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Methods for Evaluating Changes in Cartilage Stiffness Following Electromechanical Reshaping

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ABSTRACT

One common component of otolaryngological surgeries is the reshaping of cartilage. Previous studies have demonstrated the efficient achievement of this procedure through electromechanical reshaping (EMR), a technique that involves the direct application of voltage to cartilage that is mechanically deformed in a jig. Two main parameters, voltage and application time, may be regulated to achieve varying degrees of shape change. Although prior research has correlated these EMR parameters with degree of shape change, it remains necessary to correlate the same parameters with the degree of change in the mechanical properties of tissue. Once this is accomplished, an ideal balance may be determined, in which shape change is maximized while intrinsic tissue damage is minimized This study satisfies this need by providing comprehensive data on the pre- and post-EMR stiffness of both septal and auricular cartilage over a range of voltages (2-8V) with constant application time (2 min for septal, 3 min for auricular). EMR was applied using flat platinum electrodes to one of two 15 mm X 5 mm samples obtained from the same cartilage specimen, while the second sample was maintained as a control. Following a 15 min re-hydration period, the Young's modulus of the tissue was calculated for both the control and experimental sample from data obtained through a uniaxial tension test. A general reduction in stiffness was observed beginning at 3V, with the magnitude of reduction increasing at 6V.

Keywords: electromechanical reshaping, cartilage, septal, auricular, Young's modulus

1. INTRODUCTION

Commonly required in facial reconstructive surgeries, the traditional reshaping of the cartilaginous structures in the head and neck is an invasive and technically demanding procedure. Scoring, carving, suturing, or morselizing cartilage is often necessary to correct deformations in the ear, nose, trachea, and larynx.[1] If reconstruction of a missing facial structure is required, the application of these same techniques to the necessary cartilage grafts produces many challenges, such as the tissue's intrinsic resistance to reshaping and tendency to warp.[2] Several non-invasive alternatives to these methods of cartilage reshaping have been proposed and researched, such as laser, radiofrequency, and electromechanical cartilage reshaping.[3-6]

The electrochemically-driven shape-change mechanism of electromechanical reshaping (**EMR**) distinguishes it from thermally-driven methods such as laser and radiofrequency, which risk thermal injury to the tissue.[7] Accomplished through the direct application of a DC electric field to cartilage that is mechanically deformed in a jig, EMR may alter the fixed charge distribution of proteoglycans in the collagen matrix of the tissue.[7-9] This effect, as well as the hydrolysis produced by an electrolytic reaction in the cartilage, results in changes to the tissue's mechanical properties. One cause of such changes may be the tissue pH changes associated with the EMR hydrolysis reactions, as pH is well known to determine the mechanics of a tissue.[8]

Modification of a tissue's mechanical properties is necessary for it to become more conducive to shape change. However, such changes may also affect the structural integrity of the cartilage, creating a possible side-effect of the technique. Previous EMR studies have examined the mechanical response of cartilage to EMR and correlated the degree of achieved shape change with variations in the EMR parameters of voltage and application time.[7,8,10] This study contributes to existing research by focusing on the change in both septal and auricular cartilage mechanical properties, as measured by the Young's modulus, brought about by voltage application without mechanical deformation.

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2. MATERIALS AND METHODS

2.1. Specimen Preparation

Both rabbit auricular and rabbit septal cartilage specimens were obtained from New Zealand White Rabbits from a local packinghouse (Rabbit Farm, Turlock, CA). Rabbit auricular cartilage was removed with perichondrium intact from the rabbits' ears. To avoid damage to the thin tissue (0.40 ± 0.046 mm thick, average ± standard deviation), no microdissection of the perichondrium was performed on the auricular cartilage. Septal cartilage was harvested from the rabbits' crania, and the mucosa and perichondrium were removed from each specimen. For both types of cartilage, each specimen was cut into two rectangular sections approximately 15 X 5 mm (Figure 1a-b). The geometry of each section was measured using a digital electronic caliper (CD-6"CS, Mitutoyo Corp, Japan). One section was randomly designated as a control, and the other was designated for EMR treatment, producing matched pairs for the experiment.

