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Electromechanical Reshaping of Septal Cartilage

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Objectives: **This study describes the process of tissue electroforming and how shape changes in cartilage can be produced by the application of direct current (DC). The dependence of shape change on voltage and application time is explored.** ***Study Design:*** **Basic investigation using ex vivo porcine septal cartilage grafts and electromechanical cartilage deformation focused on development of a new surgical technique.** ***Methods:*** **Uniform flat porcine nasal septal cartilage specimens were mechanically deformed between two semicircular aluminum electrodes. DC current was applied to establish charge separation and electrical streaming potential. Voltage (0–3.5 V) and application time (0–5 minutes) were varied. Shape change was measured, and shape retention was calculated using analytic representation. The effect of the direction of applied current on shape change was evaluated by switching the polarities of electrodes and using parameters of 0 to 5.5 V and 5 minutes. Temperature during reshaping was monitored with a thermocouple, and surface features were evaluated using light microscopy.** ***Results:*** **Reshaped specimen demonstrated mechanical stability similar to native cartilage tissue. Shape retention strongly correlated with increasing voltage and application time. Only a small current (<0.1 A) through the tissue was measured. Temperature change was less than 2°C during electroforming, suggesting that electroforming likely results from some nonthermal mechanisms. Surface features indicated that electrodeposition may occur depending on electrode material and magnitude of the applied voltage.** ***Conclusions:*** **These findings demonstrate that cartilage can be reshaped through the process we have described as "electroforming" by generating intrinsic differences in charge separation with negligible heat production.**
Key Words: **Cartilage, electroforming, streaming potential, shape change.**

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INTRODUCTION

Cartilage serves many functional and structural roles in the head and neck, including the support of soft tissue in the ear and nose, maintenance of airway patency, phonation, and joint movement. The functional and esthetic defects in the head and neck that result from cancer surgery, trauma, or congenital malformations have led to the development of surgical techniques to reshape cartilage to recreate damaged or absent structures. Current reconstructive techniques include carving, morselizing, scoring, or suturing native cartilage grafts.[1,2] The disadvantages of these approaches include donor site morbidity from graft harvest, waste of excess graft tissue, shape memory effects, and lack of control over warping, particularly in costal cartilage tissue. Several alternative approaches to reshaping cartilage have been advocated, including enzymatic digestion in situ,[3,4] radiofrequency (RF) reshaping,[5] and laser cartilage reshaping.[6] Of these new approaches to changing the shape of native cartilage tissue, laser reshaping has received the most attention. In 1993, Helidonis et al.[6] proposed an alternative laser-assisted approach based on thermal-mediated stress relaxation to reshape cartilage. Despite clinical uses of laser technology to reshape human cartilage, this method remains investigational, and the associated biophysical changes accompanying shape change are incompletely understood.

As an extension of the work in our laboratory focused on cartilage reshaping, we have developed a technique that can be used to reshape cartilage tissue by combining mechanical deformation with the application of direct current (DC) electric fields. Although electrosurgery has been a fixture in surgery for over a century, the concept of tissue electromechanical reshaping (hereafter referred to as electroforming) is novel, and our preliminary investigation was inspired by the observation that cartilage is a

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piezoelectric material. Initially, we postulated that, similar to a conventional piezoelectric material, the application of DC voltage may result in breakdown of the fixed charge distribution in this tissue, leading to significant changes in tissue material properties.

Composed of a network of collagen, proteoglycans, and water, the cartilage matrix is innately electronegative, and this electric charge is only partially compensated by free cations that permeate the interstitial fluid. The presence of this charge distribution generates a double-layer electric field with a large electrical charge density. Within the double-layer regions, Sah et al.[7] demonstrated that cations may move freely within the matrix, and these cations separate from the fixed negative charges during mechanical deformation. This phenomenon of charge separation or streaming potential led to the development of the hypothesis that cations undergo reorganization when placed in an electric field. This study describes the biophysical behavior of cartilage during electroforming by studying the degree of shape change in porcine nasal septal cartilage grafts. In particular, we evaluated the dependence of shape change on the magnitude and polarity of the applied DC voltage and application time as well as monitoring tissue temperature and electrode deposition during this reshaping process. Characterizing the effects of applied voltage and the duration of treatment is an important step in understanding the physical processes responsible for electroforming and providing insight into how this procedure can be optimized.

