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Minimizing Superficial Thermal Injury Using Bilateral Cryogen Spray Cooling During Laser Reshaping of Composite Cartilage Grafts

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Abstract

Composite cartilage grafts were excised from New Zealand rabbit ears. Flat composite grafts (of cartilage and overlying skin graft on both surfaces) were obtained from each ear and cut into a rectangle measuring 50 mm by 25 mm (x by y) with an average thickness of approximately 1.3 mm (z), skin included. Specimens were manually deformed with a jig and maintained in this new position during laser illumination. The composite cartilage grafts were illuminated on the concave surface with an Nd:YAG laser (1,064 nm, 3 mm spot) at 10 W, 20 W, 30 W, 40 W, 50 W. Cryogen spray cooling (CSC) was applied to both exterior (convex) and interior (concave) surfaces of the tissue to reduce thermal injury to the grafts. CSC was delivered: (1) in controlled applications (cryogen released when surface reached 40°C, and (2) receiving only laser at above wattage, no CSC [representing the control group]. The specimens were maintained in a deformation for 15 minutes after illumination and serially examined for 14 days. The control group with no CSC caused injury to all specimens, ranging from minor to full thickness epidermal thermal injury. Although most levels of laser and CSC yielded a high degree of reshaping over an acute time period, after 14 days specimens exposed to 30 W, 40 W, 50 W retained shape better than those treated at 10 W and 20 W. The specimens exposed to 50 W with controlled CSC retained its new shape to the highest degree over all others, and thermal injury was minimal. In conclusion, combinations of laser and CSC parameters were effective and practical for the reshaping of composite cartilage grafts. *Lasers Surg.*

Keywords

rabbit ear; cryogen spray cooling; laser; reshaping; thermal injury

INTRODUCTION

The *lop ear deformity* is a congenital malformation of the ear where the anti-helical fold is absent. This is caused by a defect embryogenesis during the 12th to 16th week of gestation, resulting in the ears protruding prominently. Children with this deformity are often subjected to ridicule. Currently, the deformity is treated using techniques that require incisions and sutures to recreate the natural anatomy of an anti-helical fold and balance the intrinsic forces within cartilage tissue that resist deformation.

Laser-assisted reshaping of cartilage may be an alternative to these conventional techniques without the attendant morbidity of surgery [1–4]. The main advantage of using a multimode optical fiber to illuminate and generate heat in cartilage is the ability to control the space-time temperature distribution and the time-dependent thermal denaturalization kinetics, resulting in reduced cellular injury that would be experienced by using other laser treatments such as laser illumination [5]. Several studies have investigated laser-assisted reshaping of ear cartilage in porcine and rabbit ear [6–10]. While successful reshaping has been demonstrated, nonspecific thermal injury to the superficial layers continues to be a problem [11]. Moreover, the composite tissue of the ear is thin. This entails a broad penetration of laser energy deep into the tissue such that the superficial layers first penetrated by the laser are not the only layers affected. Laser energy applied directly to a concave surface continues through the skin's exterior, the cartilage in between, and finally through to the convex side of the specimen's skin.

Protection of both surfaces of skin is clinically important, however protection of the exterior skin surface is especially aesthetically important. This study approaches this problem of superficial thermal injury by using a cryogen spray cooling (CSC) system. CSC involves atomized delivery of cryogen onto the surface of the skin 40–120 milliseconds before laser illumination. With the use of CSC, cryogen spray is released over the illuminated region when the detected surface temperature reaches a user-defined value. The cryogen evaporates from the skin, drawing heat from the epidermis, and with laser heating this allows for the spatially selective thermal treatment of tissue [12–15]. The objectives of our study is to employ CSC delivery to the interior and exterior surface of the epidermis of the composite ear grafts while directly laser illuminating the exterior side.