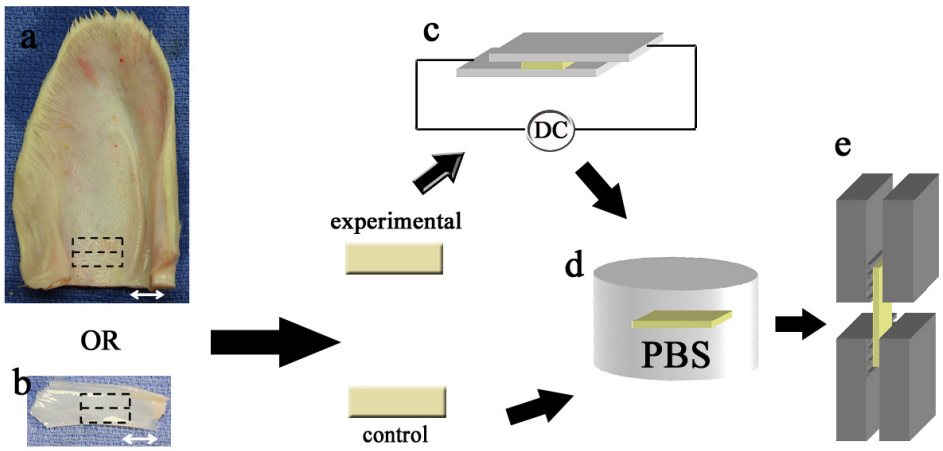

Figure 1. Scheme of experiment. **a**. Dissected rabbit ear with region used to harvest cartilage indicated. **b**. Rabbit septal cartilage with mucosa and perichondrium removed, region used to obtain two samples indicated. (Double arrow lines indicate 1 cm.) **c.** EMR applied to sample through flat platinum electrodes. **d.** Hydration in PBS. **e.** Sample placed between grips of mechanical testing device.

Samples were immersed in 1x phosphate buffered saline solution without calcium or magnesium (PBS, pH 7.4, Sigma-Aldrich). Use of this solution was significant for several reasons. Control of pH was necessary because of the pH-dependence of the mechanical properties of cartilage.[9,11] PBS at pH 7.4 reflected physiological conditions, in which collagen possesses zero or minimal fixed charge, an important consideration for the electromechanical reactions that are the basis of EMR.[9] Similarly, the addition of calcium or magnesium ions to the solution could potentially affect these reactions as well.

2.2. Voltage Application

Samples designated for EMR treatment were placed and clamped between two flat platinum electrodes (25 x 12.5 mm) insulated at the edges with a thin plastic layer to avoid short-circuits during voltage application (Figure 1c). The danger of shorting had been a common setback in pilot studies, especially when the highest voltages were used on septal cartilage. Each electrode was attached to a lead from a programmable DC power supply (Agilent Technologies, Inc., Palo Alto, CA) with custom designed software (LabView, National Instruments, Austin, TX) to control voltage magnitude and application time, and to monitor current during the experiment. The application time was held constant, 3 min for auricular samples, and 2 min for septal samples. Voltage was varied from 2 V to 8 V for 5 different voltages, 2 V, 3 V, 4 V, 6 V, 8 V for auricular cartilage, and 2 V, 4 V, 5 V, 6 V, 8 V for septal cartilage. Previously conducted research with these EMR these parameters demonstrated 20-100% reshaping in similarly shaped samples. Following

voltage application, EMR-treated samples were re-hydrated in 1x pH 7.4 PBS solution without calcium or magnesium for 15 min (Figure 1d).

2.3. Mechanical Measurements

A mechanical uniaxial tension test was performed for all EMR-treated samples and their matched controls to determine Young's moduli for all samples. Samples were secured between two custom-designed metal grips (UCI Biological Sciences Shops, Irvine, CA) precision-machined with bluntly serrated surfaces to grip the tissue and attached to a mechanical testing device (Electroforce 3200, Bose, Eden Prairie, MN). (Figure 1e) Tensional strain was applied to the sample at a constant rate of 0.01 mm/sec until the reaction force neared a maximum of 200 g. Force was recorded, and the data was analyzed to determine the Young's modulus for each sample in Excel (Microsoft Corporation, Redmond, WA).

3. RESULTS

Table 1. Summary of Young's Moduli Data

Type of Cartilage	Standard Deviation* of Control Moduli	Standard Deviation* of EMR Moduli	Voltages Producing Tissue Softening	Degree of Softening Range**
Auricular	20.4 %	21.4 %	3 - 8 V	15.6-33.0 %
Septal	34.3 %	26.4 %	5 - 8 V	5.51-46.5 %

* Standard deviation as percent of the mean, averaged for all voltage groups
** Measured as percent of the matched control Young's Modulus for each EMR-treated sample and averaged for each voltage group

The relative accuracy achieved by the experimental methods is illustrated by the standard deviation values for the sample groups in Table 1. These values are satisfactory, indicating an experiment design with credible results. However, the standard deviations for the septal data are larger than those for the auricular data, evidence of an increased variation among septal samples. The occasionally wedged shape of the septum, as well as its varying height from animal to animal, contributed to this variation. Furthermore, although a smaller standard deviation would be expected for the septal control Moduli, this value was actually larger than that for the EMR-treated samples' Moduli.

