Changes in the Tangent Modulus of Rabbit Septal and Auricular Cartilage Following Electromechanical Reshaping

Amanda Lim

Department of Biomedical Engineering,
The Beckman Laser Institute and Medical Clinic,
University of California, Irvine,
Irvine, CA 92697
e-mail: adlim@alumni.uci.edu

Dmitry E. Protsenko

The Beckman Laser Institute and Medical Clinic,
University of California, Irvine,
1002 Health Sciences Road,
Irvine, CA 92612
e-mail: dprotsen@uci.edu

Brian J. F. Wong

Department of Otolaryngology, Head and Neck Surgery
and Department of Biomedical Engineering,
The Beckman Laser Institute and Medical Clinic,
University of California, Irvine,
1002 Health Sciences Road,
Irvine, CA 92612
e-mail: bjwong@uci.edu

Transforming decades' old methodology, electromechanical reshaping (EMR) may someday replace traditionally destructive surgical techniques with a less invasive means of cartilage reshaping for reconstructive and esthetic facial surgery. Electromechanical reshaping is essentially accomplished through the application of voltage to a mechanically deformed cartilage specimen. While the capacity of the method for effective reshaping has been consistently shown, its associated effects on cartilage mechanical properties are not fully comprehended. To begin to explore the mechanical effect of EMR on cartilage, the tangent moduli of EMR-treated rabbit septal and auricular cartilage were calculated and compared to matched control values. Between the two main EMR parameters, voltage and application time, the former was varied from 2–8 V and the latter held constant at 2 min for septal cartilage, 3 min for auricular cartilage. Flat platinum electrodes were used to apply voltage, maintaining the flatness of the specimens for more precise mechanical testing through a uniaxial tension test of constant strain rate 0.01 mm/s. Above 2 V, both septal and auricular cartilage demonstrated a slight reduction in stiffness, quantified by the tangent modulus. A thermal effect was observed above 5 V, a newly identified EMR application threshold to avoid the dangers associated with thermoforming cartilage. Optimizing EMR application parameters and understanding various side effects bridge the gap between EMR laboratory research and clinical use, and the knowledge acquired through this mechanical study may be one additional support for that bridge. [DOI: 10.1115/1.4004916]

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1 Introduction

In order to correct deformities or injuries in the face, head and neck, cartilage must often be reshaped in facial reconstructive and cosmetic surgery. Cartilage deformations may result from trauma, cancer, or they may be congenital, as in the case of microtia. Traditional techniques to correct these deformities are marked by destructive modification of the cartilage through cutting, scoring, and morselization [1]. Intrinsic resistance in the cartilage to deformation often necessitates complex and invasive incisions and sutures, increasing the level of skill demanded in the surgeon [2]. Applying these methods to commonly used facial cartilage grafts can result in warping and waste of an already limited supply of tissue. To address such drawbacks to traditional surgical methods, research has focused on the development of less invasive methods that can be implemented *in situ*.

Established cartilage reshaping methods rely upon a variety of biophysical processes creating shape change. Two prominent methods, laser and radiofrequency reshaping, rely on heat generation within the tissue to facilitate shape change [3–6]. While the effectiveness of thermally induced shape change has been extensively verified, the heat generation that creates shape change comes at the expense of chondrocyte viability within the tissue. Nonthermally induced methods have also been investigated, including enzymatic digestion and sustained mechanical loading [7,8]. Another reshaping method, and the focus of this study, electromechanical reshaping (EMR), uses voltage applied to mechanically deformed cartilage to electrochemically reshape specimens (Fig. 1) [9,10]. It has been suggested that the EMR mechanism involves redox reactions initiated at the interfaces between the electrodes and cartilage, subsequently creating local and temporal alteration of pH [9,10]. Two electrode configurations for EMR have been studied: surface and needle electrodes (Fig. 1(b)) [9–11,23]. Although negligible heat generation has been demonstrated in previous studies [9], other potential compromises to native tissue properties related to EMR are present. Surface electrodes allow for a uniform treatment of a large volume of cartilage. However, they may produce extensive electrochemical damage extending along the entire tissue-electrode interface [11]. To minimize and spatially localize cartilage injury, EMR using arrays of needle electrodes has been suggested (Fig. 1(b)) [23].

