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Optimal Electromechanical Reshaping of the Auricular Ear and Long-term Outcomes in an In Vivo Rabbit Model

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Abstract

IMPORTANCE—The prominent ear is a common external ear anomaly that is usually corrected through surgery. Electromechanical reshaping (EMR) may provide the means to reshape cartilage through the use of direct current (in milliamperes) applied percutaneously with needle electrodes and thus to reduce reliance on open surgery.

OBJECTIVE—To determine the long-term outcomes (shape change, cell viability, and histology) of a more refined EMR voltage and time settings for reshaping rabbit auricle.

DESIGN, SETTING, AND SUBJECTS—The intact ears of 14 New Zealand white rabbits were divided into 2 groups. Group 1 received 4 V for 5 minutes (5 ears), 5 V for 4 minutes (5 ears), or no voltage for 5 minutes (control; 4 ears). Group 2 received an adjusted treatment of 4 V for 4 minutes (7 ears) or 5 V for 3 minutes (7 ears). A custom mold with platinum electrodes was used to bend the pinna and to perform EMR. Pinnae were splinted for 6 months along the region of the bend. Rabbits were killed humanely and the ears were harvested the day after splint removal. Data were collected from March 14, 2013, to July 8, 2014, and analyzed from August 29, 2013, to March 1, 2015.

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Conflict of Interest Disclosures: Dr Wong reports having intellectual property (licensed by Aerin Medical, Inc) that pertains to the research performed in this study. Aerin Medical, Inc has licensed the intellectual property from the University of California, Irvine, from which Dr Wong may benefit. No other disclosures were reported.

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Study concept and design: Manuel, Wong.

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MAIN OUTCOMES AND MEASURES—Bend angle and mechanical behavior via palpation were recorded through photography and videography. Tissue was sectioned for histologic examination and confocal microscopy to assess changes to microscopic structure and cell viability.

RESULTS—Rabbits ranged in age from 6 to 8 months and weighed 3.8 to 4.0 g. The mean (SD) bend angles were 81° (45°) for the controls and, in the 5 EMR groups, 72° (29°) for 4 V for 4 minutes, 101° (19°) for 4 V for 5 minutes, 78° (18°) for 5 V for 3 minutes, and 126° (21°) for 5 V for 4 minutes. At 5 V, an increase in application time from 3 to 4 minutes provided significant shape change (78° [18°] and 126° [21°], respectively; $P = .003$). Pinnae stained with hematoxylin-eosin displayed localized areas of cell injury and fibrosis in and around electrode insertion sites. This circumferential zone of injury (range, 1.3–2.1 mm) corresponded to absence of red fluorescence on the cell viability assay.

CONCLUSIONS AND RELEVANCE—In this in vivo study, EMR produces shape changes in the intact pinnae of rabbits. A short application of 4 V or 5 V can achieve adequate reshaping of the pinnae. Tissue injury around the electrodes is modest in spatial distribution. This study provides a more optimal set of EMR variables and a critical step toward evaluation of EMR in clinical trials.

LEVEL OF EVIDENCE—NA.

The prominent ear is the most common congenital anomaly of the external ear, affecting about 5% of a healthy population.¹ Correction was first described by Ely in 1881,² and since that time, optimal surgical management has been widely debated.³ Although the most common cause of a prominent ear is lack of development of the anti-helical ridge, conchal hypertrophy, a protruding conchal wall, or all of these can be causative. The broad aim of otoplasty is to reposition or reshape the ear such that it does not draw attention to itself.⁴ This process is accomplished by attempting to place the pinna in an ideal position relative to the head and to recreate the missing features if necessary.

Although Ely's initial technique involved removing cartilage and skin, a number of minimally invasive techniques to correct protruding ears have been developed during the past 50 years.^{5–10} Although these techniques have been used commonly during the past 2 decades, many of them require the use of sutures and/or cartilage scoring to weaken the cartilage at desired areas of curvature.^{9,11,12} In addition, these techniques have been difficult to master, with a steep learning curve and poor outcomes owing to suture extrusion, asymmetry, and scarring. Truly noninvasive approaches are limited, with laser-assisted cartilage reshaping^{13–17} being the least invasive, but without widespread adoption owing to the need for specialized and costly lasers.

