostbite with 12 J/cm^2 and 45 ms cooling as well as with 12, 13, and 14 J/cm^2 and 55 ms cooling. These parameters corresponded to T_m of 68.1, 58.0, 67.8, and 71.6 °C, respectively. It is important to note that this is the maximum temperature reached (following the fourth heating/cooling cycle) and that the previous three cycles produced much lower surface temperatures (10–15 °C increase per cycle). It appears that, at least from this preliminary data, maintaining the surface temperature below 70 °C using this particular LCR setup with simultaneous CO_2 spray cooling can prevent gross thermal injury. There may be an additional interaction effect between the laser and highly dynamic cooling spray which may cool the surface by convective heat dispersal. A detailed assessment of the CO_2 spray cooling effect, including histologic analysis of the site of irradiation, is required, and this is planned for future studies. Furthermore, in a live rabbit in which there is an intact healing response and ear perfusion, parameters which result in minimal injury (12 J/cm^2, 35 ms, 13 J/cm^2, 35 ms, and 13 J/cm^2, 45 ms) may be clinically acceptable (e.g., blister formation) as the standard is, after all, incisions and sutures, and would likely naturally heal over time without consequence. Long-term in vivo studies are thus required to broaden the clinically applicable dosimetry space further and to verify this possibility.

In analyzing the reshaping effect, there were no statistically significant differences in bend angle for 12 J/cm^2 and for 14 J/ cm^2, but differences were present for 13 J/cm^2 as a result of the greater bend angles found with ears treated with 13 J/cm^2, 35 ms. The most probable explanation is that, as laser cartilage reshaping is a highly complex process, irradiation of the ear over numerous sites as in these experiments may alter the overall biomechanical properties of the ear, and thus its overall shape, in a relatively unpredictable manner, especially when taken with a relatively small sample size. ANOVA of the reshaping effect in all ears treated with 35 ms cooling regardless of laser power, however, revealed no statistically significant differences (p=0.08). In clinical applications, such unpredictability may be reduced through the use of custom-designed moulages that conform to the desired shape of the patient's ear to guide the healing process.

There are several additional important limitations to this study. First, skin injury was gauged based on a visual assessment rather than histology. With burns and frostbite injury, there may be limited correlation between clinical examination and findings identified by microscopy, and, these will evolve over time in an in vivo setting. Hopefully, future investigations, including histologic analysis of irradiated tissue, will address these

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discrepancies. Second, only surface temperature at the direct site of irradiation for the thin portion of each tissue specimen was assessed. To better characterize surface temperature evolution, future experiments will aim to measure T_m at the thick portion of cartilage (six heating/cooling cycles) as well as the surface on the side opposite to the incident laser irradiation. Third, there are discrepancies in initial surface temperature among different ears as detected by the infrared camera. This is likely due to differences in ear size and surface area (and therefore heat dispersion). One potential solution would be to allow the untreated ears to re-equilibrate in room temperature over time although this may be less practical as the total treatment time interval becomes long. The differences in initial surface temperature are relatively small, likely making differences in surface temperature measurements following LCR negligible. Finally, although estimates of surface temperature immediately following irradiation provide some information on the efficacy of CO_2 spray cooling, it may not correlate with surface temperature distributions in the perfused ear and the expected evolution of thermal injury (e.g., from burns to scarring to healing).

Ultimately, there is a need to evaluate these optimal parameters in a live animal model. Specifically, frostbite and the extent of thermal injury cannot be adequately evaluated without an intact inflammatory and healing response coupled to normal physiologic function (e.g., perfusion of the ear). Thus, the purpose of this ex vivo analysis was to reduce the potentially expansive dosimetry parameter space and facilitate experimentation in a resourceful and cost-effective, animal-centered manner. Laser dosimetry, cooling duration, number of treatment cycles, and area of treatment in LCR with surface cooling result in a highly intricate thermodynamic interaction, and further investigations, especially in live animals, are necessary as modeling these processes are exceptionally complex. Currently, in vivo evaluation of laser reshaping efficacy, whole-ear biomechanics, chondrocyte viability and histologic changes, and long-term healing and tissue remodeling are in progress.

Conclusion

From these preliminary results, CO_2 spray cooling appears to be effective in cooling the skin during LCR and can thus potentially serve as an alternative to traditional cryogen spray cooling, especially in thicker tissues. The results of this study will be used to guide in vivo preclinical experiments which aim to explore the true clinical value of this new cooling modality. Despite its current limitations, CO_2 spray cooling is a promising adjunct to the growing field of cartilage reshaping and part of a paradigm shift towards more environmentally friendly and cost-effective technologies.

