In Vivo Electromechanical Reshaping of Ear Cartilage in a Rabbit Model

A Minimally Invasive Approach for Otoplasty

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Objective: To report the first successful study to date of in vivo electromechanical reshaping of ear cartilage in a rabbit model.

Methods: Ears of New Zealand white rabbits were reshaped using percutaneous needle electrode electromechanical reshaping (5 V for 4 minutes) and were then bolstered for 4 weeks. Ten ears were treated, with 2 undergoing sham procedures and serving as controls. The treatment was performed using a platinum array of electrodes consisting of 4 parallel rows of needles inserted across the region of flexures in the ear. After 4 weeks, the animals were killed, and the ears were photographed and sectioned for conventional light microscopy and confocal microscopy (live-dead fluorescent assays).

Results: Significant shape change was noted in all the treated ears (mean, 102.4°; range, 87°-122°). Control ears showed minimal shape retention (mean, 14.5°; range, 4°-25°). Epidermis and adnexal structures were preserved in reshaped ears, and neochondrogenesis was noted in all the specimens. Confocal microscopy demonstrated a localized zone of nonviable chondrocytes (<2.0 mm in diameter) surrounding needle sites in all the treated ears.

Conclusions: Electromechanical reshaping can alter the shape of the rabbit auricle, providing good creation and retention of shape, with limited skin and cartilage injury. Needle electrode electromechanical reshaping is a viable technique for minimally invasive tissue reshaping, with potential applications in otoplasty, septoplasty, and rhinoplasty. Further studies to refine dosimetry parameters will be required before clinical trials.

Published online November 5, 2012.
doi:10.1001/2013.jamafacial.2
surgical incisions.9–11 Accordingly, surgical embodiments of EMR would use percutaneous or transmucosal needles inserted into the regions of stress distribution in mechanically deformed or reshaped cartilage specimens, and the feasibility of this approach in ex vivo tissues has been previously discussed.12,13

Given that a set of voltage and time parameters has been identified that produce effective shape change in intact ex vivo auricular specimens,12 further optimization of electrical dosimetry variables and electrode configurations in vivo is needed to move this technology toward clinical use.10,12 This study aimed to evaluate the in vivo effects of EMR of ear cartilage in a rabbit model to determine the dependence of shape change, mechanical stability, and tissue injury on dosimetry (voltage and application time) and electrode configuration.

METHODS

The protocol was approved and performed under the guidance of the Institutional Animal Care and Use Committee at the University of California, Irvine. All the regulations were followed throughout the duration of the study.

EMR PROCEDURE

Ten New Zealand white rabbits (Western Oregon Rabbit Company), weighing 3.8 to 4.0 kg, were anesthetized using subcutaneously administered ketamine hydrochloride (20–40 mg/kg) and xylazine (3–5 mg/kg). The ear was positioned within the EMR jig along a line halfway between the base and tip of the ear (Figure 1). The jig encompassed 4 rows of 19 apertures to extend across the width of the rabbit ear. Apertures were set at 3.0 mm apart from one another. Platinum needles were then inserted through each aperture and through the rabbit ear and were then connected to leads from a direct current power supply (PPS-2322; Amrel) to deliver current. The EMR current was applied at 5 V for 4 minutes for 8 of 10 ears treated. In controls (2 of 10 ears), the same procedure was performed without applying current. Following EMR treatment, both ears were splinted over a gauze roll (1.5 cm in diameter) and sandwiched between two 3 × 7-cm silicone elastomeric bolsters that were sutured into place using two 3-0 polypropylene sutures. An Elizabethan collar was placed, and the animals were transferred to the vivaria for recovery.

PROCEDURES AFTER EMR

After surgery, the rabbits were monitored for overall well-being and for signs of pain or agitation; photographic documentation of the ears was obtained at regular intervals. After 4 weeks, the animals were killed with a lethal dose of intraperitoneal pentobarbital, and the splints were removed. The auricles were then harvested in preparation for the subsequent procedures listed in this subsection.

