Published in final edited form as:

JAMA Facial Plast Surg. 2014 July 1; 16(4): 245–252. doi:10.1001/jamafacial.2014.85.

In Vivo Needle-Based Electromechanical Reshaping of Pinnae:

New Zealand White Rabbit Model

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Abstract

IMPORTANCE—Electromechanical reshaping (EMR) is a low-cost, needle-based, and simple means to shape cartilage tissue without the use of scalpels, sutures, or heat that can potentially be used in an outpatient setting to perform otoplasty.

OBJECTIVES—To demonstrate that EMR can alter the shape of intact pinnae in an in vivo animal model and to show that the amount of shape change and the limited cell injury are proportional to the dosimetry.

DESIGN, SETTING, AND SPECIMENS—In an academic research setting, intact ears of 18 New Zealand white rabbits underwent EMR using 6 different dosimetry parameters (4 V for 5 minutes, 4 V for 4 minutes, 5 V for 3 minutes, 5 V for 4 minutes, 6 V for 2 minutes, and 6 V for 3 minutes). A custom acrylic jig with 2 rows of platinum needle electrodes was used to bend ears at the middle of the pinna and to perform EMR. Treatment was repeated twice per pinna, in proximal and distal locations. Control pinnae were not subjected to current application when being bent and perforated within the jig. Pinnae were splinted for 3 months along the region of the bend using soft silicon sheeting and a cotton bolster.

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Author Contributions: Dr Yau and Mr Manuel had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr Yau and Mr Manuel contributed equally to this work.

Study concept and design: Yau, Manuel, Protsenko, Wong.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Yau, Manuel.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Yau, Manuel.

Obtained funding: Wong.

Administrative, technical, or material support: Yau, Wong.

Study supervision: Manuel, Protsenko, Wong.

Conflict of Interest Disclosures: Mr Manuel is a paid consultant for Praxis BioSciences, LLC. Dr Wong has intellectual property (licensed by Aerin Medical, Inc and by Praxis BioSciences, LLC) that pertains to the research performed in this study. Electromechanical reshaping is licensed by Aerin Medical, Inc. Dr Wong also has equity with Praxis BioSciences, LLC. 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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MAIN OUTCOMES AND MEASURES—The ears were harvested the day after splints were removed and before euthanasia. Photographs of ears were obtained, and bend angles were measured. Tissue was sectioned for histologic examination and confocal microscopy to assess changes to microscopic structure and cellular viability.

RESULTS—Treated pinnae were bent more and retained shape better than control pinnae. The mean (SD) bend angles in the 7 dosimetry groups were 55° (35°) for the control, 60° (15°) for 4 V for 4 minutes, 118° (15°) for 4 V for 5 minutes, 88° (26°) for 5 V for 3 minutes, 80° (17°) for 5 V for 4 minutes, 117° (21°) for 6 V for 2 minutes, and 125° (18°) for 6 V for 3 minutes. Shape change was proportional to electrical charge transfer, which increased with voltage and application time. Hematoxylin-eosin staining of the pinnae identified localized areas of cell injury and fibrosis in the cartilage and in the surrounding soft tissue where the needle electrodes were inserted. This circumferential zone of injury (range, 1.5-2.5 mm) corresponded to dead cells on cell viability assay, and the diameter of this region increased with total electrical charge transfer to a maximum of 2.5 mm at 6 V for 3 minutes.

CONCLUSIONS AND RELEVANCE—Electromechanical reshaping produced shape change in intact pinnae of rabbits in this expanded in vivo study. A short application of 4 to 6 V can achieve adequate reshaping of the pinnae. Tissue injury around the electrodes increases with the amount of total current transferred into the tissue and is modest in spatial distribution. This study is a critical step toward evaluation of EMR in clinical trials.

LEVEL OF EVIDENCE—NA.

The prominent ear, also known as cup ear or lop ear, is a congenital malformation in which the pinna projects more than the normal distance from the skull. Auricular deformities are common, with documented incidences ranging from 5% to 15% of all newborns in the United States.¹ Overprojection of the ear may be secondary to an increase in the conchal mastoid angle, an excess of conchal cartilage, or an absence of the antihelical fold. Combinations of these anatomic variants frequently occur. Despite limited physiological consequences of having a prominent ear, it can be a source of ridicule and teasing, especially in school-aged children.

