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Electromechanical reshaping of rabbit septal cartilage: a six needle electrode geometric configuration

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ABSTRACT

Electromechanical reshaping (EMR) of cartilage is a novel technique that has significant potential for use in facial reconstructive surgery. EMR achieves permanent shape change by initiating electrochemical redox reactions in the vicinity of stress concentrations, thereby altering mechanical properties of tissue matrix. This study reports the use of a six electrode needle-based geometric configuration to reshape cartilage. Rectangular samples (24 x 12 x 1 mm) of rabbit nasal septal cartilages were bent at a right angle in a precision-machined reshaping jig. Two parallel arrays of three platinum needle electrodes were each inserted into cartilage along the bend at 3 mm from the bend line. One array served as an anode and the other as cathode. Constant voltage at 1, 2, 4, 6, and 8 volts was applied to the arrays for 2 minutes. The specimens were then removed from the jig and rehydrated for 15 minutes in phosphate buffered saline. Following rehydration, bend angles and thicknesses were measured. Bend angle increased with increasing voltage and application time. No statistically significant bending was observed below 6 volts for 2 minutes application time. Maximum bend angle of 33 ± 8 degrees or reshaping degree of 33% was observed at 8 volts applied for 2 minutes. Current flow was small (< 0.1 A) for each case. Sample thickness was 0.9 ± 0.2 mm. ANOVA analysis showed that cartilage thickness had no significant impact on the extent of reshaping at given voltage and application time. The six needle electrode geometric configuration conforms to the voltage- and time-dependent trends predicted by previous EMR studies. In the future, the reshaping properties of other geometric configurations will be explored.

Keywords: Electromechanical, reshaping, cartilage, facial reconstructive surgery, otolaryngology

1. INTRODUCTION

Head and neck cartilage tissue is a significant structural element of the nose, ears, larynx, and trachea. Congenital malformations, surgical intervention, or trauma often result in irreparable functional and aesthetic damage to cartilage. As such, an active area of research in facial reconstructive surgery and otolaryngology is effecting long-term cartilage shape change. However, reshaping cartilage in the head and neck has been a continual challenge. Conventional techniques include sculpting and suturing allografts,¹⁻³ which are subject to complications such as graft rejection and time-dependent shape reversion (known as warping). Furthermore, cartilage reshaping by manual means yields highly variable results depending on the skill of the surgeon. In 1993, Helidonis et al pioneered the technique of laser-based thermal-mediated stress relaxation to reshape cartilage.⁴ Since then, quantifiable thermal mechanisms such as laser⁵⁻¹² and radiofrequency¹³ cartilage reshaping have received considerable attention.

Electromechanical reshaping (EMR, electroforming) is a novel, noninvasive technique developed to effect cartilage shape change.^{14, 15} In EMR, direct current (DC) electrical fields are applied through the tissue specimen under mechanical stress, which results in permanent cartilage reshaping. Unlike laser and radiofrequency cartilage reshaping, the mechanism of EMR is not thermal-dependent.¹⁴ Instead, previous studies suggest that the mechanism is electrochemical: oxidation-reduction reactions are spatially localized in the tissue matrix to regions of stress concentrations, causing changes in mechanical properties of cartilage.¹⁵ In addition, the extent of reshaping as measured by bend angle is proportional to voltage and application time.^{14, 15}

The earliest EMR studies were performed using large aluminum electrodes (8 mm radius of curvature) that acted as the source of mechanical compression. A recent adaptation is the use of small platinum needle (0.3 mm diameter) electrodes that directly penetrate, but do not compress, the cartilage specimen. A distinguishing feature of needle electrode-based EMR is the ability to alter the electrical field based on variations in geometry. Thus, altering the number of electrodes, the size of electrodes, the distance between electrodes, the distance between arrays of electrodes, and the polarity of arrays of electrodes will generate an infinite number of geometric configurations, each creating a corresponding electrical field. The ability to apply the same method to noninvasively reshape cartilage to different angles by simply altering geometry makes EMR a technique with significant potential in facial reconstructive surgery and otolaryngology. This study aims to outline the unique methodology and experimental findings of EMR conducted with a novel six needle electrode geometric configuration.