Translation of EMR to clinical practice requires a better understanding of the mechanical changes that occur in reshaped cartilage as a consequence of the redox-mediated processes. In this study,

Fig. 1 Two types of EMR jigs with corresponding reshaped specimens. (a) Schematic of semicylindrical surface electrode jig with sandwiched cartilage (b) Schematic of right angle non-conducting jig with two 3 needle electrode arrays (c)–(d) Pre-EMR rabbit septal samples (e)–(f) EMR-reshaped samples corresponding to each jig after 30 min (e), or 10 min (f). (Black scale bar indicates 1 cm.)

Fig. 2 Scheme of experiment: Two samples of cartilage each were harvested from (a) a dissected rabbit ear in the region indicated with black dashed lines or from (b) rabbit septal cartilage with mucosa and perichondrium removed. (White double arrow lines indicate 1 cm.) (c) Voltage was applied to each sample through flat platinum electrodes. (d) Samples were rehydrated in PBS for 15 min. (e) Each sample was placed between grips of mechanical testing device, and a tensile force was applied.

our objective was to determine changes in cartilage mechanical properties following application of electric voltages typically used in EMR. Samples of rabbit auricular and septal cartilage were electrically treated using flat surface electrodes and mechanically tested in tensile deformation. Tangent moduli were calculated from the linear regions of stress-strain curves and compared with controls. This study adds to recent viability studies [11] and will help expand understanding of any limitations to EMR's shape-changing capability, enabling further development of clinical treatment.

2 Methods

Auricular and septal cartilage samples were harvested from the crania of New Zealand white rabbits purchased from a local abattoir. The mucosa and perichondrium were removed from septal samples through microdissection techniques. For the auricular samples, only the skin was dissected: the auricular perichondrium is tightly bound to the cartilage, and thorough removal results in cracks and potential mechanical damage to the specimen. Each cartilage specimen was cut into two immediately adjacent rectangular pieces with approximate area of $15 \times 5 \text{ mm}^2$, which were used as matched pairs, with one designated control that was not electrically modified and one experimental sample (Fig. 2). The thicknesses of auricular and septal samples were 0.40 ± 0.047 and 0.60 ± 0.11 mm thick, respectively. A total of 75 auricular and 52 septal samples were used. Phosphate buffered saline solution (PBS) maintained at pH 7.4 was used to hydrate the samples and maintain the pH within normal physiologic range.

A specimen was placed between two flat platinum electrodes which were then firmly held together by an insulated clamp to ensure full electrode contact with the tissue (Fig. 2(c)). To avoid short circuiting from contact between the electrodes, a thin, adhesive plastic film was used to cover the edges of the electrodes. The leads of a DC power supply (E3646A, Agilent Technologies, Inc., Palo Alto, CA) were connected to the electrodes. Voltage amplitude and application time were under computer control (LabVIEW, National Instruments, Austin, TX), and voltage, current, and total charge transferred in electric circuit were recorded. Auricular samples were treated with 2, 3, 4, 6, or 8 V applied for three min, while septal samples were treated with 2, 4, 5, 6, or 8 V for two min. These parameters had previously been demonstrated to produce clinically significant reshaping in a semicircular surface electrode jig [10]. At least eleven or nine auricular and septal samples, respectively, were used for each voltage parameter. An electrically insulated thermocouple was inserted at the cartilage-electrode inter-

face, and the initial and maximum temperatures were recorded. Temperature changes during EMR were measured in two to three samples from each voltage group. Immediately after voltage application, samples were rehydrated in PBS for 15 min (Fig. 2(d)).

Following rehydration, all treated samples and their matched controls were tested in uniaxial tension on a mechanical testing platform (Electroforce 3200, Bose, Eden Prairie, MN). Samples were secured by two sets of grips separated by approximately 8.6 mm. A tensile deformation was applied at a constant strain rate (0.01 mm/s) until the reaction force of the sample reached the maximum force of the load cell, or 200 g, indicating a maximum displacement (Fig. 2(e)). As a ramp displacement function was applied to reach the maximum load, displacement and reaction force data were recorded, and stress was calculated afterwards. Tests were performed without an additional preconditioning load and in air, without immersion in saline or aqueous conditions.