MATERIALS AND METHODS

Tissue Preparation

Auricles from 30 (60 individual specimens) freshly euthanized New Zealand white rabbits were obtained from an animal facility at Chang Gung Memorial Hospital (Taipei, Taiwan) in accord with the regulations of animal studies of Chang Gung University and the University of California, Irvine. Specimens were excised from the mid-lateral portion of the

auricle (Fig. 1). One composite graft (cartilage and overlying skin graft on both surfaces) was obtained from each ear and cut into a rectangle measuring 50 mm by 25 mm (x by y) with an average thickness of approximately 1.3 mm (z), skin included. To minimize dessication, all experiments were performed within 30 minutes of specimen harvest.

Laser Systems

Tissue specimens were illuminated with light from an Nd:YAG laser ($\gamma = 1.064$ mm, Sharplan lasers, Allendale, NJ). The light was delivered by a 400 mm core diameter multimode optical fiber. Spot size (~ 3 mm) was estimated using burn paper (Zap-It, Kentek, Pitts field, NH). The fiber was secured to a vertically oriented caliper for increased accuracy and adjusted to a set-point of 0 mm). The specimen was bent into a new shape (Fig. 2) using a vice that served as a jig. The interior side of the specimen formed the inner portion of the bend and its exterior side formed the outer portion. The distance between the two plates of the jig was 7 mm. The fiber delivered laser energy to the composite cartilage grafts on the inner sides of the bend. This method of bending the cartilage was selected, as it is analogous to creating the anti-helical curve in a human ear. The concave side was illuminated at ten different sites with the centers of each laser spot separated from one another by 2 mm. This resulted in an overlap of approximately 20%. The center of the first spot was oriented 3.5 mm away from the upper edge of the specimen, which was also the case for the final spot with respect to the lower edge of the specimen.

A broad range of laser parameters was systematically evaluated and a range of parameters that caused composite cartilage reshaping was determined. Laser powers of 10 W, 20 W, 30 W, 40 W, and 50 W were selected for this project as they produced the most striking results exhibiting shape change in relation to thermal injury, relative to the use and absence of CSC.

Cryogen Delivery

Cryogen Spray Cooling (CSC) was applied to both the interior (concave) and exterior (convex) portions of the bent specimens using a solenoid valve to protect the superficial tissues from excess heating. The distance from the valve orifice to the cartilage was maintained at 30 mm (Fig. 2). Of the 60 experimental specimens, Cryogen R134a (1,1,1,2-tetrafluoroethane BP = 26.2°C) delivery occurred in the following manner:

1. Controlled CSC: Cryogen being manually released when surface temperature on the outer portion of the bend reached a user-specified set-point (40°C) [16–18]. Coolant was delivered at 50–70 ml/second spurts and was ceased at 408C thermocouple reading. Ten specimens for each power level constituted a group in itself.
2. Without CSC: No cryogen applied. Laser solely used to control specimens, which consisted of the remaining 10 composite cartilage grafts.

Peak temperature was recorded from the 10 illuminated spots for each parameter pair using a double thermocouple and monitor [19]. One thermocouple was placed in contact with the surface of the concave side along with the second thermocouple that was placed at the convex side of the specimens at a site corresponding to that opposite of illumination. The temperature of both sides of the composite specimens was measured. Duration of laser

illumination spurts were conducted based on the temperature maintained in the specimens, and to keep the 40-degree constant, observation of temperature shown on thermocouples allowed administrators to determine how long laser illumination lasted on each specimen, which was no more than 5 seconds. While repeatedly illuminating tissue specimens with a laser in short time intervals, it was important to allow adequate cooling time in between each exposure—laser illumination dehydrates tissue, making it less flexible and brittle. In this study, a non-contact infrared temperature detector (Exergen Microscanner D1001, Watertown, MA) was used to confirm the return to ambient temperature.