As an alternative to laser-based tissue reshaping techniques, electromechanical reshaping (EMR) was developed as a means to shape cartilage without the use of scalpels, sutures, or heat.^{18–27} Electromechanical reshaping is a non-thermal-based method that relies on chemical reduction-oxidation (redox) reaction to alter tissue matrix structure and thus change tissue stress-strain associations. To perform EMR, a mold or jig is first used to bend cartilage to a desired shape, and electrodes are inserted into regions of increased internal stress. Direct current is applied, causing in situ redox reactions to occur without heat generation. The jig and electrodes are removed and a retainer is used to maintain the desired

bend while the tissue undergoes remodeling (Figure 1). Previous work^{23,27} demonstrated the achievement of stable shape change in vivo in rabbit auricles and identified EMR settings, voltage, and application time for effective reshaping and its effects on the tissue remodeling as long as 3 months after treatment. Although this interval provides significant information with respect to the acute and subacute elements of wound healing and remodeling, it does not provide information on long-term outcomes essential to clinical evaluation and applicability in humans. Herein, we present an expanded in vivo study that examines a longer survival of 6 months while also refining the EMR settings appropriate for auricular ear reshaping in a live rabbit model, further demonstrating the feasibility of this emerging technology.

Key Points

Question

What is the dose-response association between electromechanical reshaping (EMR) settings (5 V and 4 V) and shape change following 6 months of treatment?

Findings

At 5 V, auricle bending was significant at an application time of 4 minutes compared with an application time of 3 minutes. At 4 V, there was no significant difference in auricle bending between an application time of 4 minutes or 5 minutes.

Meaning

The most optimal EMR setting used in this study was 5 V for 4 minutes, which resulted in the most prominent bend, with a marginal 2-mm circumferential tissue injury at each electrode.

Methods

Study Design

We optimized the EMR variables through 2 iterations of the study. Fourteen New Zealand white rabbits (Western Oregon Rabbit Company) ranging in age from 6 to 8 months were divided into 2 groups. Group 1 (n = 7) received EMR with variables previously identified²⁷ for effective reshaping, including 5 V for 4 minutes (5 ears), 4 V for 5 minutes (5 ears), or no voltage application (control; 4 ears). These rabbits were killed humanely after 6 months to assess the structural integrity, cell viability, and shape retention of the ears. The ears were palpated and their mechanical behavior was video recorded. The ears were then harvested and processed for conventional histologic and cellular viability analysis using laser scanning confocal microscopy. The results from this group of animals led to refinement of EMR variables with a reduction of voltage application time for the second group. The remaining 7 rabbits (group 2) underwent EMR using 5 V for 3 minutes (7 ears) or 4 V for 4 minutes (7 ears).

The protocol was performed under the aegis of the Institutional Animal Care and Use Committee of the University of California, Irvine, and the Animal Care and Use Review Office of the US Army.

EMR Procedure

New Zealand white rabbits weighing 3.8 to 4.0 kg were injected with ketamine hydrochloride (20–40 mg/kg) and xylazine hydrochloride (3–5 mg/kg) into the thigh muscle. Once sedated, lidocaine hydrochloride, 1%, with a solution of epinephrine, 1:100000, was subcutaneously injected at the base of the ear to provide regional anesthesia proximal to the surgical site. The ears were shaved, and visible blood vessels flanking the ear were demarcated to avoid needle insertion. A custom acrylic jig was used to deform the distal third of the ear into a 90° bend and to provide guidance slots for the insertion of 20 platinum needle electrode pairs (Grass Technologies) during EMR (Figure 1). Electrodes were connected to a power supply, inserted through the jig and ear, and held in place with a triangular wedge of synthetic cork. Four EMR settings were investigated (4 V for 4 minutes, 4 V for 5 minutes, 5 V for 4 minutes, or 5 V for 3 minutes) using a power supply that was controlled using custom-written software (LabVIEW; National Instruments). After the first application of EMR, the jig was moved 2 mm distal to the first set of needle insertion points, and EMR was repeated, creating a double row of EMR-treated tissue regions. Temporal changes to voltage and current were recorded during EMR, and the total charge transfer was calculated. After EMR, the jig was removed, and the ear was secured around a cotton bolster (1.5 cm in diameter) and secured in place with 2–0 polypropylene sutures and soft silicon sheeting. The bolsters were used to retain the bend of the jig. Control ears were subjected to the same bending protocol, with the insertion of needle electrodes but without current application. The duration of the procedure lasted 50 to 70 minutes (including sedation, site preparation, etc) for both ears, although the actual reshaping time was 10 minutes or less. Data were collected from March 14, 2013, to July 8, 2014.