MATERIALS AND METHODS

Specimen Preparation

Porcine nasal septal cartilages were harvested from freshly killed pigs' crania obtained from a local abattoir (Clougherty Packing Company, Vernon, CA) as previously described.[8] Only the cranial part of the septal cartilage was used for experimentation because previous studies in our laboratory have shown that there are regional variations in biochemical, cellular, and mechanical properties in the porcine septum along the cranial- (near the nasopharynx) caudal (near the nares) axis.[9] Cartilage grafts from each septal cartilage were obtained and cut into uniform rectangular slabs ($16 \times 5 \times 2$ mm) using a custom guillotine microtome.[10]

Apparatus and Reshaping

Before electroforming, cartilage specimens were sandwiched between two semicircular aluminum electrodes mounted on a jig consisting of acrylic blocks secured by screws (Fig. 1). The radius of curvature of the jigs were 8 mm and 11.5 mm, respectively. Aluminum was selected as the electrode material because of its malleability, low cost, and electrical conductivity. Leads from a DC power supply (Model PPS-2322, Amrel, Arcadia, CA) were connected to the electrodes to establish charge separation and establish the electrical streaming potential.[7] As illustrated in Figure 1, the inner and the outer curvatures of the specimen were connected to the anode (positive) and cathode (negative), respectively. The applied current was measured and monitored using a multimeter in series with the jig (2001, Keithley, Cleveland, OH) to avoid excessive resistive heating caused by a high current density between electrodes.

Voltage was varied from 0 V to 3.5 V for a set application time of 5 minutes. Likewise, the effect of application time on shape change was studied by varying application time from 1 to 6 minutes for four different voltages (1 V, 1.5 V, 2 V, 2.5 V). To study the effect of applied current direction, the polarities of the electrodes were switched (inner curvature to negative electrode), using parameters of 0 to 5.5 V and 5 minutes. Control specimens underwent identical bending procedures without the application of voltage, thus allowing determination of the effect of mechanical deformation on shape change alone.

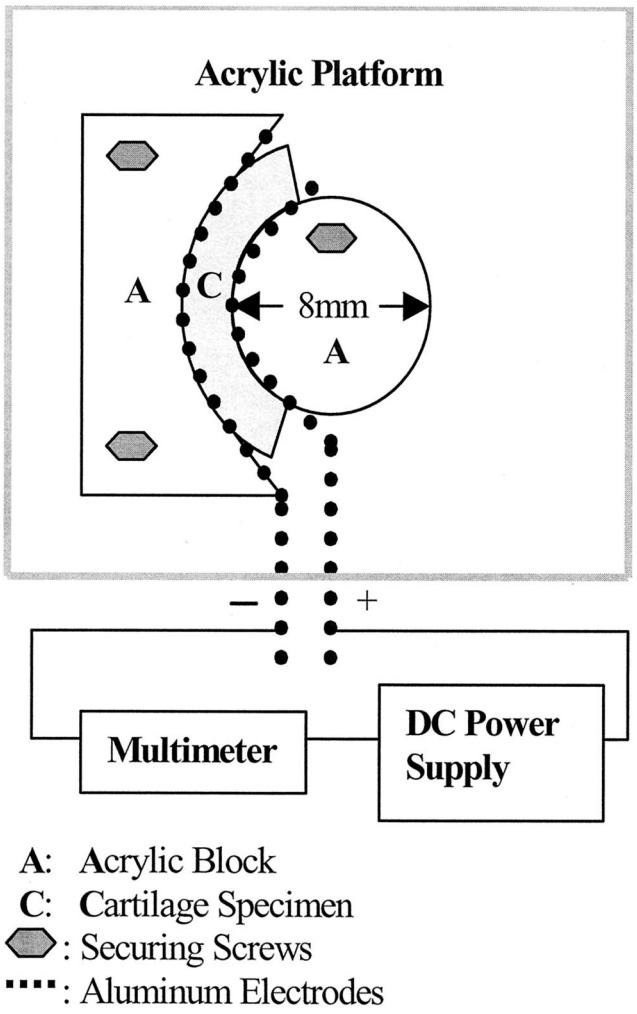

Fig. 1. Experimental protocol used to reshape cartilage.