Otoplasty corrects structural and aesthetic defects resulting from trauma, cancer, or congenital malformations. Surgical treatments have been described since the 7th century AD, although most contemporary efforts to reshape the ear build on the work by Jack Mustarde, MD, FRCS, and others.^{2,3} Modern otoplasty operations rely on suturing, cutting, and morselizing or removal of conchal cartilage. A combination of these techniques is frequently required to balance the intrinsic elastic and structural forces. Conventional surgery has disadvantages, including cost, blood loss, recovery time, scarring, and other complications.

There has been a move to less invasive methods of performing otoplasty, particularly toward non-cartilage-splitting techniques. For example, the cartilage in newborns is malleable, and splints or molds can be placed on the auricle to reshape the ears.⁴ However, nonsurgical methods in older children and adults have largely been laser based.⁵⁻¹⁰

We developed electromechanical reshaping (EMR) as a means to shape cartilage tissue without the use of scalpels, sutures, or heat.¹¹⁻²⁰ In EMR, cartilage is mechanically deformed by a jig, and needle electrodes are inserted into regions of increased internal stress. Current is applied, and in situ redox reactions occur in the tissue without heat generation. The jig is removed, and stable shape change is achieved. Electromechanical reshaping creates shape change in cartilage tissue and in composite cartilage grafts.¹¹⁻²⁰ It is a low-cost, needle-based, and simple technology that can potentially be used in an outpatient setting to perform otoplasty. Herein, we present an expanded in vivo study that refines and better identifies the dosimetry parameter space for auricular EMR in the rabbit, further demonstrating the feasibility of this emerging technology.

Methods

Study Design

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 United States Army. Thirty-six ears in 18 New Zealand white rabbits (Western Oregon Rabbit Company) were divided into the following 7 dosimetry groups: control (n = 5), 4 V for 4 minutes (n = 5), 4 V for 5 minutes (n = 6), 5 V for 3 minutes (n = 5), 5 V for 4 minutes (n = 5), 6 V for 2 minutes (n = 5), and 6 V for 3 minutes (n = 5). The rabbits were allowed to survive for 3 months following EMR. Resultant shape change and mechanical behavior were documented with digital imaging and videography. Auricular tissue was then harvested and processed for conventional histologic analysis and for cellular viability analysis using laser scanning confocal microscopy.

EMR Procedure

The method used to reshape rabbit ears was previously described and will be briefly reviewed.¹⁷ New Zealand white rabbits, weighing 3.8 to 4.0 kg, were sedated using intramuscularly injected ketamine hydrochloride (20-40 mg/kg) and xylazine (3-5 mg/kg). Once sedated, 1% lido-caine with 1:100 000 epinephrine was subcutaneously injected at the base of the ear to achieve a regional block proximal to the surgical site. The ears were shaved, and visible blood vessels were marked with ink to avoid needle insertion. A custom jig (eFigure 1A in the Supplement) was used to deform the ear at the junction of the distal third and the middle third (eFigure 1B in the Supplement) into a 90° bend and to provide guidance slots for the insertion of 20 platinum needle electrode pairs (Grass Technologies) during EMR (eFigure 1A in the Supplement). Electrodes spaced 2 mm apart were inserted through the jig and percutaneously through the ear (eFigure 1C in the Supplement). The electrodes were then connected to leads from a direct current power supply (PPS-2322; Amrel). Six voltage and application time parameters were investigated (4 V for 4 minutes, 4 V for 5 minutes, 5 V for 3 minutes, 5 V for 4 minutes, 6 V for 2 minutes, and 6 V for 3 minutes). Current delivery and data collection were controlled using a software program (LabView; National Instruments). The current parameters expanded the dosimetry values identified in pilot investigations.¹⁸ Subsequently, 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 (eFigure 1B in the Supplement). Total charge transfer was recorded. 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 mimic the 90° bend of the jig. The control ears were subjected to the same bending scheme, with the insertion of needle electrodes but without current application. The procedure was repeated on the contralateral ear. The duration of the whole procedure lasted 60 to 75 minutes, although the actual reshaping time was 10 minutes or less.