Postoperative Procedure

The splinted ears were photographed at regular intervals. The animals were observed for any change in behavior and eating patterns and were examined for any signs of local infection on the ears. Any loose sutures on the splint were replaced. The ears were continuously splinted for 6 months after EMR.

Splints were removed 1 day before the rabbits were killed, and shape change and appearance of the ears were recorded on the day of splint removal. The animals were killed humanely with an intraperitoneal injection of pentobarbital sodium (100 mg/kg). The ears were immediately shaved and palpated to assess the gross shape stability and mechanical behavior. The steady state shape was first photographed while the base of the ear was held upright by hand. Next, the ears were videotaped and palpated, first by straightening the bend of the ear and then by bending it completely to observe tissue recoil. The bend angle (outer angle) of each ear was measured from photographs using available software (Photoshop; Adobe Systems). We used a 2-tailed *t* test to determine any significant difference in bend angles between ears in the EMR and control groups. Data were analyzed from August 29, 2013, to March 1, 2015.

Microscopy and Histologic Analysis

Whole ears were excised from the crania and immediately dissected to prepare specimen samples for histologic and cellular viability analyses. Samples were obtained from the thick and thin regions of the ear. These samples were then cut in half to produce adjacent tissue sections for confocal microscopy and hematoxylin-eosin–stained histologic analysis. Each ear produced 4 specimens, including 2 for each examination type.

The specimens were prepared for hematoxylin-eosin staining and cell viability assays (LIVE/DEAD; Molecular Probes Inc) as previously described.^{23,27} To assess the viability of chondrocytes, specimens were stained with the fluorophores calcein acetomethoxy and ethidium homodimer 1 and underwent confocal microscopy (Meta 510; Carl Zeiss LSM). Digital images were recorded and analyzed. The width of tissue injury at the electrode insertion sites was measured.

Results

All rabbits tolerated EMR and survived without any local or systemic complications. No infection, hemorrhage, hematoma formation, skin slough, or soft-tissue necrosis was observed. Exudative crusts formed on the surface of the ears at the electrode insertion sites within the first day, as observed previously.^{23,27} The degree of crusting was proportional to the EMR dose (eg, higher voltage or longer application time), and crusts fell off after 1 week. When the splints were removed at 6 months, normal-appearing skin was noted to be fully present over the needle insertion sites, although dense fur growth was absent. Normal hair regrowth was observed on the rest of the ear.

Mechanical Evaluation

The ears underwent subjective evaluation for strength and mechanical changes when the splints were removed at 6 months after EMR. The ears treated with EMR did not appear grossly different from the control ears in terms of structural integrity or elastic recoil. Even after the ears were straightened for examination, they returned to their reshaped position. Despite removal of the splints 24 hours before the rabbits were killed, the overall shape and bend of the ears were maintained.

Shape Change

In general, the bend angle increased with voltage or application time variables compared with control ears (Figure 2). Control ears had some shape retention compared with native and untreated ears, although retention was less dramatic than in the EMR-treated ears. The mean bend angle increased from 81° in control ears to 125° in the ears treated with 5 V for 4 minutes (Figure 3). The mean(SD) bend angles in the 5 groups were 81° (45°) for the controls (group 1), 72° (29°) for 4 V for 4 minutes of EMR, 101° (19°) for 4 V for 5 minutes of EMR, 78° (18°) for 5 V for 3 minutes of EMR, and 126° (21°) for 5 V for 4 minutes of EMR. Using 5 V for 4 minutes produced significant shape change compared with 5 V for 3 minutes (2-tail *t* test, *P* = .003). Two rabbits were used for 4 ear control data points. One rabbit with splinted-only control ears had larger bend angles than the other rabbit, which accounted for the higher mean (SD) bend angle. Ear reshaping increased with the amount of

electrical charge transferred through the tissue, which corresponds to the voltage level and duration of treatment.