Cartilage Reshaping

Initially, tissue specimens assumed a flat shape with a deformation angle of 180°. These flat specimens were manually deformed by the jig where the grafts were essentially folded in a “U” configuration with one half directly on top of the other half. This resulted in a deformation angle of 0°. They were maintained in this shape during illumination [20–23]. Following illumination, specimens were maintained in their deformations for only 15 minutes, at which time they were removed from the jig. Specimens were serially examined at 15 minutes, 30 minutes, 24 hours, and 14 days. Calculations of angle deformation observed among each specimen within every group were consolidated to form an average mean value to represent each illumination category. This mean value was configured by use of a Scale Graduated (cat# 187–201, Mitutoyo, Kawasaki, Japan) Deformation angle was defined as the angle between the two halves of the fold measured from a lateral point of view: 180° = flat, 0° = U-configuration in jig. A smaller angle indicated more reshaping. Then specimens were visually inspected and photographed to document shape change and acute thermal damage. Specimens were kept in their isolated Petri dishes with saline dampened gauze strips and preserved at 4°C, to prevent decomposition of specimens, between recorded time slots. Upon removal, each specimen underwent a series of recordings based on particular medical stain processes—Hematoxylin-Eosin stain, Safranin-O stain, and Acinic Blue stain in order to ensure the angulation of cartilage remained intact.

RESULTS

Surface Temperature Measurements

Surface temperature was measured during Nd:YAG laser illumination and peak surface temperature was recorded from each of the illuminated spots for each group of parameters. Again, the thermocouples recorded measurements from the surface of both sides of the skin. Note that at 10 W without CSC application, obvious and immediate full thickness injury through cartilage—with carbonization—was observed within 5 seconds around the perimeter of the laser spot.

Reshaping

Specimens were serially examined and photographed from a lateral view to record shape change at 15 minutes, 30 minutes, 24 hours, and 14 days after laser reshaping. Prior to illumination, specimens were flat (deformation angle of 180°) when deformed by the jig into a U-configuration, which thus served as the deformation 0° angle for recording. Specimens were then illuminated and left to remain in the jig for 15 minutes, at which time they were

removed and immediately inspected and photographed. Results demonstrated that following the removal of a specimen from the jig, the deformation angle increased regardless of dosimetry or cryogen application. With increasing elapsed time the deformation angle increased. The time and angle at which the increase reached a limit was unable to be determined within the boundaries of this study.

For the 10 W- and 20 W-specimen groups, no more than 135° reshaping had occurred and obtained major restructuring after the projected times, nor significantly persisted throughout the 14-day time interval. This led to the conclusion that a higher wattage was to be administered for more dramatic and consistent reshaping. Specimens that received 30 W and above showed more dramatic deformation with limited skin destruction. For those receiving 30 W, an approximate 115° deformation was observed after 15 and 30 minutes. After a 24-hour period, the reshaping was recorded at about 130° after 14 days. Specimens undergoing 40 W showed a 90° reshaping after both 15 and 30 minutes. Following observation after 24 hours, those 40 W-specimens displayed a 100° reshaping and remained approximately the same at 105° after 14 days. Optimal reshaping that occurred and prolonged throughout the 14-day experimental observation period was obtained by the 50 W-specimens. A calculated $90.10 \pm 0.05^\circ$ and $91.50 \pm 2.51^\circ$ deformation was observed after 15 and 30 minutes, and with a minimal retraction of $99.70 \pm 1.89^\circ$ after 24 hours and 14 days, making a final recording of $100.30 \pm 2.78^\circ$ deformation obtained by the 50 W-specimens (Fig. 3). Deformation figures and calculations were recorded and provided in Table 1.

Thermal Injury

Thermal injury was evaluated by inspection of the tissue noting color change, epithelial slough, and frank carbonization—all recorded with a digital camera. Texture was evaluated qualitatively by touch. In general, obvious thermal injury was not observed for specimens illuminated with CSC. Control group specimens treated without CSC were the most damaged. Nearly all specimens that solely received laser treatment displayed thermal damage. Carbonization in the line of illumination was noted in all specimens. At the time of the study, texture was rough and dry with a central depression center.