During electroforming, surface temperature was measured using an insulated thermocouple and a cold-junction compensator (HH509R, Omega Engineering, Stamford, CT) placed between the specimen and electrode to determine whether any temperature rise accompanied this process. Immediately after electroforming, specimens and the securing jig were immersed in saline solution at ambient temperatures for 15 minutes in accord with protocols established for laser reshaping studies.[8] Specimens were then removed from the jig, photographed, and the distance between the two ends of specimen was measured by a digital electronic caliper (CD-6″CS, Mitutoyo Corp, Japan). The specimen deformation or shape change is described in terms of the bend angle. The bend angle was calculated using a model that assumes that specimen shape approximates an arc segment of a circle.[11] The model is based on the following equation:

$$L = [2Li \sin(\theta_{expt}/2)]/\theta_{expt}$$

where L is the distance between the ends of the bent sample, L_i is the initial length of the sample before deformation, and $\Theta_{expt.}$ is

the experimental bend angle in radians numerically determined by solving the above equation using the "goal seek function" in Excel (Microsoft Corporation, Redmond, WA). The maximal bend angle Θ_{max} was calculated by measuring the linear distance between the two ends of the specimen while it was still secured to the jig. The extent of shape retention was determined through normalizing $\Theta_{expt.}$ by the maximal bend angle Θ_{max}:

$$\text{Shape Retention} = \theta_{expt} / \theta_{max}$$

For the given reshaping jig and specimen dimensions used in this study, Θ_{max} was within the range of 2.76 to 2.88 radians. In addition, cartilage surfaces were inspected using a high-power light microscope to ascertain whether any surface feature changes such as electrodeposition occurred.

RESULTS

A photographic montage of cartilage specimen before (A) and after (B) electroforming is shown in Figure 2. Figures 3 and 4 illustrate the effect of voltage and application time on shape retention, respectively.

Figure 3 demonstrates that degree of shape retention increases gradually with voltage, reaching a plateau value of approximately 0.84 at about 1.5 V, which represents an empirical threshold for electroforming using the present apparatus and specimen size. Values were averaged for each treatment group with error bars representing the standard error of measurement.

A similar pattern is observed when shape retention is evaluated as function of increasing voltage application time (Fig. 4). With each of the four voltage studies (1 V, 1.5 V, 2 V, 2.5 V), the minimum application time required to establish near-maximal shape retention were approximately 5, 5, 4, and 1.5 minutes, respectively.

Figure 5 depicts the dependence of shape retention on electric field polarity (negative electrode to the inner curvature). The threshold value (approximately 4.5 V) was significantly higher than that (1.5 V) of the experimental protocol, as shown in Figure 3.

A representative plot of surface temperature during reshaping is depicted in Figure 6. During electroforming, surface temperature increased slowly, albeit with temperature rise of less than 2°C after 5 minutes of electroforming at 3.5 V.

During electroforming, white foam formation (small bubbles) was observed after approximately 10 seconds of voltage application. No foam generation occurred when voltages were less than 1 V. Applied current measurements were within the range of 0 to 12 mA. After electroforming and rehydration, no apparent change in dimensions or surface texture was observed. There was no char formation or any other obvious evidence of tissue injury on visual inspection. Microscopic examinations revealed minimal gray colored electrodeposition on specimen under the experimental conditions (0–3.5 V). Substantial specimen shrinkage and dark electrodeposition that likely represents electrochemical deposition of aluminum ions were observed on the specimen surface when examined under light microscopy, but only when voltage exceeded 4 V. Several reshaped specimens (removed from the jig) were stored in saline solution (4°C) for 24 hours, and the resulting shape retention was found to be approximately 80% to 90% of the original values measured immediately after rehydration.

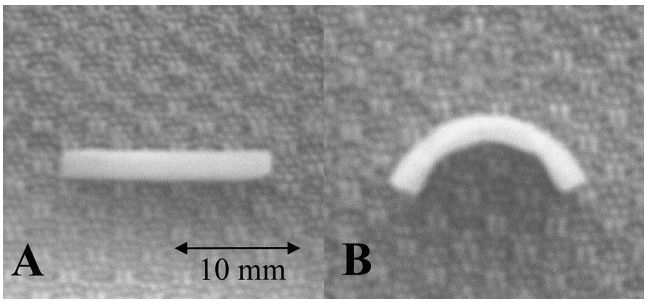

Fig. 2. Cartilage specimen before (A) and after (B) electroforming.