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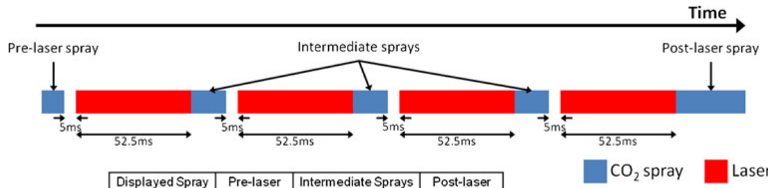
Fig. 1.

The rabbit ear is irradiated and cooled using a handpiece with spacer over transilluminated areas outlined by a cylindrical jig with numerous perforations and a fiber optic light source



Displayed Spray Duration (ms)	Pre-laser Spray (ms)	Intermediate Sprays (3 segments) (ms)	Post-laser Spray (ms)
33	6.5	5.33	10
35	10	5	10
45	10	8.33	10
55	10	11.25	11.25
65	10	13.75	13.75
75	10	16.3	16.3
85	10	15	30

Fig. 2.

Laser/cooling pulse trains per cycle used in the experiment. Note that the duration of each sub-pulse is constant (52.5 ms), while the cooling durations before, in between, and after heating are distributed based on a user-defined total cooling duration setting

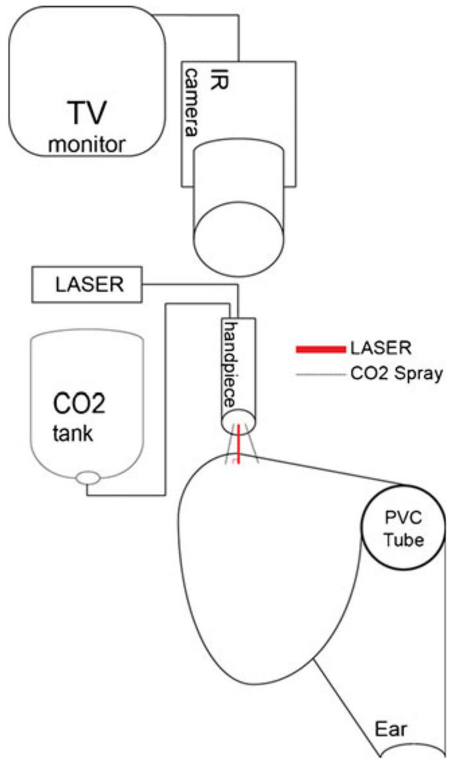


Fig. 3.

Schematic of laser/cooling spray and thermal camera setup for measuring surface temperature. The camera distance was adjusted to capture the entirety of the laser spot during irradiation. The laser and CO_2 tank is connected to a handpiece which irradiates/cools the tissue sample coiled over the cylindrical jig, while the infrared camera simultaneously records the surface spatial heat distribution

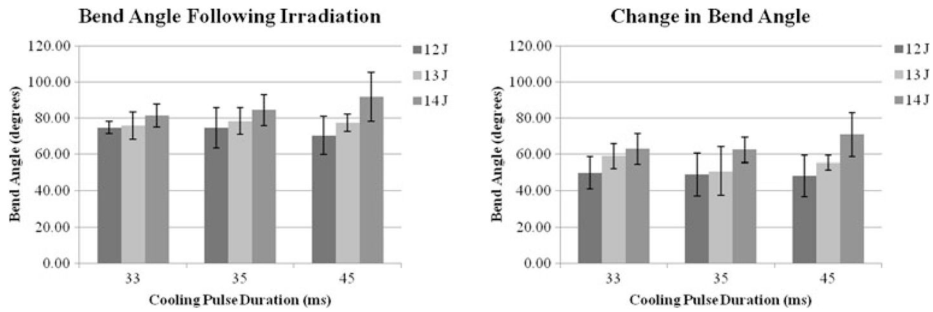


Fig. 4.

