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Ex Vivo Electromechanical Reshaping of Costal Cartilage in the New Zealand White Rabbit Model

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Abstract

Objectives/Hypothesis—Determine the effective electromechanical reshaping (EMR) parameters for shape change and cell viability in the ex vivo rabbit costal cartilage model.

Study Design—Ex vivo animal study combined with computer modeling to guide electrode placement and polarity selection.

Methods—Rabbit costal cartilages were secured in a jig that approximated the shape of the rabbit auricle framework. Finite element modeling was used to select the initial electrode geometry, polarity, spacing, and estimate dosimetry parameters. Porcine costal cartilage was utilized to refine the selection of dosing parameters. Parametric analysis was performed to determine the effect of voltage and application time on tissue shape change. Next, rabbit rib cartilage was reshaped, varying voltage and application time to identify the lowest parameters to produce acceptable shape change mimicking native auricular cartilage. Acceptable qualitative shape change was determined on a five-point Likert scale analyzed using one-way general linear analysis of variance. Confocal microscopy with live/dead cell viability analysis determined the degree of injury and the distribution of live and dead cells.

Results—The minimum acceptable deformation of rabbit costal cartilage was found at 4 V–3 minutes. Viability analysis of cartilage reshaped at 4 V–3 minutes demonstrates cell injury extending 2 mm away from each electrode with viable cells found between the electrodes.

Conclusions—The EMR parameters of 4 V–3 minutes demonstrates appropriate shape change producing grafts that resemble the native auricle and contains the viable cells adequate for clinical evaluation. The rabbit auricular reconstruction model using EMR is a feasible one.

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Brian J. F. Wong, MD, has had intellectual property licensed for the development of this technology. Dr. Wong has equity interest in Praxis BioSciences, which is commercializing this technology.

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Keywords

Costal cartilage; reconstructive surgery; tissue reshaping; otolaryngology; otoplasty; rabbit; animal model; microtia repair; trauma repair

INTRODUCTION

External ear malformations are structural tissue defects that can be congenital or acquired. Such deformities can provoke ridicule and lead to negative emotional and psychological damage in children with these malformations.¹ Although successful intervention in a noninvasive manner, such as taping or splinting, has been used in newborns for lop ear and related deformities, these methods produce varying rates of success in children older than 3 years of age due to a lack of compliance, growth alterations, and the inability to change the overall geometry and dimensions of the auricular cartilage.^{2,3} Furthermore, microtia surgery required for total and subtotal ear defects requires reconstruction with the creation of a new cartilaginous auricular framework or use of an alloplast.

Current techniques for microtia repair have evolved from the contributions of Tanzer,⁴ Brent,⁵ Nagata,⁶ and Firmin.⁷ Although these techniques advocate autologous rib cartilage framework carved from the costal synchondrosis, alternative auricular reconstruction materials include using a prosthetic or an alloplastic implanted framework. The latter, such as porous polyethylene and Silastic implants, require thicker cutaneous protective coverage and have been associated with a high rate of extrusion, respectively.^{8,9} Attachable prosthetics remain an option for certain clinical situations; however, there is great variability in the aesthetic quality of the prosthesis, and they may require frequent replacement.¹⁰ In contrast, reconstructed ears made from autogenous materials have been used successfully for more than 50 years.⁵

The reconstruction of the intricate surface topography of the external ear is a technically demanding procedure with a steep learning curve, where poor aesthetic outcome is common, even in the hands of experienced plastic surgeons, and there are greater than 40 different techniques reported in the literature.¹¹ To reach the next level in microtia repair, the development of tissue engineering solutions, and not surgical techniques, must be investigated.