2. MATERIALS AND METHODS

2.1. Cartilage specimen preparation

Rabbit septal cartilages were harvested from freshly killed rabbit heads from a specialized processing center (Rabbit Farm, Turlock, CA). To minimize differences in mechanical properties among specimens, each specimen was trimmed with a razor blade along superior, inferior, cranial, and caudal edges to eliminate parts of the specimen where thickness was not similarly uniform. Final, adjusted rectangular specimens measured approximately 24 x 12 x 1 mm. One septal cartilage yielded only one experimental specimen. Prior to conducting each experiment, specimens were pre-cut and kept on a shallow pool (approximately 2 cm deep) of 1x phosphate buffered saline (PBS, pH 7.4, Sigma-Aldrich). Soaking the cartilage specimen in solution results in a change in mechanical properties, most likely due to fluid-mediated cellular swelling.

The use of phosphate buffered saline at pH 7.4 ensures that the cartilage specimen is exposed to an external environment as closely matched to that of *in vivo* physiology (physiological pH = 7.35-7.45, buffered) as possible. Secondly, the treatment of all cartilage specimens in phosphate buffered saline at pH 7.4 prevents undesired pH-dependent changes in mechanical properties.^{16, 17}

2.2. Mechanical compression and electromechanical reshaping

Cartilage specimens were placed into and bent at a right angle in custom-designed precision-machined reshaping jigs (UC Irvine Machine Shop, Irvine, CA). The line at which the specimen becomes angled is defined as the bend axis. It is important to note that, although the jig exhibits an exact right angle, the mechanical properties of the cartilage specimen limit its ability to conform to that shape completely. As a result, the jig-secured initial bend angle is not sharp as expected, but rather has a region of curvature (Figure 1).

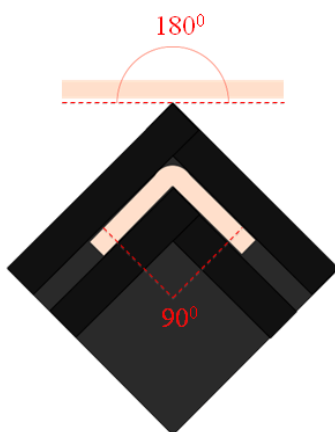


Figure 1. The cartilage exhibits a curved bend in the jig.

Three platinum needle electrodes (Grass Technologies, West Warwick, RI) were then inserted through pre-measured, drilled holes in the jig and into the cartilage specimen parallel to and 3 mm bilaterally on each side from the bend axis. The distance between holes within an array is 2 mm. Platinum was chosen for its high standard potential, which prevents undesired electrode oxidation. It is critical that none of the electrodes are in direct contact, as contact results in delivery of large currents that destroy the cartilage structure. Copper wires at the ends of the platinum needle electrodes served as conductors for the leads of the DC power supply (Model PPS-2322, Amrel, Arcadia, CA). One set of three electrodes on a given side, defined as an array, were connected to the anode (positive) of the DC power supply. The array on the opposite side was connected to the cathode (negative) (Figure 2). A custom software program (LabVIEW, National Instruments, Austin, TX) provided user-input control of the voltage and application time delivered by the DC power supply as well as measurement of the current in each experimental trial. The experimental setup must be placed atop insulating materials (e.g. towel, plastic) before each trial to prevent undesired changes in the electrical field.