Photography

With the tip pointing superiorly and inferiorly while being supported from the base, each ear was photographed and videotaped. Analysis of the degree of shape change in each ear was measured using a commercially available software package (Photoshop version 9.0; Adobe Systems, Inc).

Mechanical Response Measurement

To test the integrated mechanical response of the reshaped ears to flexural deformation quantitatively, auricular tissue was analyzed using a precision mechanical testing platform (ELF 3200; Bose Corporation). The integrated response of an intact reshaped ear to mechanical deformation (flexure) was evaluated. The ear was removed from the crania at the level of the external auditory meatus. The bottom of the ear was then embedded in a base consisting of plaster of paris, which was allowed to cure. This base was then mounted onto the upper platen of the mechanical testing platform. Ears were bent 90°, and the bottom platen of the platform was gradually raised until its edge was in contact with the deflected portion of the ear 10.0 mm away from the bend axis of the ear. A static reaction force was recorded at this moment. The upper platen moved up and down at a constant velocity of 2.5 mm/s to a maximum amplitude of 2.5 mm, flexing the ear at its bend (resulting from reshaping). A stationary load cell rated to 250 N attached to the bottom
platen measured the integrated reaction force produced by the ear in response to flexing.

Displacement and integrated reaction force were recorded during flexing. The interval where this force changes linearly with displacement was identified, and the integrated elastic modulus was calculated. The mechanical response of control (no EMR) ears was evaluated in a manner similar to that of the treatment ears. In both treatment and control ears, the initial reaction force registering at the moment of contact with the bottom platen and corresponding to the reaction to 90° bending was recorded and was subtracted from the force obtained in subsequent flexing experiments.

Microscopic Assessments

The ears were sectioned to include 2 specimens for histologic analysis and 2 specimens for live-dead fluorescent assays using confocal microscopy. The live-dead assay (Molecular Probes Inc) uses 2 dyes, calcein acetomethoxy and ethidium homodimer 1. Calcein acetomethoxy 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, becoming trapped inside 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 were prepared under minimal lighting conditions because the aforementioned dyes are photoreactive. Both dyes were mixed according to manufacturer instructions, were added to each specimen in a microvial, and were kept in a light-free environment for 30 minutes. The specimens were then washed with phosphate-buffered saline, transported in a light-free box, and immediately imaged using a laser confocal microscope (Meta 510; Carl Zeiss LSM). All the segments incorporated a region of the ear created a discontinuous cartilaginous layer spanning 0.3 to 0.8 mm. A chondroblastic proliferation was noted near the area of the cartilaginous defect. This corresponded to a superperichondral formation of cartilage around the periphery of the site where EMR was applied.

RESULTS

All the rabbits tolerated EMR and survived for the duration of the study. Initial exudative scabs at perforation sites resolved by postoperative day 11 (Figure 2); fully intact epithelium was noted in all the ears at the time of euthanasia. No infection, scarring, or bleeding complications were noted. All the treated ears showed significant shape change (mean, 102.4°; range, 87°-122°), exceeding that of control ears (mean, 14.5°; range, 4°-25°) (Table).

HISTOLOGIC EXAMINATION

Histologic examination of the electroformed sites showed an intact epidermis and adnexal structures (Figure 3). Platinum needle electrode (30-gauge) perforations in the ear created a discontinuous cartilaginous layer spanning 0.3 to 0.8 mm. A chondroblastic proliferation was observed near the area of the cartilaginous defect. This corresponded to a subperichondrial formation of cartilage around the periphery of the site where EMR was applied.

CONFOCAL MICROSCOPY

Confocal microscopy demonstrated a localized zone of nonviable chondrocytes less than 2.0 mm in diameter surrounding the point of insertion of needle electrodes in all the treated ears (Figure 3). Within this zone was evidence of fibrocartilage scar tissue. The anodic and ca-
thodic rows were similar in appearance for live-dead fluorescent assays.

MECHANICAL RESPONSE MEASUREMENT

Ears treated with EMR and control ears demonstrated statistically similar integrated elastic modulus. The mean (SD) values were 0.43 (0.05) MPa for EMR-treated ears and 0.48 (0.06) MPa for the control ears ($P < .01$).