Specimens treated with controlled delivery of CSC at low wattage (10 W and 20 W) demonstrated minor changes in coloration. These specimens that received controlled delivery of CSC produced no obvious change in the appearance of the superficial epidermis in neither the convex nor concave cartilage surface when compared with the control specimens. Specimens that were treated with CSC on both convex and concave surfaces showed thermal damage proportional to laser wattage, namely 30 W specimens showed visible discoloration without burning, 40 W-specimens increased discoloration without burning, etc. Texture of the skin surfaces of specimens in the 30 W, 40 W, and 50 W-groups were also observed to have shown slight alteration, yet no singing appearance by illumination (Fig. 4). Conclusively, all specimens in the control revealed carbonization as a result of thermal damage.

DISCUSSION

Cartilage possessed considerable shape memory as demonstrated in preliminary studies where continued placement of a cartilage specimen in a jig following illumination increased shape retention when compared to specimens that were immediately removed from their jigs. These previous studies that focused on the usage of controlled CSC reshaping of cartilage to produce fixed results as opposed to continuous CSC application can support the development of the experiment at hand [10]. Statistical analysis of this study is presented by listing power settings in relation to time. Lower power settings, 10 W and 20 W, with continuous CSC produced no significant changes, as degree angle of reshaping increased over the 14-day observation period. Higher wattage produced significant cartilage reshaping and allowed considerable shape memory to persist without severe external damage. In accordance to the normal angulation degree/measurement of the antihelix, our study was attuned to achieving those results with the 50 W-test group [24]. Best results were obtained and shown in specimens receiving 50 W with controlled CSC, where 100° reshaping was achieved and recorded after 14 days. With these results, a non-invasive method of cartilage reshaping was achieved, along with minimal observed thermal damage. Depending on the subject, in regards to thermal damage as a result of laser application, recovery can occur in vivo over a period of time—naturally in accordance to the type of treatment. The minimal thermal damage observed in this study is believed by the study's conductors to surpass over time. As opposed to conventional surgical reshaping methods that are known to leave scarring and/or permanent treatment markings, controlled CSC for laser treatment can be administered for reshaping composite tissues/cartilage. Nonetheless, the study holds the recorded degrees of reshaping 2 weeks after procedures and further study on the prolonging of cartilage reshaping should follow.

Most cutaneous CSC laser treatments are performed with the objective of generating heat below the specimen surface, with the superficial layers maintained at a safe temperature by the CSC delivery. Usually for these cutaneous applications, both laser and CSC are delivered to the same surface of the target tissue [16,25–31]. The study at hand used a novel approach in that CSC was applied to cool the graft on both sides of the specimens to where laser energy was to be delivered. In this arrangement, excess damage due to overheating was avoided in the superficial tissues on both concave and convex surfaces of the specimens. CSC application to both the convex and concave surfaces of the specimens allowed the laser illumination to more effectively heat and reshape the cartilage since it escaped the over-cooling interference of CSC delivery. Graft viability was optimized by preserving both layers of the skin; one was incised and reapproximated after illumination, while both were protected with CSC in the non-control groups. However, excess cryogen delivery may prevent the cartilage from reaching adequate temperatures for shape change and must specifically depend on preparation, primarily balancing, factors: (1) heat-generation produced by a laser with evaporative cooling and (2) the heat-sink effect created by CSC which was a challenge and required both careful experimentation and mathematical modeling to optimize.

Higher powers were executed for achieving optimal reshaping because the specimens were so thin, and profound cooling was applied and occurred throughout its thickness. This is

suggested by the data showing the use of “controlled CSC delivery” in this study. CSC delivery was set to reach levels so high as to have prevented the cartilage from reaching adequate temperatures to result in shape change. These high levels of CSC made the addition of more laser power feasible in an attempt to reshape the cartilage beneath. The time and angle at which the increase reaches a limit is unknown within the boundaries of this study. An improvement to this study would include extending the long-term observation time of the specimens to allow investigation into when, if at all, the deformation angle becomes fixed into its final and permanent position. This would aid in ruling out the possibility of complete reversion to original shape when enough time is allowed to elapse.