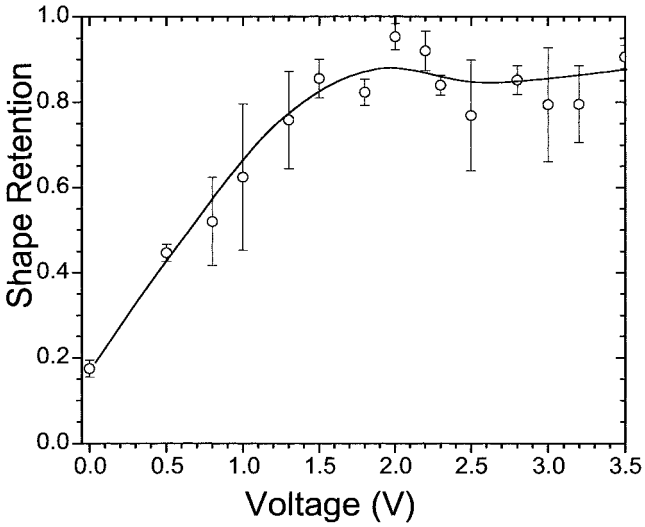

Fig. 3. Shape retention as a function of applied voltage (± SE).

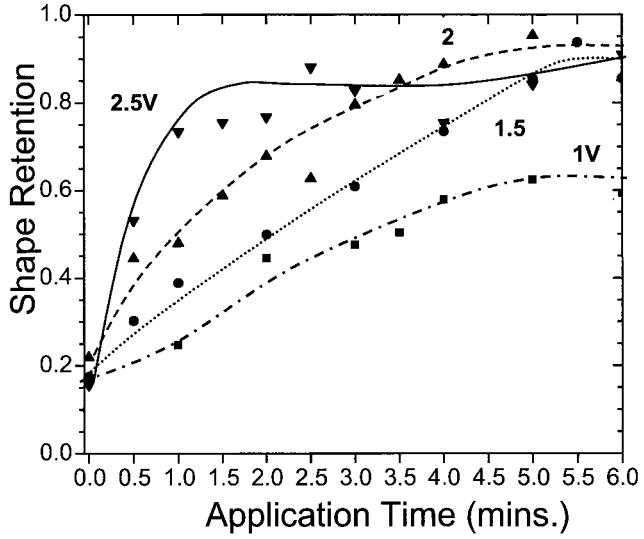

Fig. 4. Shape retention as a function of application time using four different voltages.

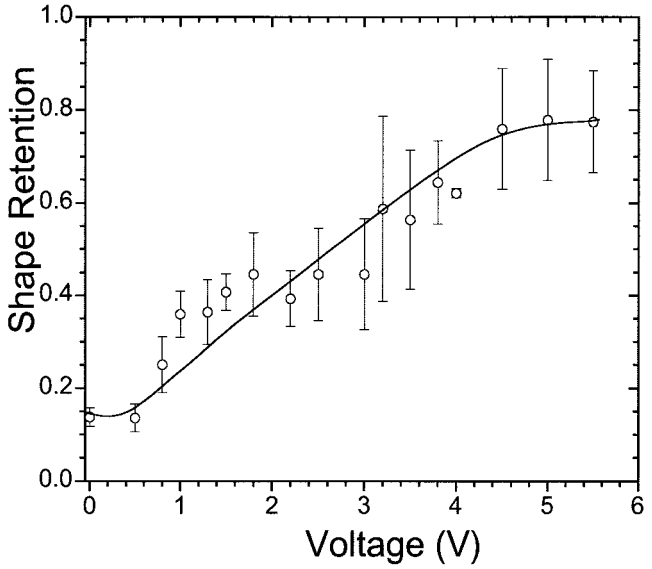

Fig. 5. Shape retention as a function of applied voltage with polarities of electrodes reversed (±SE).

DISCUSSION

Electroforming is a novel technique that can be used to reshape cartilage. Our motivation for studying cartilage electroforming was based on a number of advantages electroforming has over other methods of cartilage reshaping including the following: 1) it is readily available at low cost; 2) it is technically simple and can conceivably be performed using a battery power source; and 3) the current can be easily controlled to avoid thermal injury, which may occur in other procedures such as laser and RF-mediated reshaping.