Electromechanical reshaping (EMR) is a technique that has been shown to permanently change the shape of cartilage via active stress relaxation in the region of externally applied deformation. Low amplitude electric voltage is applied to needle electrodes strategically inserted into locations of stress concentration in mechanically deformed specimens.^{12,13} The electric field, created in the vicinity of needle electrodes, initiates local redox reactions that produce relaxation of the internal stress without thermogenic denaturation of the tissue.^{13–17}

EMR has been examined *ex vivo* in porcine costal cartilage tissue producing successful reshaping results.¹² This study aimed to determine the optimal EMR parameters for shape change and cell viability in the *ex vivo* rabbit costal cartilage model for auricular reconstruction, as this is the first step required before proceeding to *in vivo* animal studies.

MATERIALS AND METHODS

Tissue Preparation

Fresh rib cartilage ($n = 12$) was harvested from freshly euthanized New Zealand White rabbits used in other Institutional Animal Care and Use Committee-approved protocols, and the surrounding muscle, fat, connective tissue, and perichondrium was meticulously removed. A custom-created dual-blade cutting device was used to standardize the thickness and height of the tissue to 1.10 mm and 2.50 mm, respectively. Each specimen was then cut to a length of 25 mm. To meet these measurement criteria, the costal cartilage of rib 6 was the only candidate. A similar process with identical dimensions was performed utilizing porcine tissue ($n = 21$) prior to the utilization of rabbit cartilage, as this tissue is readily obtained in bulk and is low cost, and thus is ideal for pilot investigations.

Reshaping Protocol

To reshape the tissue, the cartilage was mechanically deformed within a custom, fired, ceramic jig that recreated the shape of the rabbit auricle framework (Fig. 1). The geometry, polarity, spacing, and initial dosimetry of the treatment were identified utilizing previously developed finite element models implemented in the COMSOL Multiphysics programming environment (COMSOL, Los Angeles, CA) (Fig. 2).¹⁶ The model simulated the electric field between electrodes positioned in cartilage and allows selection of the voltage-producing target electric field of 2 kV/cm in the regions of stress concentrations. Previously, we have demonstrated that the value of 2 kV/cm produces both acceptable shape change and cell viability, and this is the best available estimate for the electric field intensity at which EMR occurs.^{18,19} Based on these numerical simulations, a pattern of 4 platinum needle electrodes (F-E2M-48; Grass Technologies, West Warwick, RI) connected to a direct current power supply (E36446A; Agilent Technologies, Inc., Palo Alto, CA) was inserted into the cartilage through perforations in the jig set at 4 mm apart with alternating electrical polarity (Fig. 3). A dosimetry starting point of 7 V and 3 minutes was selected based on the numerical simulation as well as work from a previous study.¹² Following EMR, these electrodes were removed, and the jig containing the reformed tissue was placed in a phosphate-buffered solution (pH 7.4) for 15 minutes prior to removal and digital photography of the specimen (Canon EOS 1000D; Canon, Lake Success, NY).

First, using porcine costal cartilage, parametric analysis was performed to narrow the range of dosimetry parameters and determine the effect of voltage and application time on shape change. Three samples per parameter were studied. Although keeping time constant (3 minutes), voltage was varied (3, 4, 5, 6, 7 V) until a voltage-time parameter produced a poor amount of shape change relative to the initial 7 V-3 minutes dosimetry. A reduced parameter set producing acceptable reshaping was identified at 2 V, 3 V, and 4 V. This refined parameter set consisted of a total of 4 rabbit cartilage reshaping groups: 4 V for 3 minutes, 3 V for 3 minutes, 3 V for 2 minutes, and 2 V for 2 minutes. This was done to identify the lowest parameters required to produce acceptable shape change mimicking native auricular cartilage. Control trials utilized porcine specimens that were inserted into the jig, perforated with platinum electrode needles, and had no current delivered for 3 minutes.