Postoperative Procedure

The splinted ears were photographed at regular intervals. The animals were observed for any changes in behavior and eating patterns and were examined for any signs of local infection on the ears. Loose sutures on the splint were replaced. The ears were continuously splinted for 3 months following EMR.

Microscopy and Histologic Analysis

Splints were removed 1 day before euthanasia, and shape change and appearance of the ears were recorded on the day of splint removal. Euthanasia was accomplished with intraperitoneally injected pentobarbital sodium (100 mg/kg). The ears were immediately photographed, videotaped, and palpated to assess the gross mechanical behavior and shape stability. Ears were held at the base and the root directed superiorly, allowing the tips to fall with gravity. The resting bent shape was recorded, and the ear was then palpated in 2 ways, first by straightening the bend of the ear and letting it fall back down and then by bending it completely to observe tissue recoil. The analysis of static shape change was measured from photographs using available software (Photo-shop 9.0; Adobe Systems). Statistical analysis with regression correlations was performed with a spreadsheet (Excel; Microsoft Corporation).

Whole ears were excised from the crania and immediately dissected to prepare specimen samples for histologic and cellular viability analyses. Because rabbit ear cartilage is thicker anteriorly and thinner posteriorly, samples were obtained from both regions to determine if EMR is affected by tissue thickness. In each tissue region, a sample for confocal microscopy and hematoxylin-eosin histologic examination was removed from adjacent regions. Therefore, each ear resulted in 4 specimens, 2 for confocal microscopy and 2 for histologic examination.

The specimens were prepared for hematoxylin-eosin staining and cell viability assay (LIVE/DEAD; Molecular Probes Inc) as previously described.^{18,19,21-23} To assess the viability of chondrocytes, specimens were stained with the fluorophores calcein acetomethoxy and ethidium homodimer 1 and were imaged with a confocal microscope (Meta 510; Carl Zeiss LSM). Digital images were recorded and analyzed. Cartilage thickness and the width of tissue injury were 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. The degree of crusting was proportional to EMR dosimetry (eg, higher voltage or longer application time), and crusts fell off after 1 week (eFigure 2A in the Supplement). When the splints were removed at 3 months, normal-appearing skin was noted to be fully present over the needle insertion sites (eFigure 2B and C in the Supplement), although dense fur growth was not present at the needle insertion sites. Normal hair regrowth was observed on the rest of the ear.

Mechanical Strength

The ears were subjectively evaluated for strength and mechanical changes when the splints were removed at 3 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 (eFigure 2C in the Supplement), they returned to their reshaped position. Despite removal of the splints 24 hours before euthanasia, the overall shape and bend of the ears were maintained.

Shape Change

In general, the bend angle increased as the voltage or application time parameters increased. The bend angle increased from 55° in the splinted-only control ears to 125° in the ears treated at 6 V for 3 minutes. The mean (SD) bend angles in the 7 dosimetry groups were 55° (35°) for the control, 60° (15°) for 4 V for 4 minutes, 118° (15°) for 4 V for 5 minutes, 88° (26°) for 5 V for 3 minutes, 80° (17°) for 5 V for 4 minutes, 117° (21°) for 6 V for 2 minutes, and 125° (18°) for 6 V for 3 minutes. The EMR-treated ears had greater shape change than the control ears (**Figure 1**). The bending of auricular cartilage increased with total charge transfer. The control ears had some modest shape change compared with native ears, but it was less than that in the EMR-treated ears. Ear reshaping increased with the amount of electrical charge transferred through the tissue, which corresponds to the voltage level and duration of treatment (eFigure 3A in the Supplement). We observed a mild positive linear correlation of the charge transferred to the bend angle, with a regression correlation of 0.08. Shape retention and the bend angle of histologic specimens increased with increasing specimen thickness for each EMR voltage and application time setting, with a weakly positive correlation (eFigure 3B in the Supplement).