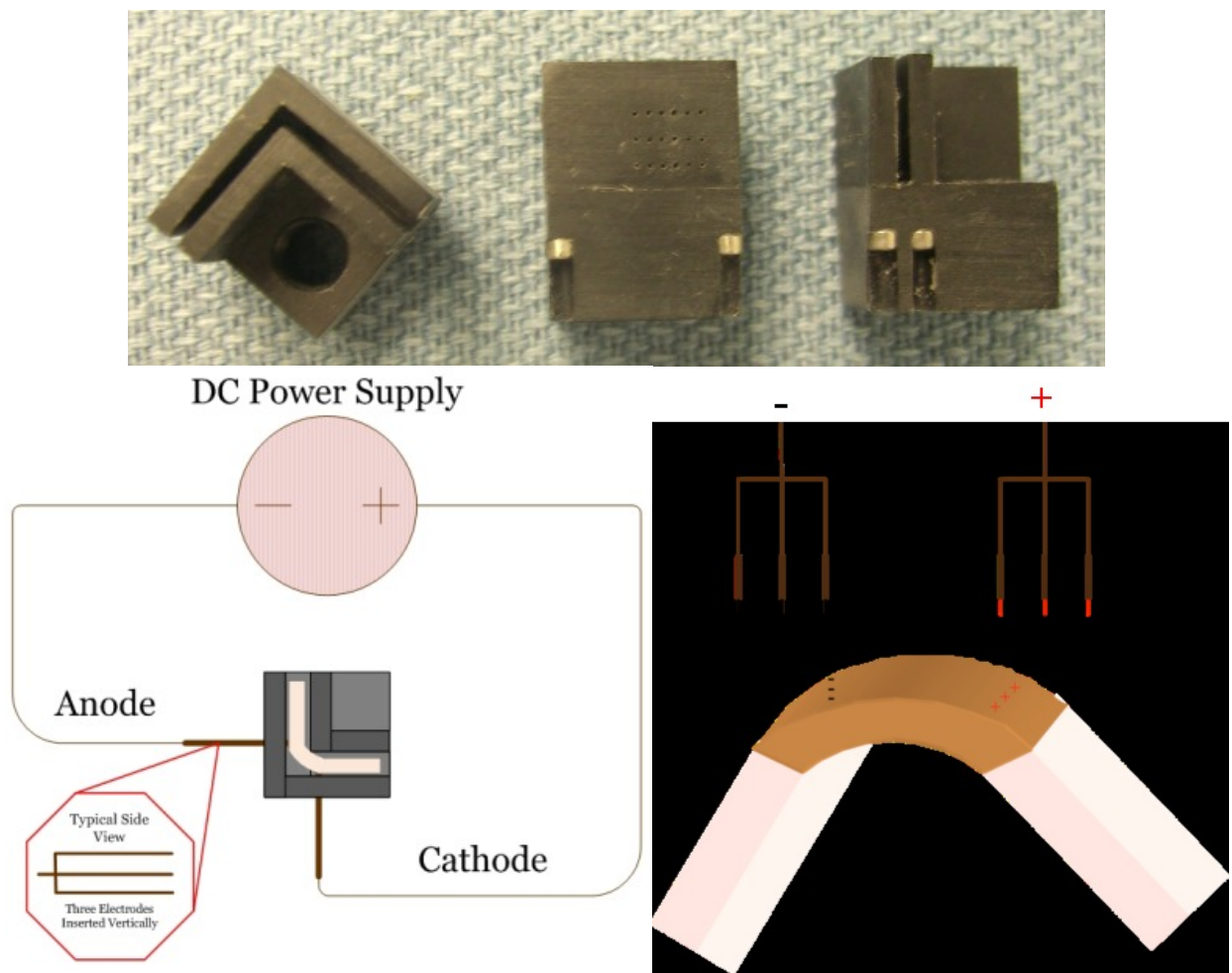


Figure 2. Precision-machined jig (top) and circuit diagram with jig setup (bottom left) and polarity of electrodes (bottom right). Each side of the bend axis has 6 arrays of 3 holes each. The distance between two adjacent arrays is 2 mm.

Once the needle electrodes were inserted and removed, the ability to identify which row of electrode insertion sites on either side of the bend axis corresponded to the positively or negatively charged array was uncertain. Knowledge of the location of the cathode and anode is of interest for a separate live-dead assay staining experiment. Thus, for future identification, one edge of each sample was trimmed off. The trimmed edge was designated as facing outwards and that half-plane of the bend axis was connected to the anode. The untrimmed half-plane was connected to the cathode (Figure 3). Voltages of 1, 2, 4, 6, and 8 V as well as control (0 V) were applied for 2 minutes.