Analysis of Shape Change

Three individuals blinded from the study qualitatively rated the degree of shape change on a simple five-point Likert scale. Because greater voltage-time application correlates with greater shape change, as reported from earlier studies^{12,18} and confirmed by our results, 7 V–3 minutes was given a score of 5 and ranked as very acceptable, whereas those that resembled the control were deemed unacceptable and assigned a score of 1. Scores of 4, 3, and 2 were categorized as good, acceptable, and poor based on comparison to resemblance of the initial parameter (Fig. 4). Statistical analysis of the scorers result was performed using one-way general linear model analysis of variance (ANOVA) to determine if the means tabulated from each voltage group were different between voltage groups. If the result of ANOVA shows that each voltage produced entirely different results ($P < .05$), then a Student paired t test was used to see which EMR voltage groups were statistically significant ($P < .05$) based on the scores noted in the blind study.

Viability

The distribution of live and dead cells after EMR was imaged using fluorescent laser scanning confocal microscopy. The technique, as described by Choi et al.,^{20–24} determined the degree of injury generated around the electrodes in the reshaped rabbit rib cartilage. Immediately following the 15-minute rehydration period in EMR protocol, the rabbit tissue with the lowest parameters that had achieved acceptable shape change (4 V–3 minutes) was sectioned lengthwise through the center of electrode insertion sites. The specimen was then stained with calcein-acetoxymethylester and ethidium homodimer-1 (Molecular Probes, Eugene, OR) and visualized with a confocal microscope (LSM 510 META; Carl Zeiss, Jena, Germany).

RESULTS

Throughout the application of electrical current, the evolution of gas at the anode and cathode needle-tissue interface was observed, as had been described in previous EMR studies.^{12,16,18} In all experiments, there was increased transparency and opacity surrounding the area around the anode and cathode (1 - to 3-mm diameter) with increasing application time and voltage.

A total of 33 rib specimens were modified, and 3 specimen were controls that were not undergoing EMR treatment. Generally, the shape change of rib grafts increased with increasing voltage and application time. The control specimen maintained a small amount of shape memory from the jig and platinum needle perforations.

Maximum shape change of the porcine cartilage tissue was identified at the initial dosimetry setting of 7 V for 3 minutes. This amount of shape change was classified as “very acceptable,” and was given a Likert score of 5. At 5 V, shape change was characterized as “good” and given a Likert score of 4. “Acceptable” shape change was noted at 4 V, with 3 V determined as poor shape change and assigned a Likert score of 2. The Likert score of 1 was reserved for shape change similar to that of the control. Rabbit rib cartilage was reformed at 4 V and 3 V (for 3 minutes) and at 3 V and 2 V for 4 minutes following porcine trials. At 4

V–3 minutes, the rabbit rib cartilage produced the most acceptable reshaping, whereas 3 V–4 minutes, 3 V–3 minutes, and 2 V–4 minutes, in decreasing order, produced less acceptable shape change (Fig. 5).

The result of ANOVA between the scores marked by the 3 blinded raters showed no significant difference ($P = .8697$), and therefore the rating of shape change after EMR is presumed to be consistent among the 3 blinded raters. The result of ANOVA among EMR dosimetries does not show any significant difference, and therefore no paired t test between EMR dosimetries were done. As such, the highest EMR dosimetry (4 V–3 minutes, $n = 3$) was not significantly different from the lowest EMR dosimetry (2 V–4 minutes, $n = 3$) based on the rater's scores on the Likert scale.

Viability

Viability analysis of cartilage reshaped at 4 V–3 minutes demonstrated cell injury extending 2 mm in diameter away from each electrode, with viable cells found between the electrodes (Fig. 6).

DISCUSSION

We demonstrated that EMR has the ability to achieve acceptable shape change of the rabbit rib cartilage while maintaining cell viability for potential use in the in vivo auricular reconstruction animal model. Previous work by our group has demonstrated efficacy of EMR in reshaping native rabbit auricular cartilage in vivo as a model for otoplasty surgery. This study represents the first step toward an in vivo assessment of EMR in orthotopically transferred costal cartilage for use in a rabbit model of microtia repair. Although alternatives in tissue reshaping are available, including laser and radiofrequency-mediated reshaping,^{25,26} EMR allows for a low-cost, technically simple, and nonthermogenic method of sustaining shape change. This spatially selective treatment modality has the potential to reshape cartilage structures of the face in situ, or as demonstrated within this study and others, with rib grafts.¹²

Although this study successfully reshaped rib cartilage, one concern in developing the animal model was the frequent breaking or fracture of cartilage samples with flexure, which occurred while inserting the specimen into the mold. This breaking of samples occurred when the cartilage came from older rabbits with advanced ossification of the costal margin. Ossification of costal cartilage in the mature and older rabbit is an observation we were surprised to observe.