Shape Retention and Memory

Our results illustrate that cartilage can undergo significant shape change by way of electroforming, and we have correlated this effect with applied voltage, application time, and electric field polarity. The experiments that focused on examining the effect of application time on shape change were performed using four different voltages to demonstrate another correlation: the time dependence (minimum time required to reach maximal reshaping) of this process. As shown in Figure 4, for higher voltages, shorter application times are required to reach maximal shape change. Given the trends established in this study, further analysis using larger parameter sizes will likely provide additional information on the combined effects and relative contributions of voltage and application time on shape retention and will facilitate the development of a rigorous model to describe this behavior.

In addition, a memory effect was observed in these studies, as illustrated by the slight decrease in shape retention after 24 hours. Therefore, clinical implementation of this technique will likely require "overbending" of the cartilage graft to compensate for this effect. Because shape memory, like shape retention, varies with spatial location of cartilage and with the time interval allowed for the release of interlocked forces (native stress relaxation), future studies using longer observation periods will be crucial in determining the degree of overcorrection needed for achieving the desired clinical result.

The assessment of actual shape change using closed-form analysis or numerical solutions is difficult and has prompted our use of the simple circle model to estimate shape change using easily measured linear dimensions. The jig was carefully designed so that the inner and outer surfaces of the specimen were in close contact to the rigid semicircular electrodes. However, a variation in the maximal bend angle Θ_{max} (2.76–2.88 radians) was introduced by the slight differences in specimen dimensions (primarily thickness) and the pressure exerted during mechanical deformation by the securing screw assembly. To compensate for this variation, shape retention, instead of shape change, was calculated by normalizing the final shape acquired after rehydration by the initial secured shape before electroforming. The accuracy of linear measurements was further enhanced by the use of a digital electronic caliper.

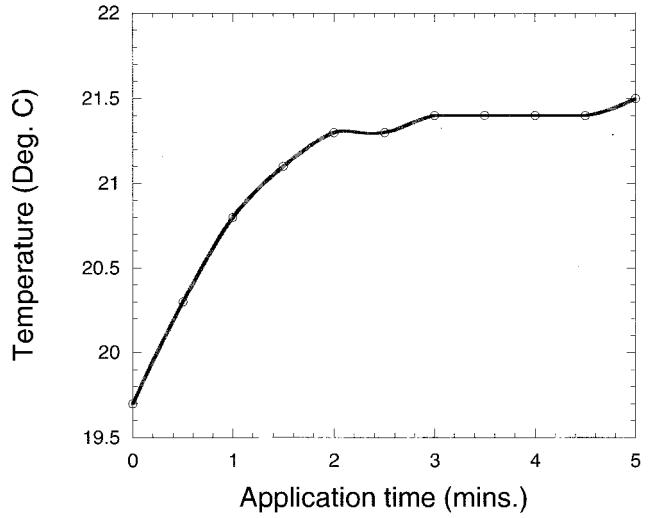

Fig. 6. Temperature as a function of application time using 3.5 V.

Temperature Measurement

As shown in Figure 6, surface temperature rose slightly from 19.7°C (ambient) to 21.5°C after 5 minutes voltage application (3.5 V), an increase of 1.8°C. Other temperature measurements were made using voltages lower than 3.5 V, and the changes in temperature over a 5-minute time span were less than 1.8°C. However, when voltage was raised to 20 V, significant heating was observed. This is not surprising because the corresponding increase in current (I) (from less than 0.1 A at 3.5 V to about 1 A at 20 V) leads to more than a 100-fold increase in power (power = $I^2 \times R$, where resistance R stays relatively constant in this case). In this study, temperature measurements have shown that very little heat is generated during this process when voltage is low and the resistance of the cartilage matrix is large (>1 KΩ). Therefore, unlike RF[4] and laser cartilage reshaping, shape change after electroforming likely results from some nonthermal mechanisms.