COMMENT

In this pilot study, we demonstrated that EMR can be used to alter the shape of the ear in an in vivo rabbit model. The low cost, technically simple, and nonthermogenic features of EMR make this modality an appealing alternative to other minimally invasive systems, such as laser and RF-mediated reshaping. To advance toward clinical trials, the safety and efficacy of EMR on living tissue must be assessed.14 This study demonstrated that EMR technology can be applied along the entire width of the auricle to create shape change, without complications of major skin injury, necrosis, or infection. Skin injury merits discussion because the electrodes were in contact with the skin and cartilage during the procedure. A major concern was that EMR performed without the use of an insulation layer covering portions of the needle electrode not in contact with cartilage might lead to cutaneous injury and adverse outcomes. On inspection and with histologic analysis, we found no sustained injury, despite the observation of crusting and serous exudates immediately after EMR was performed. Based on this information, we conclude that epithelial injury with the present protocol will not be a major hurdle for using this technology.

The dosimetry parameters were derived from prior ex vivo investigations12 as a starting point for producing effective shape change in live rabbit ears. Future experiments will further examine the dependence of shape change on dosimetry in vivo, with the broad goal of developing methods to optimize the electrical dosimetry, as well as needle electrode design and placement. Geometry of electrode placement, voltage difference, and application time would affect the viability of the chondrocytes and the shape change.

On subjective assessment of ear mechanical behavior, manual inspection of each treated auricle demonstrated that shape change after splint removal was maintained and that qualitatively the reshaped segments had elastic behavior similar to that of native nontreated adjacent tissue. We observed that the thicker, anterior portion of each ear sustained more consistent and robust shape change than the adjacent thinner regions, and we attribute this to the increased tissue mass here that is available for the electrochemical changes that accompany the EMR effect.

Histologic analysis of the ear showed patterns promising to remodeling that lead to permanent shape change with EMR. Normal tissue regions that were 2.0 mm away from the electrodes showed no signs of necrosis, fi-
Brotic changes, or other damage. Regions near the vicinity of each electrode (<2.0 mm away) showed chondrocyte proliferation. The cells were most dense by the needle sites and where curvature was greatest. Needle insertion sites there were replaced with a fibrotic process or remained unchanged, indicating that a longer study period is warranted to observe complete closure. The toxic pH gradient created by the EMR procedure seems spatially limited and perhaps may be mitigated in part by blood flow through vascularized tissues. Blood does not flow through cartilage tissue, and this fact is exploited when performing EMR.

Assessing chondrocyte viability using laser confocal microscopy and live-dead fluorescent assays is a well-established technique,15,16 aiding the optimization of EMR dosimetry and geometry of electrode placement. Confocal microscopy identified some regions of tissue injury, which were spatially limited to the immediate vicinity around each needle electrode up to 1.2 mm away. The tissue injury surrounding each electrode insertion was consistent among the specimens, but these regions were small and represented a fraction of the total tissue volume in the specimen. In addition, the visualization of chondrocyte neogenesis coincided with the histologic re- 
volution on the long-term in vivo response of cartilage tissue (and overlying skin) to EMR.

In conclusion, EMR altered the shape of the rabbit auricle, providing good creation and retention of shape, with limited skin and cartilage injury. Needle electrode EMR is a viable technique for minimally invasive tissue reshaping, with potential applications in otoplasty, septoplasty, and rhinoplasty. Further studies to refine dosimetry parameters and to assess reshaping over time with the auricular splints removed will be required before clinical trials.

Accepted for Publication: October 31, 2011.
Published Online: November 5, 2012. doi:10.1001/2013jamaface.2

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Conflict of Interest Disclosures: Dr Wong has intellectual property (licensed by Aerin Medical, Inc, and by Praxis BioSciences, LLC) that pertains to the research performed in this study. He also has equity with Praxis BioSciences, LLC. Aerin Medical, Inc will license the intellectual property from the University of California, Irvine, from which Dr Wong may benefit.

Funding/Support: This study was supported by grant ERT 44380-29302 from the National Institutes of Health (Dr Wong).

REFERENCES