Fluorescent confocal microscopy utilizing a live/dead assay allows for the assessment of chondrocyte viability, which aids in the optimization of EMR dosimetry. Microscopy assessing the specimen with the least voltage–time application while still maintaining acceptable shape change (4 V–3 minutes) allowed for the greatest number of viable cells to be visualized between needle perforations. Imaging identified regions of tissue injury spatially limited to the 2-mm diameter region around each needle electrode. This is similar to previous ex vivo EMR studies.^{18,27} Cell injury, however, is not unique to EMR and is noted in the utilization of laser and radiofrequency for achieving shape change.^{26,28,29} As

with these other emerging technologies, in EMR, shape change comes at the expense of tissue injury, but spatially, selectivity makes this tradeoff a viable option for potential surgical procedures.

The Likert scale categorization of acceptable shape change is by design subjective. Using results from porcine tissue, we devised a 5-point scale based on curvature. The results obtained in the assessment of shape change in rabbit cartilage, although consistent for the 3 blinded independent reviewers, did not show any significant differences among EMR voltage (2 V, 3 V, 4 V)–time (3 minutes, 4 minutes) settings. The shape change noted between rabbit trials and dosimetries, however, must be approached for the purpose of future experimental roles of EMR, which is that of creating a sustained shape change in a costal graft for auricular reconstruction. For future auricular reconstruction animal studies utilizing the New Zealand White rabbit, the most appropriate change in costal cartilage shape is best reproduced at 4 V–3 minutes.

The goal of this study was to determine the effective EMR dosimetry parameters for shape change and cell viability in the ex vivo rabbit costal cartilage model. Further studies are required to more quantitatively assess shape change in a complex geometry and better localize needle placement for a more ideal auricular shape. Current experiments are optimizing the electrical dosimetry as well as needle electrode design and placement for in the in vivo animal model.

CONCLUSION

The EMR dosimetry parameter of 4 V–3 minutes demonstrates appropriate shape change and cell viability in the ex vivo rabbit costal cartilage model. The acceptable sustained auricular resemblance contains the viable cells adequate for clinical evaluation. The rabbit auricular EMR reconstruction model is feasible.

Acknowledgments

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Fig. 1.

The upper curve of the base of the rabbit auricle is the shape that is being emulated by use of costal cartilage and electromechanical reshaping. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

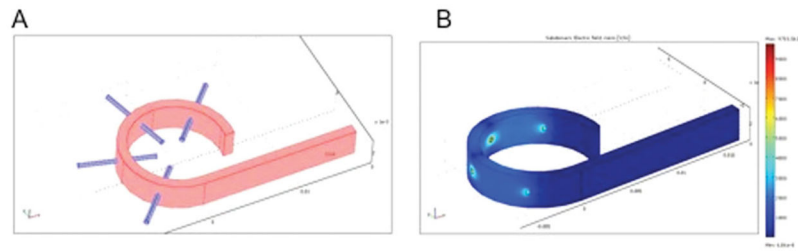


Fig. 2. Computer modeling aided in guiding electrode placement and polarity selection. (A) Geometry of cartilage sample bent in the reshaping jig with needle electrodes used for numerical simulation of electric field distribution. (B) Distribution of electric field. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



Fig. 3.