Fig. 3 Typical strain-stress curve with specified linear region indicated with markers (O)

Fig. 4 Current profiles over time for auricular and septal samples. (a) Current at higher voltages corresponds to the left scale. (b) Current at 2V corresponds to the right scale.

These conditions were chosen because mechanical preconditioning extends the duration of the experiment, causing dehydration in the absence of saline immersion. In addition, comparisons of EMR-treated samples with controls as well as sample groups treated at different EMR voltages were the priority, rather than measurement of exact mechanical properties after EMR. The tangent modulus for each sample was determined from the linear regions of strain-stress curves graphed using Excel (Microsoft Corporation, Redmond, WA), as shown in Fig. (3).

3 Results

3.1 Observations During Voltage Application. No noticeable changes in visual appearance were observed in the tissue during or after the experiment when lower voltages (2–4 V) were applied. However, beginning at 5 V for both septal and auricular tissue, subtle tissue color changes, and gas bubble formation were noted. For septal tissue, this effect was more dramatic: during the voltage application gas bubble formation was readily evident, and the tissue specimens became translucent yellow. After rehydration, the septal cartilage retained its yellow tint but otherwise returned to its more opaque pre-EMR appearance. Similar color change, though much subtler, was observed with auricular cartilage: Slight reduction in opacity was observed, and yellowing was confined to

the edges of the specimen. Gas formation (bubbles) in auricular specimens was also minimal, even at the highest voltage (8 V).

The electric current for both auricular and septal cartilage rose rapidly during the first several seconds of voltage application followed by a gradual decline, which is consistent with previous observations (Fig. 4) [10]. Small temperature elevations in both septal and auricular samples of approximately 1 °C at voltages up to 5 V were observed (Fig. 5). At 5 V, the temperature rise in auricular samples was negligible as well, while in the septal samples, the average temperature rise was 10.6 °C. In septal samples, temperature and peak current increased with applied voltage more rapidly than in the auricular specimens (Fig. 5). A temperature rise of 40 °C and average maximum current of about 460 mA were recorded for septal samples at the highest voltage of 8 V. In contrast, the temperature and current maxima for auricular samples were approximately 20 °C and 216 mA at the same voltage (8 V), albeit with a longer three min application time.

3.2 Cartilage Tangent Modulus After Voltage Application. The native auricular cartilage was significantly stiffer than septal: 27 ± 0.66 MPa vs. 11 ± 0.62 MPa, respectively (average $\pm$ standard error for controls). Both tissues softened modestly with increasing applied voltage (Fig. 6). Control values for both tissues are the average of all matched-controls for all experimental groups. Analyzed using a two-sample t-test, data depicted in Fig. 6(a) show a statistically significant softening of voltage-treated septal cartilage at 5 V ($p = 0.0060$). Auricular cartilage began softening at a 3 V threshold ($p = 0.0017$), but the moduli measured following EMR at 3 V and 4 V were not statistically different ($p = 0.19$). Since a significant thermal effect was present at higher voltages, the data corresponding to voltages above 5 and 4 V for auricular and septal cartilage, respectively, are not presented. However, samples continued to soften at these higher voltages.

4 Discussion

The advantages of EMR as a cartilage reshaping technique include: (1) low component costs; (2) relatively simple device design; (3) diminished thermal threat to the tissue; and (4) controllable parameters with predictable results [9,10,21]. While previous studies have demonstrated the feasibility of cartilage EMR, the mechanisms of action for the electrochemically mediated shape change effect are not completely understood. In 1991, Lai et al. introduced the triphasic theory of cartilage, describing it as a mixture of a water phase, an ion phase, and a solid phase composed of proteoglycans, collagen and other proteins [12–14]. The interplay between the electrostatic, osmotic and hydrolic phases account for the complex mechanical behavior of cartilage. In contrast to thermally-mediated laser or radiofrequency reshaping, redox chemistry is at the center of EMR, beginning with the formation of an electrochemical cell when voltage is applied to

Fig. 5 (a) Maximum current for septal samples ($n = 5$), EMR application time = two min and auricular samples ($n = 5$), EMR application time = three min. (b) Maximum change in temperature for septal and auricular samples ($n = 2$ to 4 for each data point). Error bars indicate $\pm$ standard error.