Histologic Evaluation

As previously noted in the 3-month trials,^{23,27} small circular defects in the cartilage approximately the size of the electrodes at the needle insertion also persisted 6 months after treatment (Figure 4). Changes in the cell distribution and extracellular matrix rearrangement were observed in control (0 V) and EMR-treated ears. Cartilage of control specimens remained intact, with the presence of chondrocytes encased in lacunae surrounding the needle-electrode insertion sites. New chondrocytes could be seen forming a layer next to the mature cartilage near the electrode insertion site (black arrowhead in Figure 4A). In contrast, EMR-treated ears distinctly retained some of the shape and curvature of the jig in group 1 (4 V at 5 minutes [Figure 4C] and 5 V at 4 minutes [Figure 4E]). Overall, group 2 ears (Figure 4B and D) retained less of a bend after histologic processing. In all EMR-treated specimens, sites of needle-electrode insertion surrounding the apex of the bend are characterized by a small gap of missing cartilage. Focal chondrocyte injury with fibrosis could be seen at the electrode insertion site (pink arrowheads in Figure 4B and D). Fibrocartilage can be seen forming on the outer surface of the cartilage (yellow arrowheads in Figure 4C and E) in histologic specimens with significant shape retention and bending. In contrast, the inner surface of the bend showed a dense tissue matrix (light blue arrowheads in Figure 4C and E). Control and treated ears displayed signs of chondroneogenesis near the vicinity of needle insertion sites.

Confocal Microscopy and Chondrocyte Viability

Chondrocyte viability was determined using a well-established cell viability assay and laser scanning confocal microscopy. A montage illustrating the distribution of live and dead cells around the needle electrode site for controls and those specimens treated with 4 or 5 V of EMR is depicted in Figure 5. A clear and distinct demarcation between viable cells (green) and the absence of red fluorescence at the electrode insertion sites can be seen. The extent of this tissue damage is very small: on the order of 2 to 3 mm for 4 V for 4 minutes and 5 V for 4 minutes. Reducing the application time by 1 minute when using 5 V or 4 V resulted in less tissue injury, ranging from 0.4 to 2.2 mm for 5 V for 3 minutes and 0.5 to 1.9 mm for 4 V for 4 minutes. This circumferential zone of injury (range, 1.3–2.1 mm) corresponded to absence of red fluorescence on the cell viability assay. In some specimens, the presence of viable cells in the center of the electrode insertion site was identified. In addition, living fibroblasts with green fluorescence were present in the subdermal soft tissue. Chondrocyte injury occurred only at the needle insertion sites, and the width of tissue injury was independent of total charge transfer for this narrow set of variables (Figure 3). In control ears, no surrounding tissue injury was observed apart from defects in the cartilage where the needles were placed.

Discussion

Previous work focused on the outcomes of EMR 3 months after reshaping in rabbit ears.^{23,27} For most preliminary studies, 3 months is a very practical time to chart the acute and

subacute phases of wound healing. In addition, these previous studies demonstrated a dose-response relationship between EMR and shape change and defined voltages that resulted in adequate shape change of rabbit ears. The present study optimizes those voltages and times and expands the information on tissue remodeling, apoptosis, and necrosis in the setting of a longer survival period (6 months).

The acute changes in the skin that we observed during recovery and in the week after the EMR procedure were identical to those observed in the previous studies, namely, limited alopecia and the formation of exudate.^{23,27} During the longer 6-month interval, we did not observe any tissue necrosis, further alopecia, or significant compromise to cartilage integrity on visual inspection. Because the costs of long-term survival studies are high, the numbers of animals in the study were small, and we did not perform mechanical analysis, which was performed in the original study demonstrating the feasibility of EMR *in vivo*.²³

In terms of the application to clinical therapy, this longer-term survival study demonstrates that EMR is at least clinically feasible because shape change persists and no overt identifiable negative structural change is seen (ie, structural collapse, necrosis, or hematoma formation or organization). Bolsters to guide wound healing will not be sutured onto a human ear and attached for days on end. Instead, a removable splint that would hold the ear to the desired flexure would likely be worn while the ear heals. This splint would act like a retainer akin to clear aligners for teeth, where wound healing and remodeling is just as important for shape change. In otoplasty, no consensus exists for how long bandages and head dressings remain in place. Also, sutures in essence act like permanent splints, constantly countering deformation forces of the antihelix.