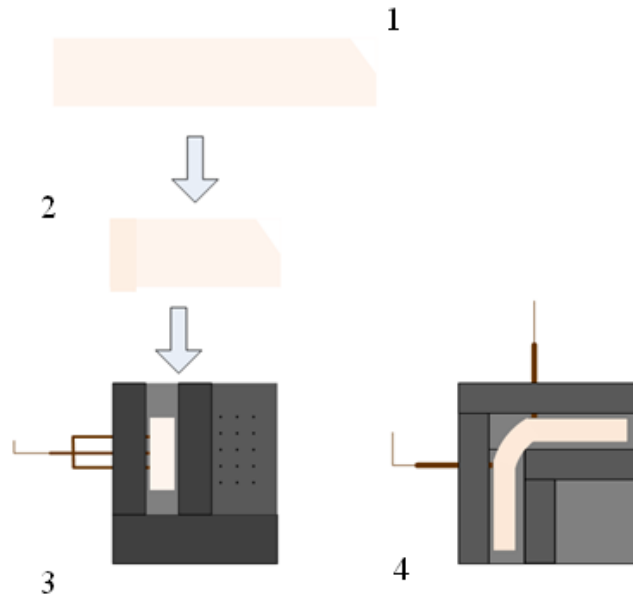


Figure 3. (1) Cut edge indicates side of positive electrode insertion; (2) Cartilage is bent at nearly a right angle and placed in jig; (3) Side view; (4) Top view.

The jig-specimen setup may be expanded to encompass a thermocouple at the apex of the bend angle. The thermocouple, with its measurements incorporated into the user-input software, can be used to measure temperature changes at the site of reshaping reactions. Previous studies utilized this setup to detect minimal temperature changes during EMR.¹⁴ Though not employed in this study, it is nevertheless worthwhile to outline this component of the protocol (Figure 4).

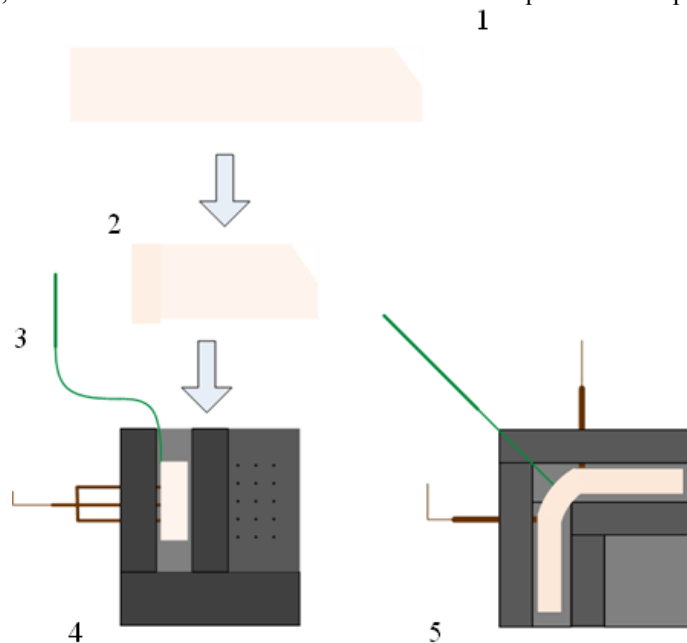


Figure 4. (1) Cut edge indicates side of positive electrode insertion; (2) Cartilage is bent at nearly a right angle and placed in jig; (3) Thermocouple; (4) Side view; (5) Top view.

2.3. Rehydration and measurement

Immediately after current delivery ceases, needle electrodes are removed from the jig-cartilage apparatus and the apparatus is placed in a bath of phosphate buffered saline at pH 7.4. The sample is rehydrated in the jig for 15 minutes, after which it is removed from the bath and from the jig. It is then placed adjacent to a ruler, photographed, flipped over along its width, and photographed again. Measurements of post-reshaping angle and cartilage thickness are performed by ImageJ (NIH, Bethesda, MD). Dimensions for each photograph are calibrated by the image of the ruler (Figure 5). Rehydration presumably restores the cartilage specimen to a more natural state.