Fig. 6 Cartilage tangent moduli plotted as average $\pm$ standard error. * indicates $p < 0.001$ in a two-sample t-test with control data. (a) Septal data, EMR application time = two min, with $n \geq 9$ for each voltage value, and $n = 52$ compiled control values. (b) Auricular data, EMR application time = three min, with $n \geq 11$ for each voltage value, and $n = 75$ compiled control values. Moduli above 5 and 4 V were omitted due to excessive temperature elevation during EMR.

cartilage through metal electrodes, leading to hydrolysis [10]. Likewise, fixed and free ions, particularly the fixed negative charges of proteoglycans, as well as glycosaminoglycans and proteins may also participate in redox reactions. Disruption to the internal stability of this liquid environment in cartilage that constitutes 70 to 80% of its wet weight may cause many mechanical changes [15]. In particular, such reactions may produce stress relaxation in the tissue, facilitating shape change and alteration of cartilage mechanical properties [9].

Measurements of tangent modulus after application of DC voltage with the amplitude and application times used in previous EMR studies were performed in rabbit septal and auricular cartilage. Auricular cartilage grafts, which were approximately half as thick as septal grafts but had intact perichondrium, received longer voltage application times. The selection of application time was based on analysis of previous EMR experiments where approximately 2 mm-thick porcine septal grafts required either longer application time or higher voltage to achieve the same degree of shape change as a 1 mm-thick rabbit septum [9–11]. In contrast to previous studies, where reshaping was performed using either semicylindrical or 90 deg jigs [9–11,21], this study used flat surface electrodes to ensure consistent measurement of the tangent modulus under tension.

The results of this study indicate that, for EMR voltages higher than 2 V, the mechanical change in cartilage was reflected by a reduction in tangent modulus (Fig. 6). A basic mechanical property, tissue tangent modulus is governed by many of the chemical components cited above as part of proposed EMR mechanisms. The aforementioned hydrolysis reactions have two mechanically important results—water loss and pH changes. The latter is specifically characterized by the production of hydrogen ions (H^+) at the anode and hydroxide ions (OH^-) at the cathode [10]. Water content and fluid movement, which would be altered by hydrolysis, are known to influence tensile cartilage properties [16]. Altered through redox reactions as well, pH and ion concentrations change the fixed charge distribution which profoundly modifies the mechanical properties of cartilage tissue [13,16–18]. Individually, pH determines the net charge of collagen, normally neutral at physiological pH, while ionic concentration also impacts the osmotic pressure of cartilage, which in turn affects tensile stress in the tissue's collagen network [15,17,18]. While pH changes may induce dramatic mechanical changes in cartilage, such alterations are often reversible [19,20]. Even after the 15-minute rehydration period; however, EMR-treated samples did not return to their native state, instead exhibiting a reduction in tangent modulus. The total sum of the various, putative electromechanical changes described above as taking place during EMR may have contributed to these irreversible changes in the mechan-

ics of the cartilage. The evidence of the mechanical change of cartilage in this study may be part of the means through which cartilage becomes more conducive to shape change accomplished through EMR.

