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Minimally Invasive Ear Reshaping With a 1450-nm Diode Laser Using Cryogen Spray Cooling in New Zealand White Rabbits

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Abstract

Background—Otoplasty is the current standard of care for treating prominent ears, a psychologically and sometimes functionally disabling disorder. The technically demanding procedure carries many risks such as poor aesthetic outcome, need for revision surgery, and need for general anesthesia. This study investigates the use of laser irradiation combined with cryogen skin cooling and stenting to reshape cartilage in the ears of New Zealand white rabbits.

Methods—In this prospective, randomized, internally controlled animal study, the right ears of 9 rabbits were mechanically deformed with a jig and then irradiated with a 1450-nm diode laser combined with cryogen skin cooling (14 J/pulse with cryogen spray for 33 milliseconds per cycle and a 6-mm spot size). The left ear served as the control. The ears were splinted for 1, 3, or 4 weeks. The rabbits were then given a lethal dose of intravenous pentobarbital, and the splints were removed and ears examined and photographed. Light and confocal microscopy were performed on the specimens.

Results—Shape change was observed in all 9 treated rabbit ears, while none of the control ears (stenting alone) showed significant change. Qualitatively, reshaped ears were stiffer after 4 weeks of splinting than after 1 or 3 weeks. None of the rabbits showed evidence of skin injury nor did they show signs of postprocedural pain. Findings from histologic analysis in the treated areas showed evidence of an expanded chondrocyte population in the region of laser irradiation, along with some perichondrial thickening and some fibrosis of the deep dermis. Confocal microscopy revealed minimal cellular death at 1 week and none thereafter.

Conclusions—Cartilage reshaping using laser energy can be performed safely transcutaneously using cryogen spray cooling in rabbits. This animal model has similarity to human ears with

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regard to skin and cartilage thickness and is a stepping stone toward developing minimally invasive laser auricle reshaping in humans.

Laser cartilage reshaping (LCR) has been an area of interest since it was first reported by Helidonis et al¹ in 1993. Since that time, numerous studies have demonstrated successful cartilage shape change with various lasers in the rabbit and porcine models.²⁻⁵ However, developing this technology for use in humans remains a challenge because optimal dosimetry is difficult to ascertain, and the efficacy of the procedures relies on spatially selective heating of the tissue.

Clinical applications of LCR have focused on correcting septal deformities⁶ and protuberant ears.⁷ Septal reshaping relies on the surgeon to first deform the deviation using a modified speculum and then target regions of increased internal stress with a laser. No attempt is made to spare the mucoperichondrium from thermal injury because the laser irradiation spots are small, heal well, and are not visible. Re-shaping the auricle using LCR presents a significant challenge because laser energy must be delivered either transcutaneously (with risk of skin injury) or via a series of incisions (negating the advantages of LCR over classic otoplasty operations). Fortunately, protecting the epidermis from injury during laser irradiation is well established in dermatology and generally involves the use of cooling devices, mechanisms, and/or agents that are applied to the tissue surface milliseconds before laser irradiation. Early investigations by our group demonstrated the efficacy of using cryogen spray cooling in particular to protect the epidermis during laser reshaping of composite auricular cartilage grafts.⁸ We demonstrated that the prudent selection of laser and cryogen dosimetry could lead to the establishment of a significant subsurface temperature elevation at the level of the cartilage in the rabbit ear. Later, Mordon et al⁹ demonstrated effective shape change in rabbit ears using an erbium:glass laser with a chilled sapphire window contact cooling system. Shortly thereafter, Trelles and Mordon⁷ reported the successful use of this same laser system in correcting protuberant ears in 8 patients and demonstrated stability of results for up to 6 months of follow-up. Whereas Mordon's group used contact cooling as a means to protect the skin, we have focused our efforts on the use of cryogen spray cooling (CSC) and, in the present study, demonstrate the efficacy of a 1450-nm diode laser with CSC to heat auricular cartilage and thus reshape rabbit ears in vivo.