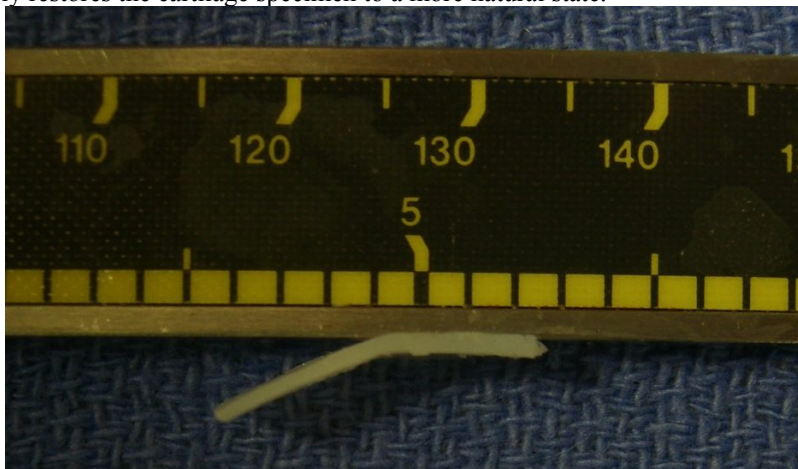


Figure 5. The cartilage specimen rests against a ruler. Measurements of the specimen are taken using the ruler as a standard source of calibration.

Post-reshaping angles are measured using the angle tool, which employs the construction of two lines sharing a common point. Each line is drawn as parallel as possible to the outer edge of the cartilage specimen on one end of the bend axis. The common point is adjusted to be in the plane of the bend axis while maintaining the two lines along the outer edge (Figure 6). The post-reshaping angle is measured twice – one for each photograph – per specimen.

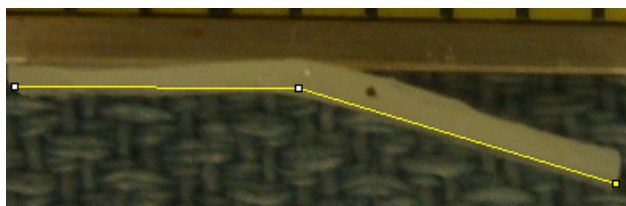


Figure 6. Example three-point angle measurement using ImageJ.

Cartilage thicknesses are measured at the thickest point of the specimen image, the thinnest point of the specimen image, and the location of the bend axis. The thickness measurements are repeated with the second image of the flipped specimen for a total of six thickness measurements (Figure 7).



Figure 7. Photographs of both sides of the same cartilage specimen are taken. For each side, the midpoint, maximum, and minimum thicknesses are measured and averaged.

Post-reshaping angles and cartilage thicknesses are tabulated and averaged in Excel (Microsoft Corporation, Redmond, WA). The actual measure of reshaping is defined as the bend angle, or degree of permanent deviation from the horizontal plane, calculated as $180^\circ - \text{post-reshaping angle}$.

3. RESULTS

2.1. Bend angle

Statistical analysis comparing means and standard errors of bend angles was performed. No statistically significant ($p < 0.01$) reshaping was observed below 6 V for 2 minutes application time. A maximum bend angle of 33 ± 8 degrees or reshaping degree, defined as the ratio of the measured bend angle to the maximum possible bend angle (90 degrees), of 33% was observed at 8 V applied for 2 minutes. The results as a function of voltage are plotted below (Figure 8).

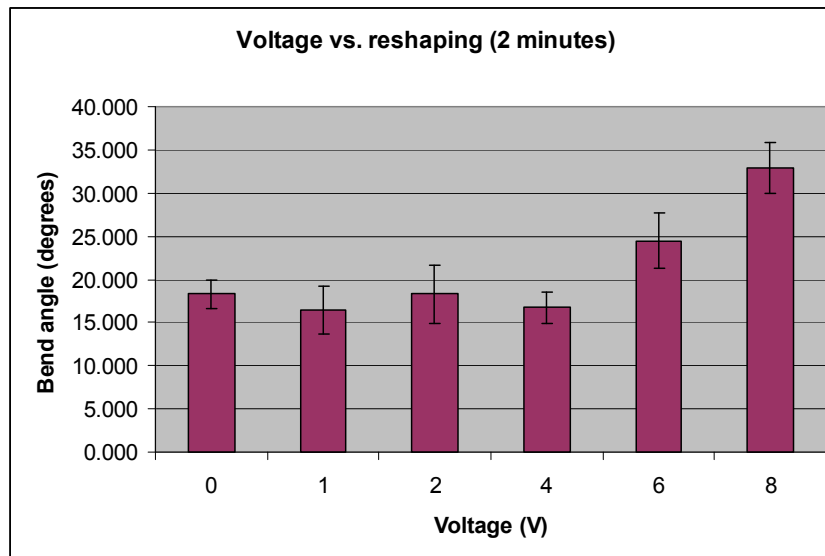


Figure 8. Rabbit septal cartilage reshaping as a function of voltage. $N = 13$ (0 V); 8 (1 V), 7 (2 V), 8 (4 V), 10 (6 V), 8 (8 V).