Shape change of rabbit auricular cartilage as measured by bend angle from longitudinal axis following laser irradiation (*left*) and change in bend angle from before to after laser irradiation (*right*). The *asterisk* (*) indicates differences in bend angle which were statistically significant by single-factor analysis of variances

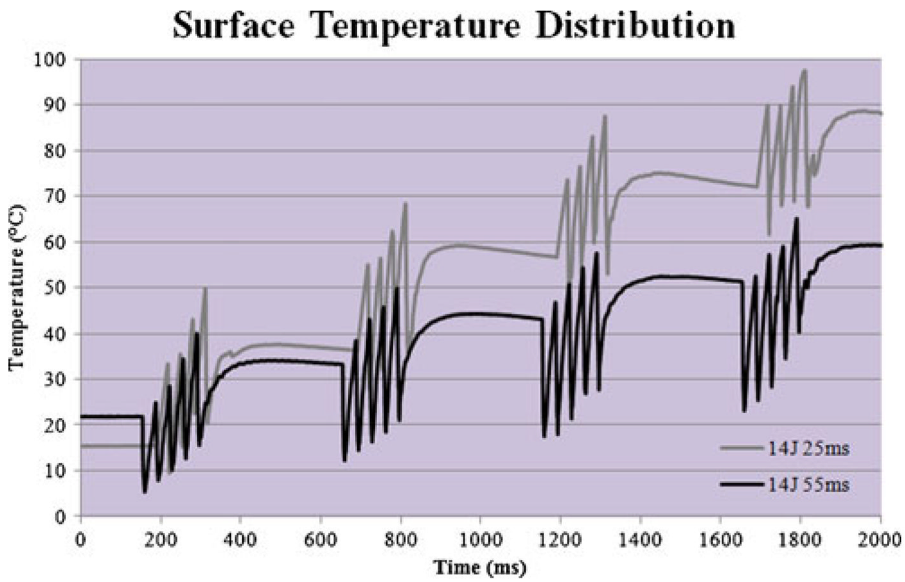


Fig. 5.

Sample surface temperature distribution (in degrees Celsius) as a function of time. Oscillating portions of the curve indicate alternations between cooling and laser heating phases of each cycle

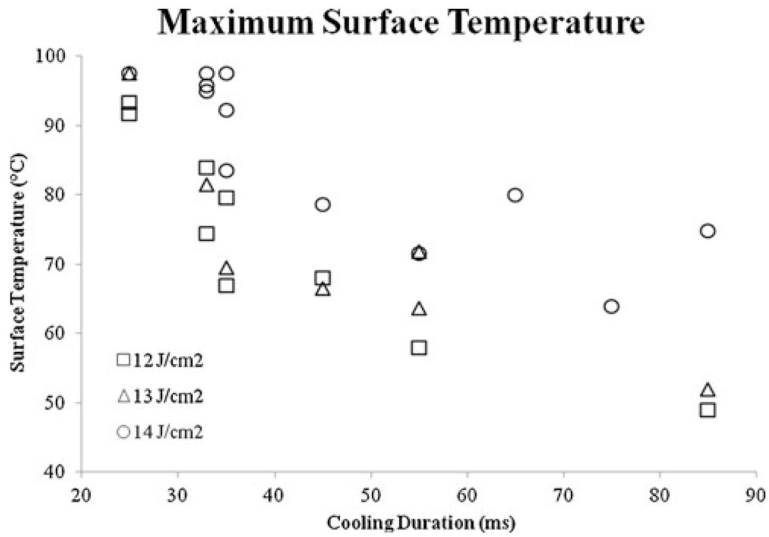


Fig. 6.

Maximum surface temperature produced by given user-defined laser and cooling parameters. Note that temperature decreases with decreasing laser fluence and increasing cooling duration; regression analyses of cooling duration with surface temperature were statistically significant for all heating parameters with $p<0.001$

Table 1

Auricular skin thermal injury following laser irradiation with carbon dioxide spray cooling

		Laser fluence (J/cm^2)		
		12	**13**	**14**
Cooling duration (ms)	33	Moderate direct	*Severe direct*	*Severe direct*
		No opposite	*Moderate opposite*	*Severe opposite*
	35	Minimal direct	Minimal direct	*Severe direct*
		No opposite	No opposite	*Severe opposite*
	45	***No injury***	Minimal direct	Severe direct
			No opposite	No opposite
	55	***No injury***	***No injury***	***No injury***
	65	**No injury**	**No injury**	**No injury**
	75	**No injury**	**No injury**	**No injury**
	85	**No injury**	**No injury**	**No injury**

Parameters in italics indicate known clinically impractical parameters (too much skin injury), while italicized and bolded parameters indicate potentially promising parameters. Parameters in bold only appear to produce no gross thermal injury but may potentially be associated with frostbite