A custom, fired, ceramic jig that recreated the shape of the rabbit auricle framework was created with perforations at every 4 mm. Electrodes alternated between anode and cathode. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

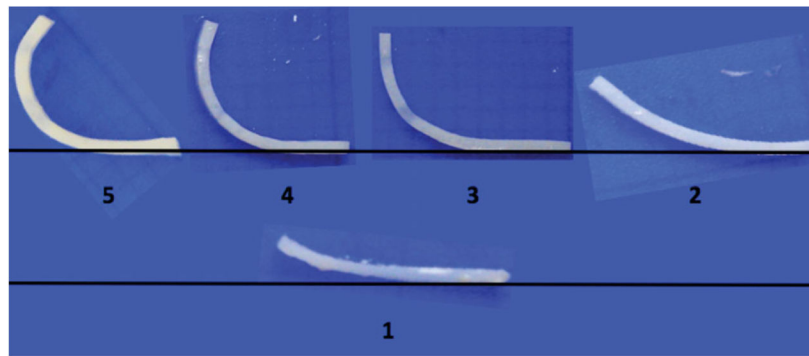


Fig. 4. Using results from porcine tissue, a five-point scale based on curvature was devised for independent assessment and qualitative comparison of rabbit costal cartilage shape change to that of porcine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

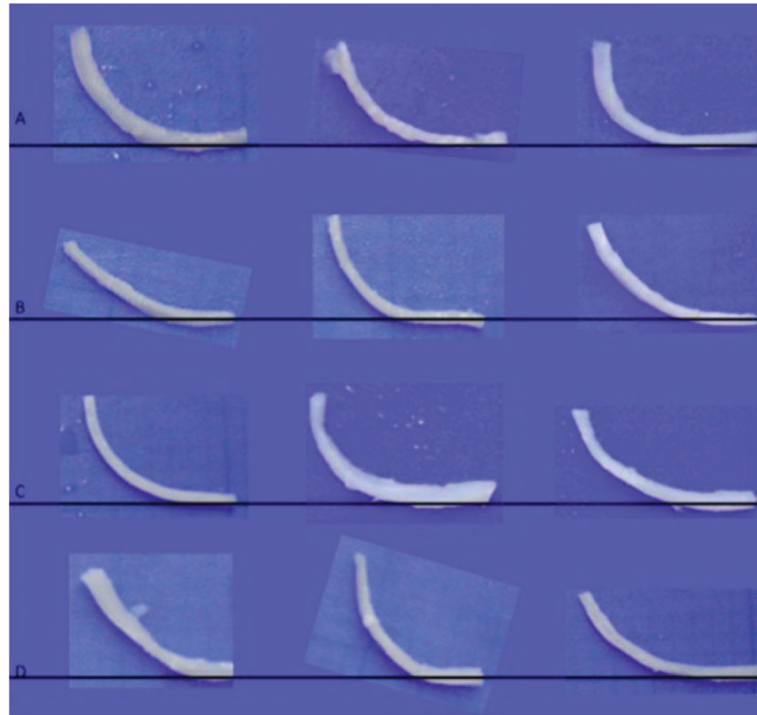


Fig. 5. Electromechanical reshaping at parameters of: (A) 4 V–3 minutes, (B) 3 V–3 minutes, (C) 3 V–4 minutes, and (D) 2 V–4 minutes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

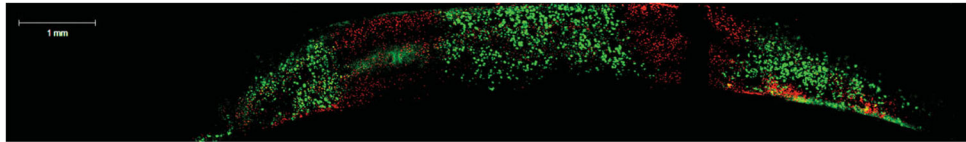


Fig. 6. Distribution of viable (hyperintense) and nonviable (hypointense) cells in rabbit costal cartilage using live/dead fluorescent assay and confocal microscopy immediately after electroforming at 4 V for 3 minutes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]