2.2. Effect of cartilage thickness on bend angle

Septal cartilage specimen thicknesses attained from different rabbit heads is highly heterogeneous (sample thickness 0.9 ± 0.2 mm, $N = 116$). As a result, it is possible to satisfactorily determine the effect of cartilage thickness on the extent of electroforming. Electroformed bend angles of cartilage specimens from different rabbits treated for 4 minutes at 2, 8, and 10 V were statistically analyzed using ANOVA. It was shown that, within the same voltage and application time conditions, different cartilage thicknesses did not have a statistically significant ($p < 0.01$) effect on reshaping degree, defined as θ/θ_M , where θ is the measured bend angle and θ_M is the maximum possible bend angle, or 90 degrees (Figure 9). These findings suggest that similar bend angles can be achieved by EMR, independent of varying cartilage thicknesses, and demonstrate the validity of comparing bend angles of cartilage specimens from different rabbits treated with the same voltage and application time.

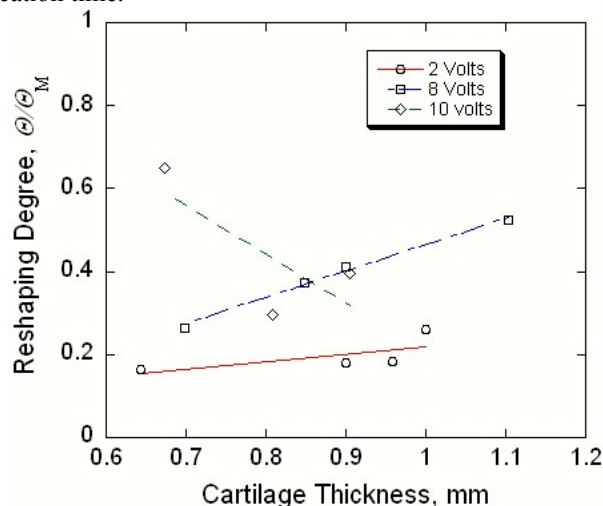


Figure 9. Cartilage thickness and its effect on the extent of reshaping as measured by bend angle.

2.3. Current profile

The current profile for each EMR specimen was measured throughout the application time. In each correctly performed experiment, the current was small (< 0.1 A). Three possible current profiles were attained at varying voltages independent of application time, each of which are worthwhile to mention.

2.3.1. Current measured at 1-3 V

The EMR current measured at voltages between 1 and 3 V has a negative magnitude and a constant slope (Figure 10). This is interpreted as a current that travels in the reverse direction due to the high standard potential of the platinum electrodes. Consequently, bend angles attained with voltages between 1 and 3 V are minimally different from the control. The total charge delivered is small, on the order of -0.001 mC.

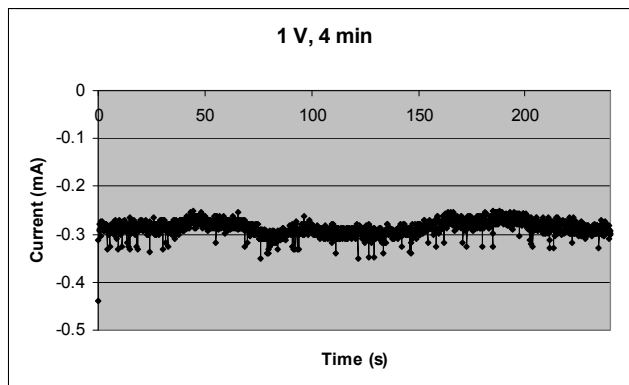


Figure 10. Negative EMR current measured at 1 V for 4 minutes.

2.3.2. Current measured above 3 V

The EMR current measured at voltages above 3 V has a very characteristic shape (Figure 11). There is an increasing slope, which peaks at a maximum current and is followed by a less steep decreasing slope that continues asymptotically at the initial current magnitude. Greater voltages result in greater maximum current values. The total charge delivered is small, on the order of 0.01 mC.