The electrical doses that were investigated represent the exploration of a narrowed variable space based on findings from previous *in vivo* works.^{23,27} Rabbits that underwent EMR with 4 V for 5 minutes or 5 V for 4 minutes showed the most shape change compared with the animals that received lower, shorter application times of the same voltage but at the expense of a modest increase in tissue injury around insertion sites. An increase of 1 minute—from 4 minutes to 5 minutes at 4 V or from 3 minutes to 4 minutes at 5 V—widened the cross-sectional tissue injury by a mean of 0.25 mm per electrode (Figure 3). Some control ears maintained shape retention and resulted in a mean of 81° compared with EMR-treated ears, in which the bend angle varied from 72° to 126° depending on the EMR variables. More importantly, shape retention is greater with a longer splint duration compared with the previous study with a 3-month splinting duration using the same dose of 4 V for 4 minutes (3 months produced 60° [15°]; 6 months produced 72° [29°]) or 5 V for 4 minutes (3 months produced 117° [21°]; 6 months produced 126° [21°]).²⁷ This study shows that shape change has a dose-response relationship, whereby applying 4 V for 5 minutes or 5 V for 4 minutes can achieve better reshaping of rabbit ears than 4 V for 4 minutes or 5 V for 3 minutes.

At the microscopic tissue level, our results (Figure 4) are consistent with previous histologic outcomes determined at 3 months,²⁷ with tissue modification approximately 2 to 3 mm in diameter at the insertion sites. These affected regions are filled with scar tissue or, in some cases, possibly regenerating chondrocytes. The surrounding peripheral tissue remains unharmed. We found a distinctive difference in cell morphologic features between the outer

surface of the auricular cartilage experiencing tension (yellow arrow heads in Figure 4) and the inner surface of the auricular cartilage experiencing compression (light blue arrowheads in Figure 4) in histologic specimens with shape retention. This difference was not noted in the previous study at 3 months.²⁷

Use of the cell viability assay with confocal microscopy echoes similar findings to histologic hematoxylin-eosin staining and provides valuable information on the extent of cell viability around the electrodes. However, preparing specimens to obtain precise cross-sections at the electrode sites is technically challenging and limits the true extent of injury of an electrode insertion site. Often, the specimen integrity is compromised owing to simply the action of the scalpel blade or, as we are dealing with, a complex flexure-curvature across the needle electrode insertion sites. Because imaging within 1 focal plane of the region of interest with confocal microscopy is also challenging, cross-sectional tissue injury data could not be obtained for every single specimen. For the limited data we have, the extent of tissue injury very clearly mirrors that observed in the previous *in vivo* studies in rabbits^{23,27} and in numerous investigations performed on *ex vivo* cartilage tissues.^{18,22,26,28}

Although we found a clear indication of tissue injury at needle electrode insertion sites, in some instances we observed the presence of live cells (green fluorescence) (Figure 5C and D). In general, fibroblasts do not survive the cartilage tissue preparation we used for confocal microscopy. In the limited cases in which we can compare these midline distributions of green cells on confocal microscopy with histologic specimens, we did see what appears to be chondrocyte populations, and these cells appear to be more similar to chondrocytes than fibroblasts.

Another interesting point that is unique to our study compared with the previous *in vivo* findings is that the total charge transfer is independent of the greatest injury length (Figure 3). In previous studies, the greatest width of tissue injury increased with escalating charge transfer *in vivo* and *ex vivo*.^{23,27} In Figure 3, we note that EMR settings used in the first phase of this study (4 V for 5 minutes and 5 V for 4 minutes) produced the largest width of tissue injury made by the electrodes. One possible explanation for this finding is a possible overlap of tissue injury with an adjacent electrode, which produces a measurement that is double the true length for a given electrode insertion site. Discerning whether the measured distance was owing to 1 or 2 needle electrodes is difficult because no characteristic boundaries (green fluorescent cells) were seen to delineate separation. If regions being measured did not overlap, then the total charge transfer would increase with tissue injury diameter made by the redox reactions in the vicinity of the electrode. Likewise, the EMR settings for application time and voltage were reduced in the current study and the broad dose-response approach used in the previous investigations was not used. Lack of dependence on charge transfer may reflect these variables, achieving a largely equivalent clinical outcome.