Histologic Evaluation

After histologic processing, the curvature of the reshaped specimen was still evident (eFigure 4 in the Supplement). The sites of needle insertion surrounded the apex of the bend. Focal chondrocyte injury with fibrosis could be seen at the electrode insertion site. Surrounding the insertion sites, areas of new cartilage formation (neochondrogenesis) were observed. The perichondrium was thickened with a layer of fibrosis at the subdermal-supraperichondral region. The diameter of chondrocyte injury zones increased with the dosimetry (Figure 2) and ranged from 1.5 to 2.5 mm. The diameter of this region increased with total charge transfer to a maximum of 2.5 mm at 6 V for 3 minutes. The perforation diameter was consistent, with a needle diameter of 300 μ m. The injury was similar at anode and cathode electrode sites, and the different electrode sites could not be identified from

histologic findings alone. When the tissue injury diameter substantially exceeded the electrode diameter, the discrete needle insertion sites could not be identified on histologic examination (Figure 2F and G) because the region of tissue injury and fibrosis seemed to be confluent.

Confocal Microscopy and Chondrocyte Viability

Chondrocyte viability was determined using the cell viability assay and laser scanning confocal microscopy. In the assay, viable cells stained green, while dead cells without intact cell membranes fluoresced red. At the electrode insertion sites, localized chondrocyte death was present as diffuse red fluorescence. In addition, living fibroblasts with green fluorescence were present in the subdermal soft tissue. Viable chondrocytes (green fluorescence) encased within their lacunae were present in the surrounding cartilage (Figure 3). The extent of chondrocyte injury was modest, ranging from 1.5 to 2.5 mm, and occurred only at the needle insertion sites. This injury diameter was similar to that estimated using light microscopy. In control ears, no surrounding tissue injury was observed. The tissue injury increased with total charge transfer (Figure 4).

Discussion

Before this work, EMR had been performed in several fresh ex vivo tissues, including cartilage and tendon, and in composite specimens such as the intact auricle.¹¹⁻¹⁷ One in vivo study¹⁸ of EMR has been published, which demonstrated the feasibility of creating shape change without cutaneous tissue injury or significant adverse consequences. In the present study, a broader range of dosimetry parameters was examined to understand shape change dependence on electrical parameters. Shape change correlates with total charge transfer and dosimetry, while the tissue injury is spatially limited to clinically acceptable levels (2.5-mm-diameter regions of injury). Electromechanical reshaping is a low-cost, needle-based technology that relies on in situ redox reactions to produce shape change. The nonthermogenic nature of this reshaping modality^{11,12} is appealing compared with other nonsurgical methods of tissue shape changing that use laser or radiofrequency technology to heat and then reshape tissue. This study shows that shape change has a dose-response relationship, whereby applying 4 to 6 V can achieve adequate reshaping of rabbit ears.

The dosimetry parameters that were investigated were based on data obtained from pilot in vivo work¹⁸ as a launch point and on variables obtained from ex vivo studies.^{13-16,19} After removal of the bolsters at 3 months, the control and EMR specimens manifested shape change. The control ears showed a mean of 55° in shape change compared with the EMR-treated ears, in which the bend angle varied from 60° to 125° depending on the dosimetry parameters. Clearly, 4 V for 4 minutes is inadequate in this animal model.

Future experiments will determine the effects of shape change as determined by different geometric configurations of the electrode placement, which may enhance the shape change effect. Previous work has shown that the anode and cathode electrodes must be reasonably close to one another to minimize impedance.¹⁰⁻¹⁴ It seems that shape change depends on the charge transferred, and it appears that voltage and application time are not key determinants (eFigure 3 in the Supplement). This is consistent with our understanding of EMR as a

Faradic process, as reduction or oxidation processes occur at the electrodes.¹² The charge transferred depends on the electrical and geometric properties of the tissue (ie, thickness of the specimen, amount of cutaneous or dermal tissues, salinity, water content, etc). Recording the charge transferred might be a means to monitor and control this process. However, an increase in EMR dosimetry above a certain limit may result in gradual saturation of shape change because the collagen matrix may become degraded by the products of electrochemical reactions. In this study, the linear shape change– charge transferred dependence, as well as the mild amount of tissue injury, suggest that the present dosimetry is well below the saturation level. The moderate amount of data variation may be attributable to charge that was dissipated to the skin and subcutaneous structures, as well as variability in the tightness of the bolsters when they were placed. The intersection of the regression curve indicates that in control ears without the application of current an anticipated shape change would be 61°, which is likely from the placement of the bolsters (Figure 2A).