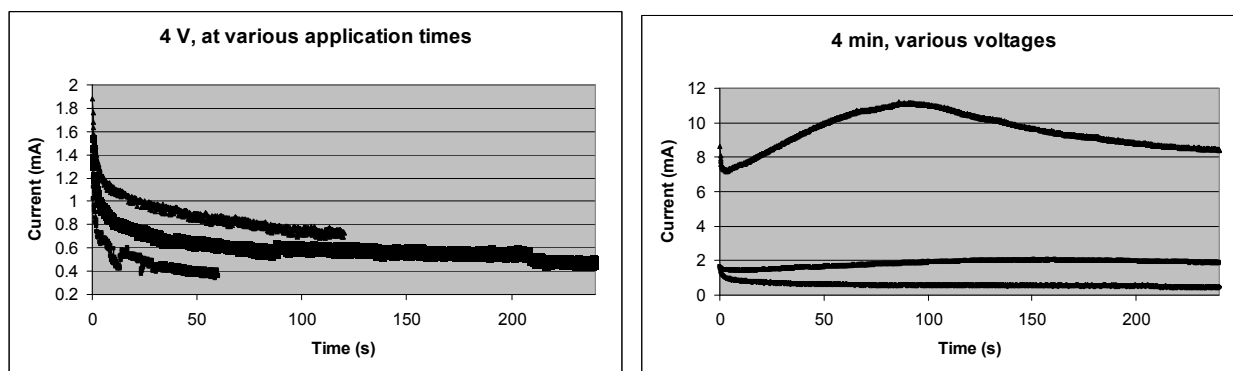


Figure 11. EMR current measured at 4 V at various times (from top to bottom: 2 minutes, 4 minutes, 1 minute) (*left*) and for 4 minutes at various voltages (from top to bottom: 8 V, 6 V, 4 V) (*right*).

2.3.3. Erroneous currents

Whenever electrodes are in direct contact with one another, an erroneous current profile is measured (Figure 12). The erroneous current profile is characterized by a current value that has (1) a constant slope with (2) significantly large magnitude. The total charged delivered is extremely high, on the order of 10 mC. Knowledge of the erroneous current profile is useful for detecting needle electrodes that are in direct contact, indicating the need to repeat the experiment.

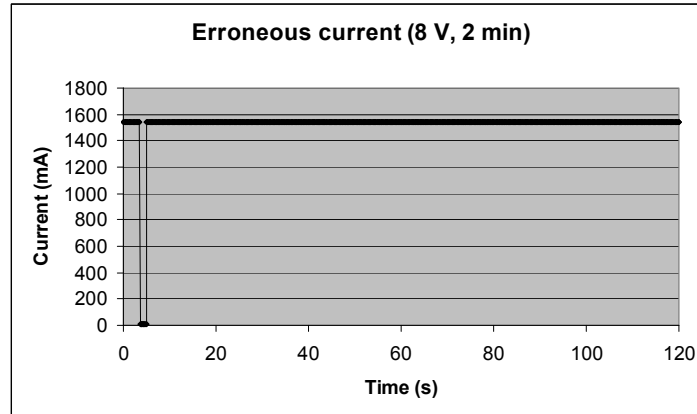


Figure 12. Erroneous EMR current. Note the constantly large magnitude of current measured.

4. DISCUSSION

4.1. Voltage and Application Time

Compared to this study, previous EMR studies used higher voltages and application times, leading to greater reshaping, as measured by bend angle. Correlation of previous study findings with mathematical models established voltages and application times above which there is too much tissue damage. Using the same mathematical models as a guide, this study utilized voltages and application times that would not result in the same degree of tissue damage. As such, bend angles measured in the six needle electrode geometric configuration are lower than reported in previous studies, but are still clinically feasible while preventing extensive tissue death.

Overall, the six needle electrode geometric configuration for EMR conformed to previously established trends in voltage and application time. Generally, as voltage and/or application time is increased, the bend angle following rehydration increases in direct proportion.

4.2. Applicability and Risks

Given the relatively simple and inexpensive implementation of EMR, it would be highly beneficial to explore the nature of various electrical fields leading to various reshaping results. One effective method is alteration of the geometric configuration of the needle electrodes. In choosing an optimal geometry, there are three main concerns: (1) the practical applicability of the geometry, including any risks in implementation; (2) the costs associated with the geometry; and (3) the effects of the geometrically unique electrical field on the tissue. A greater variety of geometric configurations will be studied in the future.