METHODS

The protocol was approved and performed under the aegis of the Institutional Animal Care and Use Committee (IACUC) at the University of California, Irvine. We maintained strict adherence to IACUC rules and guidelines throughout the duration of this study.

Nine New Zealand white rabbits (Western Oregon Rabbit Company, Philomath, Oregon) weighing approximately 5kg were anesthetized with ketamine hydrochloride (4-5 mg/kg) and xylazine (0.2 mg/kg) given intramuscularly. The ears were then shaved closely to remove as much hair as possible. The right ear was placed over a hollow cylindrical jig with an 8×10 array of 6-mm-diameter perforations. A light source was placed in the jig, and when the ear was wrapped around it, the perforations transilluminated the ear, providing a guide to target laser administration.⁹ A clinical 1450-nm diode laser with CSC (Candela Smooth-Beam with 6-mm-spot diameter; Candela Corp, Wyland, Massachusetts) was used to irradiate the specimen. The initial dosimetry parameters were based on pilot investigations that used ex vivo auricles obtained from the crania of rabbits killed for other protocols. This work tested the upper limits of the laser without causing significant skin injury on the ex vivo ears. To minimize the risk of injury, the laser dosimetry was set at 14 J/pulse with cryogen spray for 33 ms/cycle. Laser spots were separated from one another by 2

mm, and a total of 4 rows of 5 to 6 spots (depending on ear size) were placed on the midportion of each ear (**Figure 1**). A reference suture corresponding to a notch on the jig was placed on each treated ear to allow identification of each laser spot at a later date and to maintain registry with histologic sectioning. Six cycles (at a rate of 1 Hz) were administered on the more medial (thicker) aspect of the ear, while 4 cycles were used on the thinner more lateral portions.

Following laser treatment, both ears were then splinted over a gauze roll (approximate diameter of 1cm) and sutured into place using two 3-0 nylon sutures in horizontal mattress fashion (**Figure 2**). The contralateral ear was not treated with the laser but was, however, shaved, prepped, and splinted in an identical fashion. The procedure lasted approximately 10 to 15 minutes per rabbit, and no rabbits required intubation or additional doses of anesthetic agents. An Elizabethan collar was placed, and each animal was allowed to recover in the operating room while connected to cardiopulmonary monitors. The 9 rabbits were then randomly assigned to 3 groups that were splinted for either 1, 3, or 4 weeks.

Postoperatively, the rabbits were observed on a daily basis to ensure the splints were in place. No signs of thermal injury to the skin or superficial infection were observed. All rabbits demonstrated appropriate levels of activity without signs of pain or agitation, and the splints remained intact for the designated treatment times (**Figure 3**).

At the end of each designated splinting period, the splints were removed and the ears photographed. Each animal was given a lethal dose of intravenous pentobarbital, and the auricles were harvested from the treated and untreated ears for further photography, measurement, and histologic analysis.

Analysis of the degree of deformation of each ear was measured using photo analysis software (Photoshop CS3, version 10.0; Adobe Systems Inc, San Jose, California). For conventional light microscopy, a segment of specimen incorporating a laser irradiation site was fixed in formalin for 24 hours, dehydrated via serial ethanol immersions, and then embedded in paraffin. Blocks were then sectioned with a microtome and stained with hematoxylin-eosin. Slides were examined for evidence of (1) thermal injury to skin and cartilage; (2) chondrocyte proliferation, and (3) overall thickness of the matrix.

For chondrocyte viability analysis, the skin and perichondrium were carefully dissected off the fresh cartilage specimens, which was then analyzed using the LIVE/DEAD assay system and confocal microscopy as previously described.^{10,11} The LIVE/DEAD assay (Molecular Probes Inc, Eugene, Oregon) uses 2 dyes, calcein-AM, and ethidium homodimer-1. Calcein-AM emits 517-nm (green) light when excited by light at 494 nm. It is a membrane permeable esterase substrate that diffuses into the cytoplasm and is hydrolyzed, after which it becomes unable to cross an intact plasma membrane, therefore becoming trapped inside LIVE/viable cells. Ethidium homodimer-1 has a high affinity for nucleic acids; however, it is unable to cross an intact cell membrane. Therefore, it concentrates predominantly inside DEAD/nonviable cells with porous cell membranes. Ethidium homodimer-1 emits 617-nm (red) light when excited by light at 528 nm.