A reduction in Young's Modulus, indicating tissue softening after EMR was observed for both auricular and septal cartilage, beginning at 3 V or 5 V, respectively. As demonstrated from the values in the last column of Table 1, this softening was significant, up to 33.0 % for auricular and 46.5% for septal. Comparing this data for auricular versus septal cartilage, it was observed that auricular cartilage began softening at a lower voltage but its maximum amount of softening was less than that of septal cartilage. These differing voltage-softening relationships show that the responses of auricular and septal cartilage to EMR are not identical.

4. DISCUSSION

Continuing EMR research draws the technique closer to its potential clinical application as an innovative option in head and neck surgery. Existing studies have inspected possible explanatory mechanisms, EMR's mechanical and electrical effect on tissue, the ability of the technique to reshape cartilage, and the relationship between EMR parameters and degree of reshaping. [7,8,10] Building on these prior investigations and their methods, the current study focused narrowly on developing a means of quantifying any changes in tissue stiffness that might accompany EMR shape change.

4.1. Proposed EMR mechanism and its effects on tissue stiffness

Different features of the proposed EMR mechanism correlated with the observed tissue-softening demonstrated by the data from this experiment. The electrochemical reactions associated with EMR include water electrolysis and reduction, which both dehydrate the tissue and influence its pH by creating hydronium or hydroxide ions.[8] Both water loss and pH are known to affect the mechanical properties of tissue. One such property, stress relaxation has been shown to increase

as EMR voltage increases in porcine septal cartilage.[8] In addition to redox chemistry, the proposed mechanism for EMR has also included changes in the cartilage matrix, such as disrupted inter- or intra- molecular proteoglycan or collagen bonds. The latter is especially pertinent, because the uniaxial tension test used for this study depended on the tensile strength of the cartilage provided by collagen. This effect on molecular structure, as well as water loss and pH, may have contributed to the softening of the cartilage at most EMR voltages (Table 1).

4.2. Design of EMR jig

The flat platinum electrodes used in this experiment were a departure from previously-used jigs, which provided mechanical deformation to the samples in studies focused on evaluating the degree of reshaping.[7,8,10] Mechanical deformation is a necessary component for any shape change to occur during EMR, while the application of voltage induces changes that lengthen the memory effect of the deformation. Early studies implemented concave and convex semi-cylindrical aluminum electrodes to deform flat cartilage into rounded specimens.[7,8] More recent studies have relied on platinum needle electrodes piercing through cartilage deformed by a right angle jig.[10,13] Platinum needle electrodes minimize tissue damage and electrodeposition of the metal on the tissue. For clinical application, use of needle electrodes also minimizes the invasiveness of an EMR procedure, requiring less of the tissue to be exposed. Adaptation of these exact jigs for this mechanical study posed two concerns: A reshaped specimen would have a complex geometry, complicating the calculation of an accurate Young's modulus, and a specimen treated using needle electrodes would not be completely exposed to the reactions of EMR. While not completely reflecting the conditions of EMR, the use of flat platinum sheets as electrodes was a means of targeting preliminary information on EMR-induced changes in tissue stiffness.

4.3. Specimen selection

Use of matched controls was a key component of this study. Several variables concerning cartilage specimens were difficult to control, such as random variations in size among different rabbit septa. The effect of this specific variation was evident in the higher standard deviations for septal data in Table 1. Taking a control from each specimen accounted for some of these variations and allowed a more accurate calculation of the percent change in Young's modulus.

Specimens were also chosen from both auricular and septal samples to investigate whether EMR must be modified to accommodate different cartilage types. While both the elastic cartilage of the auricle and the hyaline cartilage of the septum are composed of Type II collagen, only elastic cartilage also contains bundles of elastin fibers.[12] Despite this structural difference, the data show that both hyaline and elastic cartilages are softened by EMR (Table 1).

4.4. Further research

Other means of evaluating the effect of EMR on inherent tissue properties include histological and chondrocyte viability analyses. Providing biological, rather than mechanical information, these investigations will not face any detriment from the mechanical deformation required for EMR and are currently being pursued.

5. CONCLUSION

Data produced in this research has demonstrated the success of the methods implemented, as well as a preliminary understanding of the mechanical side effects of EMR on cartilage stiffness. It was determined that an overall effect of post-EMR softening is observed for both septal and auricular cartilages beginning at a minimum voltage and then increasing with increasing voltage. However, the lack of mechanical deformation in the experimental apparatus makes the experiment an incomplete study of the mechanical effects of EMR, because lack of mechanical deformation precludes any shape change. Incorporating mechanical deformation into a mechanical study provides a challenge, for the geometries of reshaped specimens are non-uniform, making uni-axial tension tests difficult. Nevertheless, a basic methodology for assessing the mechanical changes in cartilage following EMR has been established and will be important in studies to determine which EMR parameters will maximize shape change without unnecessarily softening the tissue.

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