It is important to consider the applicability of the needle electrode geometry. The practically ideal geometry is one that can be surgically implemented safely and precisely. This is intuitively defined by the following parameters.

4.2.1. Number of electrodes

The obvious advantage of a large number of electrodes is the diverse number of geometries and electrical fields that can be generated, leading to a wide spectrum of reshaping effects. However, too many needle electrodes inserted into the specimen increase the potential for direct physical trauma and electrical damage (discussed in section 4.3). Direct physical damage can result in chondrocyte death by mechanical disruption of the extracellular matrix or the cell itself. Until viability studies are performed, there is no data on the sustainability of chondrocytes in EMR specimens, and, more importantly, whether viability is an important factor for cartilage reshaping. Furthermore, a large number of electrodes may cause crowding, making manual dexterity a significant factor in creating the desired geometry.

Conversely, having too few electrodes limits the number of reshaping geometries. At the same time, placement of electrodes and direct physical damage to the specimen become less significant considerations.

4.2.2. Size of electrodes

The use of large electrodes delivers current with a greater surface area but limits the number of geometries that can be implemented. Potential sites of application (e.g. ear, nose, trachea) have relatively small surface areas and are therefore unsuited for large interventional devices. Large electrodes are also associated with the risk of more extensive physical and electrical damage.

Small electrodes are overall a more desirable choice, as they permit a greater number of geometric configurations while minimizing traumatic injury to the specimen. This is likely the parameter with least variability due to the limited varieties of market platinum electrodes.

4.2.3. Distance between electrodes within an array

A greater distance between electrodes results in a more surface area-expansive electrical field, albeit weaker in intensity in central regions. There is also the risk of being unable to predict the electric field's behavior as it diffuses outwards. In addition, electroforming with electrodes separated by greater distances requires greater voltage and application times, leading to thermal damage. Nevertheless, an advantage of this greater separation is ease of placement, decreasing the significance of manual dexterity.

A smaller distance between electrodes makes the geometry difficult to construct and, depending on the current delivered, may concentrate the electrical field at various points. The latter concern results in greater damage to the tissue specimen regardless of voltage and application time.

4.2.4. Distance between arrays of electrodes

The precision-machined reshaping jigs were specifically constructed to accommodate different distances between arrays of electrodes (2, 4, 6, 8, 10, 12 cm). The considerations are related to those for the distance between electrodes – variations in area of electrical field and geometry implementation.

4.2.5. Polarity of arrays of electrodes

The polarity of arrays of electrodes is the most complex parameter, as the different electrical fields generated must be analyzed by mathematical modeling, which is beyond the scope of this study. Nevertheless, auxiliary studies on electrical fields derived from different polarities have been and will continue to guide the selection of optimal geometries for cartilage reshaping.

4.3. Costs

Compared to alternative methods for cartilage reshaping (i.e. laser), EMR is relatively inexpensive. The setup is simple, consisting of standard electrical hardware and computer software that is readily affordable and available. Furthermore, the simplicity of the setup suggests that it can potentially be reduced in size as well.

4.4. Tissue effects

Delivery of current to cartilage results in electrical damage, perhaps also mediated by redox reactions. Highly concentrated electrical fields (high voltage, high application time, decreased distance between electrodes and distance between arrays of electrodes) tend to cause more chondrocyte death at the site of delivery when compared to less concentrated electrical fields. The mechanism and significance of electrical damage is not known. It is speculated that the effectiveness of EMR depends on at least some tissue death that leads to changes in mechanical properties conducive to reshaping.

The degree of electrical damage can be assessed by viability and histological studies, which are planned for the future.

5. CONCLUSION

This study demonstrates a new and effective geometry for electromechanical cartilage reshaping. The results form the foundation for further investigation into parameters dependent on geometric configuration, including the number of electrodes, the size of electrodes, the distance between electrodes, the distance between arrays of electrodes, and the polarity of arrays of electrodes. Moreover, the development of a standardized, repeatable protocol serves as the basis for other EMR and cartilage reshaping studies, such as viability studies and determination of biophysical properties.

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