Specimens were cut in serial cross-section with a razor blade and prepared under minimal lighting conditions because the aforementioned dyes are photoreactive. Both dyes were mixed according to manufacturer instructions and added to each specimen in a micro-vial and kept in a light-free environment for precisely 30 minutes. The specimens were then washed with phosphate-buffered saline, transported in a light-free box, and immediately imaged using a confocal scanning laser microscope (Meta 510; Carl Zeiss LSM, Peabody, Massachusetts).

RESULTS

Each rabbit tolerated the surgery and recovery without mishap or incident. The treated ears splinted for 1 week demonstrated a bend averaging 86° (**Figure 4A**). The 3- and 4-week groups showed significant shape change as well, averaging 106° and 75°, respectively (**Figure 4B and C**). There was not a statistically significant difference between these groups; however, qualitatively, the ears splinted for 4 weeks were obviously stiffer in response to simple flexure than the specimens splinted for shorter intervals. The mean bend angle across all treatment groups was 89° (99.5% confidence interval, 63°-115°). This represented approximately 30% to 50% unfurling of the ear from its splinted shape. None of the control ears demonstrated shape change, and all returned to their native shape immediately after splint removal.

Findings from a histologic analysis of the 3- and 4-week splinted specimens showed that treated ears had a substantially expanded chondrocyte population along with increased thickness of the cartilage at the sites of laser irradiation. The perichondrium was also thickened and accompanied by adjacent fibrosis in the deep layers of the dermis (**Figure 5A**). The specimens splinted for only 1 week did not show significant histologic findings other than shape change. The control ears showed no significant abnormalities or changes from native tissue (**Figure 5B**).

Confocal microscopy was performed on all groups. The specimens splinted for 1 week showed viable chondrocytes with a weak background signal, indicating the possibility of very few dead or dying cells in the central irradiated portion (**Figure 6**). The weeks 3 and 4 treatment groups showed only viable cells and no evidence of dying chondrocytes.

COMMENT

This study demonstrates the feasibility of LCR of the ear in an animal model using a 1450-nm diode laser in combination with CSC. As a commercial clinical laser system was used to accomplish reshaping without any custom hardware or software modifications to the native operating system, it may be possible to rapidly translate and apply this technology to reshape protuberant ears in human subjects, albeit “sutured in place splints” would be replaced with either silicone rubber mouldages or head-bands as in traditional otoplasty operations. We currently are pursuing a clinical trial with this instrumentation. By transcutaneously heating the cartilage framework of the pinna with limited injury to the over-lying skin, shape change can be achieved without incisions. The feasibility of this technology is further supported by findings from anatomical studies that show that the thickness of the skin, subcutaneous tissues, and auricular cartilage in the rabbit are similar to that of the human ear—especially in the pediatric population.¹² Cryogen spray cooling allows protection of the skin from severe thermal injury while facilitating substantial subsurface temperature elevations, at least above threshold values required for cartilage shape change. Heating cartilage results in a change in the material properties of this tissue that facilitates shape change and is manifested by accelerated stress relaxation.¹³⁻²⁷

As in the study by Mordon et al,⁹ rabbit ears underwent shape change with LCR, albeit with 2 major differences. First, we opted to reshape the central region of the rabbit pinna, which is about halfway between the tip of the ear and its connection to the bony external auditory canal. The skin and cartilage of the rabbit ear in this area is substantially thicker than that at the tip of the ear where Mordon et al⁹ performed their reshaping study. The thicker skin requires a more robust application of both cryogen and laser energy because more light must penetrate deeper beneath the subsurface. Thicker cartilage also requires a larger region of tissue axially (ie, in the direction of laser light propagation) to be heated to the threshold

temperatures needed for shape change.^{20,21} Simply put, this is a more challenging task, and the central region of the rabbit ear reshaped herein is more similar to the geometry of the human ear.