This study utilized two thermocouples and a monitor to measure the high temperatures reached during illumination of both surfaces. For future studies it is recommended that more be developed with the use of a thermopile instead of a thermocouple when studying similar cases. Observation was made during experimentation that the thermo-couple would measure the temperature of actual laser beam at times. Placement of the thermocouple on both concave and convex surfaces of specimens corresponding to the site of illumination theoretically places the wires only directly in the laser line of fire. Although the temperature tended to agree with the results of the study, they could theoretically have been due to the direct heat of the laser energy alone, not the heating of the actual tissue. The structure of a thermopile could eliminate this problem as the use of a series of connected thermocouples would fully log the temperature of entire surface of specimen. A thermopile is highly recommended for future study and experimentation. Future studies include histology of ex vivo specimens under various laser and CSC parameters. At different wattage of laser application, specimens treated with different modes of CSC will be compared. One anticipated observation is that high laser power creates a drastic decrease in tissue viability.

CONCLUSION

This project aimed to explore combinations of laser and CSC parameters that were unlikely to cause permanent injury in an ex vivo model while still being effective and practical for the reshaping of cartilage. Direct high-power laser illumination of cartilage with CSC delivery to both sides of the superficial surfaces allowed rapid heating while minimizing injurious temperature elevations on both sides of the superficial epidermis. Specimens that underwent 50 W with controlled CSC were found to produce significant reshaping with a minimal amount of superficial thermal injury. As ex vivo specimens were administered under the experiment's storage environment of 4°C, future in vivo subjects to undergo like procedures will be treated and protected under those regulations that are to be developed at that time. This study's parameters will be used as a starting point for future in vivo studies, in which subjects will be closely monitored and recorded for reaction of treatment that may stem further research and application to clinical patients.

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Fig. 1.
A composite cartilage graft (50 mm×25 mm×1.3 mm) was excised from the mid-lateral portion of the rabbit auricle, skin included.

Fig. 2.

The device used with the jig to (1) keep the shape of cartilage in specimens and (2) obtain double-sided cooling during laser illumination.

Fig. 3.

A 50 W-specimen shows to have produced the ultimate favorable deformation angle that measured 90° after the initial elapsed time slot of 15 minutes. Further, at same wattage after 30 minutes post-illumination, the deformation angle remained at 90° . After 24 hours, a small deformation angle of approximately 100° was measured. Deformation angle was maintained at about 100° and nonetheless produced a favorable angle, as with other specimens of this group, that closely measured to that calculation throughout the remaining 14 days.

Fig. 4. The histopathological reshaping of composite cartilage grafts using a Hematoxylin-Eosin stain (above), Alcian Blue stain (center), and a Safranin O stain (bottom) was utilized to display the resulting exterior angulation after 14 days with the experimental 50 W-group. No significant thermal damage to internal and external skin due to illumination was recorded as a result of laser treatment.

TABLE 1

Laser Reshaping Listed in Degrees of Composite Cartilage Grafts Under Controlled CSC in Various Parameters

Power	15 minutes	30 minutes	24 hours	14 days
10 W (n = 10)	145.6 $\pm$ 2.7	146.0 $\pm$ 2.8	150.5 $\pm$ 2.4	154.1 $\pm$ 3.1
20 W (n = 10)	135.7 $\pm$ 3	136.1 $\pm$ 2.9	140.2 $\pm$ 3.7	141.6 $\pm$ 3
30 W (n = 10)	115.0 $\pm$ 2.9	115.4 $\pm$ 2.4	122.9 $\pm$ 4.0	130.2 $\pm$ 3.3
40 W (n = 10)	97.3 $\pm$ 2.9	98.5 $\pm$ 1.7	100.8 $\pm$ 2.3	105.0 $\pm$ 3.4
50 W (n = 10)	90.1 $\pm$ 2.1	91.5 $\pm$ 2.5	99.7 $\pm$ 1.9	100 $\pm$ 2.8