Expanding the specimen numbers would be ideal, but execution was cost prohibitive and limited the amount of subjects. As such, the control ears produced a notably higher mean bend angle compared with previous studies.^{23,27} One reason for this larger variance could be owing to the fact that the 4 designated control ears were assigned to only 2 rabbits; assigning

control ears to 4 separate rabbits could possibly decrease this variance. Our measurements show that the 2 largest control ear bend angles were obtained from the same rabbit. Another reason for the variance in bend angle is the minimal time (24 hours) allotted for the control ears to equilibrate from the time the bolsters were removed to the moment the rabbits were killed. Allowing the control ears to equilibrate unbolstered for a longer period may have demonstrated greater recidivism, but we wanted to be consistent with the protocol from the 2 previous studies.^{23,27}

On palpation, treated ears were able to maintain their reshaped geometry, and no qualitative decrease in thickness along the bend was observed (Video). In some specimens, palpation suggested that the cartilage was thicker or dense, although no objective data supported this hypothesis; these observations may be related to fibrosis of the skin–soft-tissue envelope surrounding the flexure. Palpation was performed by the same investigator (C.T.M.) who participated in previous studies^{23,27} to maintain consistency. A detailed investigation on quantifying the mechanical behavior of treated composite ears will need to be performed to achieve amore accurate measure but is beyond the scope of the present study. At the very least, video data showing bent and straightened ears returning to their equilibrated shapes 6 months after EMR demonstrate the stability of ears after treatment.

Electromechanical reshaping has been shown to be an effective means to shape cartilaginous tissue without the use of scalpels, sutures, or heat.^{18,21–23,26,27} Electromechanical reshaping is a low-cost, simple technology that has the potential to be used in the outpatient setting as a minimally invasive otoplasty technique. This study demonstrates the optimal voltage and time variables to achieve long-term, clinically relevant degrees of cartilaginous shape change without causing significant tissue injury. The application of EMR to long-term shape change holds promise as a future method in correcting deformities such as the protruding ear in humans.

Conclusions

Electromechanical reshaping at 4 or 5 V produced adequate reshaping while keeping the tissue injury at each electrode within a few millimeters. Using 5 V for 4 minutes provided the most significant shape change. A longer splint duration of 6 months generally produced greater bend angles compared with a shorter splint duration of 3 months. This study provides a more optimal set of EMR variables and can be regarded as a milestone toward evaluation of EMR in clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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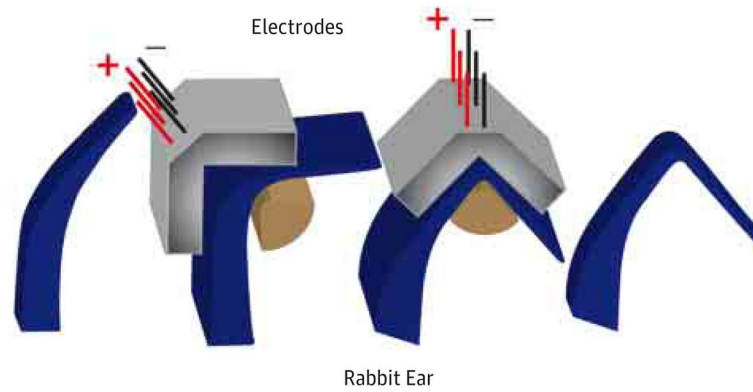


Figure 1. Electromechanical Reshaping Procedure

A custom acrylic and foam jig is attached to the ear with positive and negative needle electrodes holding the shape of a 90° bend. Low-level direct current is applied for a short period. Electrodes and moulage are removed to prepare for bolstering.

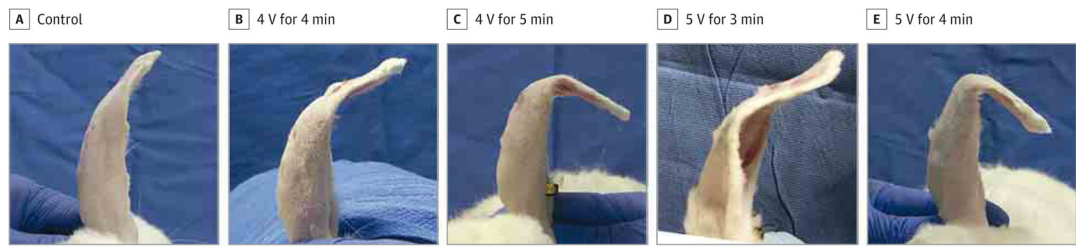


Figure 2. Rabbit Ears After Electromechanical Reshaping (EMR) and 6 Months of Splinting
Representative photographs depict results of each EMR dose. The control ears underwent no EMR.