As in previous studies,^{13,16-19} bolsters were used to stabilize shape change for the duration of therapy. During this period, the cartilage undergoes repair and remodeling, including fibrosis in areas of tissue injury and neochondrogenesis. In clinical circumstances, long-term balancing of the native spring within conchal cartilage after otoplasty is accomplished by the placement of retention sutures. The use of ear compression dressings, headbands, or similar appliances following surgery serves a complementary function, but the major load-bearing structures are the internal sutures in conventional surgical otoplasty. This animal model used an external bolster or splint to accomplish this task. If the use of EMR is translated to humans, a splint or moulage would need to be worn for a prolonged period; this would be no different from conventional otoplasty, with some surgeons advocating splinting for longer than 12 weeks.⁴ The exact duration would need to be empirically determined in humans, but we can gain insight from the results of this study, which demonstrate mature fibrosis after 3 months. Obviously, downsides exist to prolonged intermittent use of bolsters. In this study, the bolsters were removed and the ears photographed 24 hours before euthanasia. During the period without bolsters, the behavior of the animals was recorded, and the ears were palpated. The rabbits showed no abnormal behavior in response to the re-shaped ears, although they touched their ears more frequently. After 24 hours, the ears were reexamined with videography and palpation, the rabbits were euthanized, and no gross changes were observed in the mechanical structure of the ears. At this time, the ears were less bent than at 24 hours earlier, when the bolsters were first removed. The 24-hour period without bolster attachment was arbitrarily picked and could be varied in future experiments to determine if the duration of splinting and splint removal affects the overall shape and structure of the ears. Long-term studies will also need to be performed to ascertain the degree of shape change that can be achieved with reduced reliance on bolsters or splints.

On palpation, EMR did not seem to soften or weaken the specimens. Obviously, objective studies of the mechanical strength and elasticity will need to be performed to verify this observation. However, quantifying the postoperative long-term mechanical changes is a difficult task because the geometry of the ear cannot be described with a closed-form mathematical equation, nor can rigorous mechanical analysis be readily performed on a complex composite structure such as the pinna. Previous work with ex vivo cartilage specimens demonstrated a small reduction in tissue elasticity following EMR.²⁰

Light microscopy shows areas of localized tissue injury at the needle insertion sites, where electrolysis occurs. Surrounding these small, discrete, and well-circumscribed regions are normal cartilage, epidermis, dermis, adnexal structures, and subcutaneous tissues. Continued loss of hair at sites of needle insertion was observed at 12 weeks, which would be expected given that EMR shares much in common with traditional electrolysis for hirsutism treatment. Remodeling and repair were present, and new cartilage formation, focal calcification, and scar formation were observed. Cartilage is an avascular tissue and when injured may undergo chondrocyte loss, depletion of proteoglycans, calcification, fibrosis, and (in rare instances) chondroblast repopulation,²³⁻²⁶ and the present observations follow suit. Both chondrocyte loss and new cartilage formation were observed at all needle insertion sites in each EMR sample (Figure 2). As a general trend, these areas of new cartilage formation were most common where the curvature of the specimen is the greatest. Better shape retention was noted in thicker specimens. Treated thicker tissue may have more new cartilage formation, resulting in stronger tissue after healing. In less curved samples and regions, there is less new cartilage formation and more fibrosis at the needle insertion sites, which may be the histologic basis for less curvature and shape change in these specimens. In addition, areas of calcification along the perichondrium are observed. Depending on the amount and type of remodeling (ie, calcification, neochondrogenesis, or fibrosis), the mechanical properties of the treated samples may differ. Additional stains such as periodic acid–Schiff, Alcian blue, or safranin O can be used to better evaluate the extracellular matrix and to analyze proteoglycan changes that occur after EMR, but this was beyond the scope of the present study. In animal osteoporosis investigations, chondrogenic progenitor cells migrated from uninjured regions to repopulate the injured cartilage matrix after chondrocyte death from mechanical injury.²⁷ We believe that similar activities occur after EMR. Future studies will investigate biological and chemotaxic markers that are altered after EMR to determine the molecular changes that occur in tissue during this process.