Cartilage matrix components likely participate in the electrochemical reactions accompanying EMR, and one such mechanically vital molecule, elastin, distinguishes the two main types of cartilage in the head and neck—hyaline and elastic cartilage. While both types contain the tensile component of Type II collagen, only elastic cartilage also has elastin, which additionally contributes to tensile strength, an especially relevant consideration for this mechanical, tension-based study. Furthermore, elastin has greater deformability and stability to pH and thermal changes [21]. Although EMR can reshape both types of cartilage, prior EMR studies have typically focused on the more uniform hyaline cartilage found in the rabbit septum. Including auricular cartilage, and thus elastic cartilage, in this study was essential for determining if EMR mechanical effects would differ between the two cartilage types. When a mechanical change was observed in either auricular or septal samples in this study, it was a reduction in stiffness (Fig. 6), demonstrating that both types of cartilage may be softened through EMR. No effect was observed for either tissue types at low voltages: mechanical properties do not change, as the electric field at the tissue-electrode interface is likely insufficient to initiate the relevant electrochemical reactions. Low and even negative electric current observed at 2–3 V indicates the absence of electrochemically-generated changes. More than twice as stiff as septal cartilage but treated for a longer time, native auricular rabbit cartilage began softening at a lower voltage threshold of 3 V, compared to 5 V for septal. Higher voltage applications on septal cartilage showed qualitatively greater gas evolution during EMR. The softening of auricular cartilage at a lower voltage threshold could be due to a less homogenous collagen network, but the stability of elastin may be evident in the lack of significant change in softening for 3–4 V (Fig. 6) and even 6 V, when a temperature increase of $\sim 10^\circ C$ was observed. This observed stability could also be the product of each auricular sample's intact perichondrium, a dense, fibrous tissue shown to significantly contribute to the tensile mechanical properties of both septal and costal cartilage under these measurement conditions [22,23]. Other differences between elastic and hyaline cartilage contributing to the chemical differences between the two types may include the increased glycosaminoglycan (GAG) content and permeability of septal cartilage, [24] which may have influenced the EMR reactions, though we suspect these subtle differences to have little impact. Specimen thickness is another possible factor which must be taken into account for the observed reduction in the voltage threshold where tissue softening is first observed. In thinner samples, such as those of auricular

cartilage, the diffusion of redox byproducts through a relatively short distance (thickness) or even the entire sample produces a higher degree of mechanical modification.

Applied voltage and application duration are two main factors varied during all previous and current EMR studies, yet without first imposing a mechanical deformation, cartilage shape change cannot occur. This reveals a major, though not unwarranted, limitation to this study. While purporting to be an investigation of the effect of EMR on the mechanical properties of cartilage, this study omitted the creation of actual shape change in the samples. Through the use of flat platinum electrodes, samples maintained their original flat, rectangular prismlike shape. Yet, while this experiment was not a pure reflection of EMR, the exclusion of dramatic shape change was indispensable: the flat samples facilitated mechanical testing, the ultimate focus of the study. Related to this concern, flat, rather than needle electrodes were used because the former treat almost the entire surface of the cartilage, spreading the mechanical effect of EMR throughout the tissue. In contrast, in needle electrode EMR, the most dramatic effects of EMR are spatially concentrated adjacent to the electrodes [11]. Reshaped specimens of nonuniform geometry or mechanical changes due to EMR would preclude practical mechanical testing in samples that already presented challenges due to the inherent thinness of the specimens. Such sample dimension restrictions will also require creep indentation testing for compression moduli to be measured in future studies. In cartilage, an anisotropic material, collagen is the source of tensile strength, while proteoglycans provide compressive strength. Since proteoglycans are suspected to play a key role in the EMR mechanism, compression tests to examine changes in proteoglycan strength will be invaluable in upcoming studies that will continue to investigate the mechanical characteristics of EMR-treated tissue. While lack of mechanical deformation and compression testing decrease the thoroughness of this study, the data presented still provide essential, directed insights on how applied voltage itself affects cartilage, as well as highlighting directions for future EMR biomechanical studies.

5 Conclusion

The results of this study suggest that the electrochemical reactions that assist EMR-produced shape change in cartilage also change its mechanical properties by reducing its stiffness. This was a general result observed in both elastic and hyaline cartilage. While EMR may create an undesired mechanical change in the cartilage to induce shape change, much more destructive, traditional techniques such as suturing and morselizing certainly do as well. Regarding the electrochemistry of EMR, the mechanical changes in cartilage in direct contact with either the anode or cathode alone remain to be determined. This is of special interest due to a recent study demonstrating decreased viability in the anodic region [11]. Due to the thinness of this rabbit cartilage, the effects of the opposite polarities could not be separately determined using flat electrodes in this study. Clinically, needle electrodes, rather than the surface electrodes used, are the most minimally invasive means of applying EMR, minimizing any threats to chondrocyte viability [25]. Mechanical studies involving needle electrodes have yet to be performed. Despite this lack of data, the existing data provide insight into optimal EMR parameters when degree of reshaping is correlated with different voltage and time parameters. For example, for auricular cartilage, 4 V may be chosen over 3 V because 4 V produces higher reshaping without further reduction in stiffness. Such an understanding of the optimization of EMR parameters further cements the road to the clinical implementation of EMR.

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