Proposed Mechanisms

Although the molecular events that accompany electroforming are not known, on the basis of the results of this study and previous investigations on laser cartilage reshaping, we propose several mechanisms. The first mechanism is that electroforming results in the loss of water through hydrolysis, an electrolytic reaction that transforms water into free hydrogen and oxygen molecules. Hydrogen ions are reduced to form hydrogen gas at the cathode. At the same time, hydroxide ions are oxidized to produce oxygen gas at the anode. The observation of foam–bubble formation combined with a lack of significant temperature rise at the interface between the cartilage and the electrodes while current is supplied supports this hypothesis. It is well known that water content greatly influences the biomechanical properties of cartilage[12] because the mechanical properties of this tissue result from the interaction of water molecules, cations (Na^+ and Ca^{2+}), and negatively charged macromolecules (proteoglycan, collagen). It is believed that internal stress reduction during laser reshaping is caused by a bound-to-free transition of water molecules in the matrix;[13,14] water loss produced by hydrolysis may produce the same changes in the matrix, leading to accelerated mechanical stress relaxation. This mechanism is compatible with our macroscopic observations, which have shown the dependence of shape change on voltage and application time. An increase in voltage creates a stronger electric field that in turn accelerates hydrolysis, thus enhancing shape change. Likewise, an increase in application time allows further loss of water from the cartilage matrix, leading to greater reshaping.

A second possible mechanism is that protein electrophoresis occurs during electroforming. When cartilage specimen is bent into a semicircular arc, the inner and outer curvatures of the specimen undergo compression (increase in stress) and expansion (decrease in stress), respectively. Because the distribution of proteoglycan is the major determinant of the internal stress within cartilage matrix, a bent specimen has a higher density of proteoglycan molecules at the inner curvature and the lower density at the outer curvature. When voltage is applied, migration of negatively charged proteoglycan molecules toward the positive electrode increases the density of proteoglycan at the inner curvature, thus stabilizing the bent configuration. When the polarities are reversed, there are less proteoglycan molecules at the inner curvature, resulting in less shape change for a given voltage (Fig. 5). In conventional gel electrophoresis, where distance traveled by protein fragments is dependent on both applied voltage and running time, a higher voltage or longer application time will likely result in a larger proteoglycan accumulation at the inner curvature and therefore greater shape change.

The third mechanism involves the alteration in intrinsic molecular structure within cartilage matrix, including depolymerization of proteoglycan molecules or collagen molecules, disruption of bonds between the collagen and proteoglycan subsystems, and bound-to-free water transition. When triggered by the application of electric field, these processes could lead to temporary disruption and formation of bonds, alteration of internal stress, and permanent molecular reorganization.

Electrodeposition

Our initial consideration of aluminum as the electrode material was mainly for practical reasons: 1) low cost and ready availability, 2) high conductivity, and 3) high malleability. However, one concern with aluminum is that it has a low standard electrode (reduction) potential (-1.7 V) among metals with high conductivity. The standard electrode potential correlates with the likelihood of a metal to undergo oxidation and electrodeposition. A low potential material like aluminum would produce more electrodeposition than metals with higher standard electrode potentials such as silver (0.8 V) and gold (1.5 V). Although the effect of electrodeposition on cartilage reshaping is not fully understood, it is unlikely that electrodeposition plays a primary role in shape change because our observations indicate that substantial shape change can occur with minimal deposition when low voltages such as 2 V are used. Therefore, we opted not to use gold or silver electrodes in this study so as to minimize expenses. There are reasons, however, to believe that electrodeposition exerts a complementary effect on cartilage reshaping. One possible mechanism is that metal deposition changes the extrinsic or intrinsic structure of the cartilage matrix, providing stabilization to the bent configuration specified by the jig. Few studies have focused on the effect of electrodeposition on tissue, yet there are several therapeutic treatments based on the use of metals, such as the use of gold injection therapy for early active rheumatoid arthritis and other synovial inflammatory diseases.[15] Studies on the use of other electrode materials and viability of specimens are currently underway to evaluate the values and optimize the process of cartilage electroforming.

CONCLUSION

This study introduces a novel method of reshaping cartilage. This is the first documentation that describes a nonthermal, electrically-mediated technique to reshape cartilage. Our selective measurement of the change in shape, temperature, and surface features creates a solid foundation for future studies of electroforming. We plan to expand our investigations on a range of electrical and mechanical properties as our interests in cartilage electroforming and tissue engineering grow.

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