Similar to prior published results,^{18,19} EMR causes a 1.5-mm to 2.5-mm region of cellular injury at the site of electrode insertion. As with thermal cartilage reshaping methods, laser-based⁵⁻¹⁰ and radiofrequency-based^{28,29} shape change is obtained at the expense of chondrocyte loss. However, the loss of chondrocytes from EMR is limited to the needle insertion sites and herein did not exceed 2.5 mm per electrode. Future studies will need to optimize the number of electrodes necessary to produce the desired shape change, further reducing the risks of tissue injury.

Otoplasty is often performed in the pediatric population. Studies^{30,31} show a minimal effect on the growth of the auricle after traditional methods of otoplasty. Hence, EMR could be performed in the pediatric population and may carry risk factors similar to otoplasty methods of cartilage splitting or abrading. Unlike in the adult population, EMR-driven otoplasty in children may require anesthesia or moderate sedation, and splints would likely need to be worn longer and for greater daily intervals than with conventional otoplasty operations, in which retention sutures form an internal brace.

Conclusions

This study demonstrated a dose-response relationship between the charge transferred and shape change in needle-based EMR of pinnae. Electromechanical reshaping produced titratable results of auricular reshaping in live adult New Zealand white rabbits. This low-cost reshaping method is potentially adaptable to office-based treatments. It is simple to perform and in theory is repeatable to enhance successive results. Little morbidity was observed in these studies, except for acute crusting and relative alopecia at the needle insertion sites. In humans, this splint would likely be replaced with custom-fitted silicon moulage, mimicking the appropriate shape of an ideal ear, although it is conceivable that surgeons could stock a series of low-cost preformed moulages and select the proper size and shape for each individual. In addition, needle-based EMR likely has potential applications in septoplasty, rhinoplasty, and airway applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This work was supported by grant W81XWH-09-1-0602 from the Department of Defense Deployment Related Medical Research Program and by grants DE019026, HL103764, HL105215, and EB015890 from the National Institutes of Health (Dr Wong).

Role of the Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Shown are a control and 5 dosimetries.

Figure 1. Representative Examples of Ears at 3 Months After Electromechanical Reshaping
Shown are a control and 5 dosimetries.

A, Control ear shows no significant cartilage injury at the site of electrode insertion. B-G, All treated samples demonstrate evidence of empty lacunae, cellular remodeling, and fibrosis. Yellow arrowhead indicates calcification, and black arrowhead indicates neochondrogenesis. Scale bar is 1 mm.

Figure 2. Histologic Findings Showing Increasing Cellular Injury With Increasing Dosimetry
 A, Control ear shows no significant cartilage injury at the site of electrode insertion. B-G, All treated samples demonstrate evidence of empty lacunae, cellular remodeling, and fibrosis. Yellow arrowhead indicates calcification, and black arrowhead indicates neochondrogenesis. Scale bar is 1 mm.

Shown are areas of nonviable chondrocytes (red) where the needle electrodes were inserted. Healthy living chondrocytes (green) surround these distinct regions (green). A-F, Treated samples. G, Control ear. Scale bar is 1 mm.

Figure 3. Confocal Microscopy With the Cell Viability Assay (LIVE/DEAD; Molecular Probes Inc)

Shown are areas of nonviable chondrocytes (red) where the needle electrodes were inserted.

Healthy living chondrocytes (green) surround these distinct regions (green).

A-F, Treated samples. G, Control ear. Scale bar is 1 mm.

Total charge transferred is compared with the greatest diameter of cartilage injury.

Figure 4. Total Charge Transferred

Total charge transferred is compared with the greatest diameter of cartilage injury.