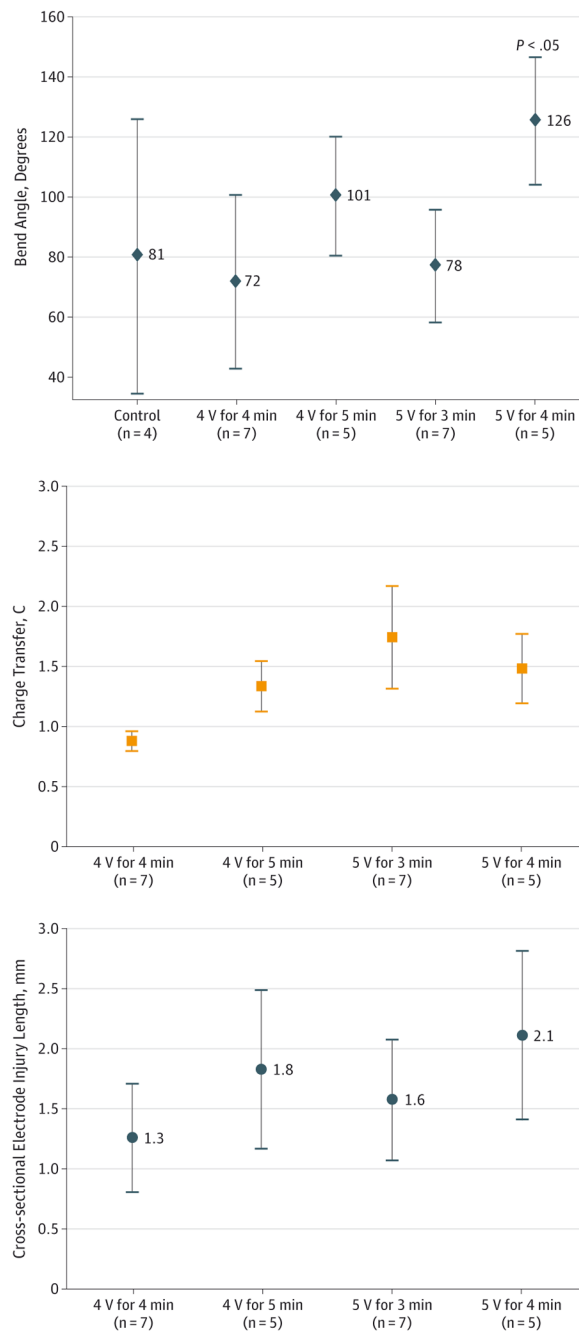


Figure 3. Association of Bend Angle With Electrode Tissue Injury and Total Charge Transfer
 Data are expressed as mean (SD). C indicates coulomb; diamonds, bend angle; and error bars, 1 SD.