Using a CSC system to protect the skin from major thermal injury is the second major difference between our work and that of Mordon et al.⁹ who used a contact cooling system based on simple heat exchange devices. Anvari et al.^{28,29} demonstrated that significant subsurface temperature elevations could be generated using infrared laser irradiation and CSC and coined the phrase “spatially selective heating,” which is an integral component of the mechanisms of LCR.³⁰ Cryogen spray cooling removes massive amounts of heat from the tissue surface as the cryogen undergoes a phase change from liquid to gas at the tissue surface. When laser dosimetry and cryogen spray duration are selected appropriately, spatially selective heating can be achieved, and this is ideal for use in auricular LCR.

Finally, our previous work³¹ and that of others⁹ have observed that heating cartilage using laser dosimetry adequate for LCR may result in stimulation of chondrocyte regeneration with the formation of new cells and new cartilage tissue matrix. This phenomenon was observed in the present study as well, suggesting that in addition to acute changes in the tissue related to shape change, a later secondary effect is the formation of new cartilage. Histologic findings of clonal chondrocyte expansion and a thickened perichondrium (Figure 5B) are consistent with the observations that ears splinted 4 weeks prior to harvest are noticeably stiffer than those harvested at 1 or 3 weeks. The orthopedic literature is replete with studies and case reports of irreversible and undesirable thermal injury to articular cartilage generated by both laser and radiofrequency devices. In contrast to these collective observations, in facial cartilage enveloped by a dense layer of mesenchymal stem cells, such profound injury is not observed. In fact, we see just the opposite effect, ie, chondrocyte proliferation, in this animal model. This may, however, be a phenomenon that is unique to the elastic and hyaline cartilage tissues of the face.

In conclusion, laser cartilage reshaping in tandem with CSC can be used to reshape ears in a rabbit model. Because the axial dimensions of the midportion of the rabbit ear are similar to the dimensions of the human ear in certain regions, it should be possible to accomplish this task with this device in patients as well. Cryogen spray cooling protected the skin surface from significant thermal injury, while facilitating the creation of adequate subsurface temperature elevations to achieve shape change. New cartilage tissue formation was observed as well, suggesting that the process is not entirely destructive in nature.

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Figure 1.

The ear to be treated is placed over a transilluminating template, which acts as a guide for laser treatment. The ear is held in place by an assistant while the surgeon applies the laser. *Transilluminated laser target locations. HP indicates laser handpiece.

Figure 2.
The treated ear and control ear are sutured over a gauze roll (diameter, 1 cm) using 2 nylon sutures placed in horizontal mattress fashion through-and-through the ear.

Figure 3.
Typical appearance of a rabbit postoperatively with a protective collar in place and both ears splinted.

Figure 4. Digital images of representative ears from the 1-week (A), 3-week (B), and 4-week (C) treatment groups. Although no statistical difference in bend angle is present between these 3 groups, the quality of the cartilage firmness and recoil increased with duration of splinting.

Figure 5.

A, Photomicrograph of a specimen from the treated ear of a rabbit from the 3-week group (hematoxylin-eosin, original magnification $\times 100$). As noted, the chondrocyte population is expanded with a thickened matrix, which is more eosinophilic. The perichondrium and surrounding tissues also demonstrate some thickening and fibroplasia. B, Photomicrograph of an untreated ear showing no abnormality (hematoxylin-eosin, original magnification $\times 100$). The chondrocyte population has the typical appearance of a natural ear, and there is no evidence of shape change or fibroplasia.

Figure 6.

Scanning confocal micrograph of a specimen from the treated ear of a rabbit in the 1-week group (original magnification $\times 40$). The yellow emission among the LIVE green cells hints toward recent cellular death in the area (possibly at deeper layers). No obvious dead cells were visualized; however, they may have been present immediately after treatment and had undergone degradation by the 1-week interval.