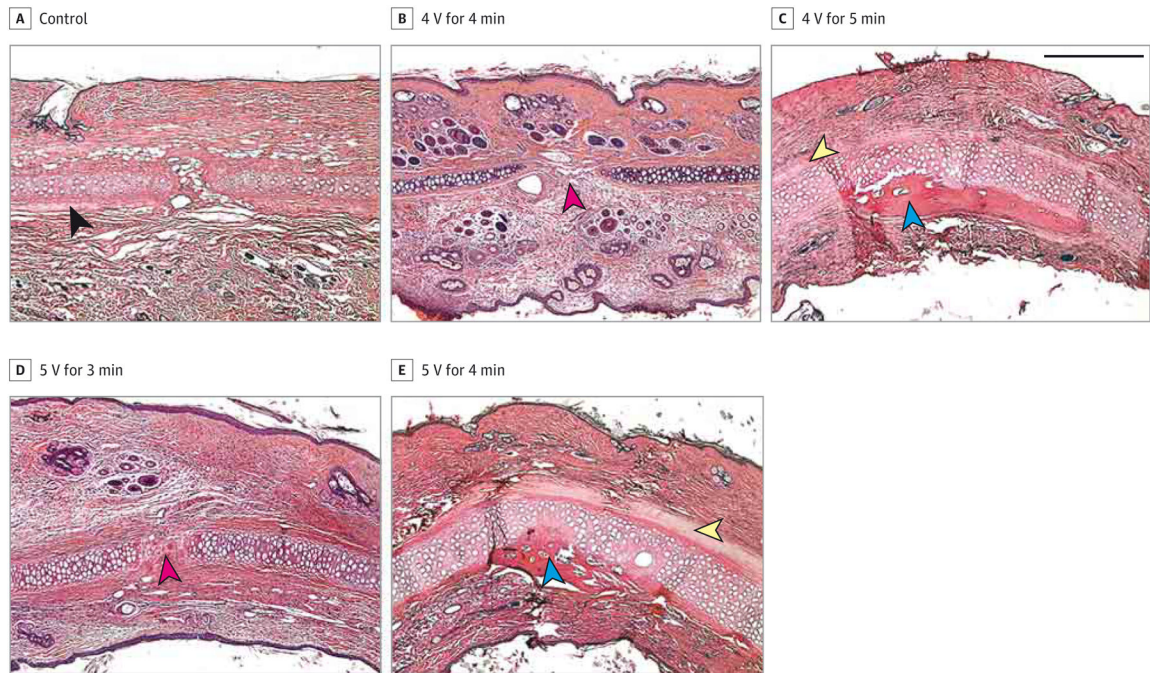


Figure 4. Histologic Findings of Increased Cellular Injury With Increased Electrical Dose
Solid black arrowhead indicates area of chondroneogenesis; pink arrowheads, areas of fibrosis at needle electrode insertion sites; yellow arrowheads, formation of fibrocartilage; and blue arrowheads, dense extracellular tissue matrix at needle electrode insertion sites (hematoxylin-eosin; scale bar indicates 0.5 mm).

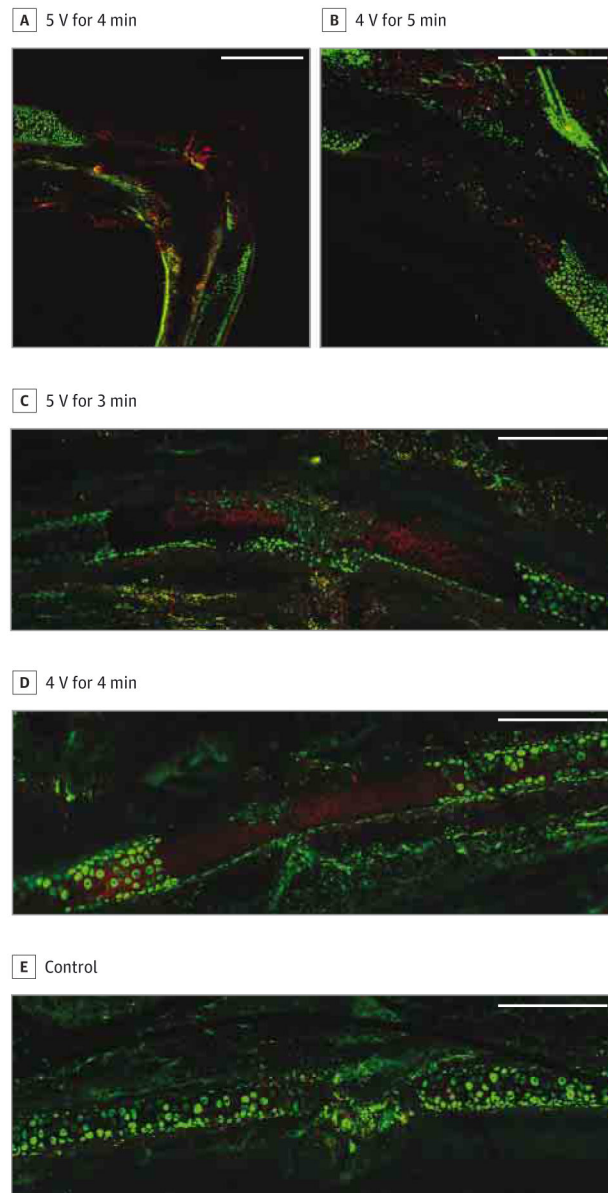


Figure 5. Confocal Microscopy With the Cell Viability Assay

Areas of nonviable chondrocytes (diffuse red) are shown where the needle electrodes were inserted. Healthy living chondrocytes (green) surround these distinct regions. A and B, Treated samples of group 1 (scale bar, 0.5 mm). C and D, Treated samples of group 2 (scale bar, 1 mm). E, Control sample from group 1 (scale bar, 1 mm) (stained with calcein acetomethoxy and ethidium homodimer 1).