Association of Electrochemical Therapy With Optical, Mechanical, and Acoustic Impedance Properties of Porcine Skin

Wesley J. Moy, PhD; Erica Su, BS; Jason J. Chen, BS; Connie Oh, BS; Joe C. Jing, PhD; Yueqiao Qu, MS; Youmin He, MS; Zhongping Chen, PhD; Brian J.F. Wong, MD, PhD

IMPORTANCE The classic management of burn scars and other injuries to the skin has largely relied on soft-tissue transfer to resurface damaged tissue with local tissue transfer or skin graft placement. In situ generation of electrochemical reactions using needle electrodes and an application of current may be a new approach to treat scars and skin.

OBJECTIVE To examine the changes in optical, mechanical, and acoustic impedance properties in porcine skin after electrochemical therapy.

DESIGN, SETTING, AND PARTICIPANTS This preclinical pilot study, performed from August 1, 2015, to November 1, 2016, investigated the effects of localized pH-driven electrochemical therapy of ex vivo porcine skin using 24 skin samples. Platinum-plated needle electrodes were inserted into fresh porcine skin samples. A DC power supply provided a voltage of 4 to 5 V with a 3-minute application time. Specimens were analyzed using optical coherence tomography, optical coherence elastography, and ultrasonography. Ultrasonography was performed under 3 conditions (n = 2 per condition), optical coherence tomography was performed under 2 conditions (n = 2 per condition), and optical coherence elastography was performed under 2 conditions (n = 2 per condition). The remaining samples were used for the positive and negative control groups (n = 10).

EXPOSURES Platinum-plated needle electrodes were inserted into fresh porcine skin samples. A DC power supply provided a voltage of 4 to 5 V with a 3-minute application.

MAIN OUTCOMES AND MEASURES Tissue softening was observed at the anode and cathode sites as a result of electrochemical modification. Volumetric changes were noted using each optical and acoustic technique.

RESULTS A total of 24 ex vivo porcine skin samples were used for this pilot study. Optical coherence tomography measured spatial distribution of superficial tissue changes around each electrode site. At 4 V for 3 minutes, a total volumetric effect of 0.47 mm³ was found at the anode site and 0.51 mm³ at the cathode site. For 5 V for 3 minutes, a total volumetric effect of 0.85 mm³ was found at the anode site and 1.05 mm³ at the cathode site.

CONCLUSIONS AND RELEVANCE Electrochemical therapy is a low-cost technique that is on par with the costs of suture and scalpel. The use of electrochemical therapy to create mechanical and physiologic changes in tissue has the potential to locally remodel the soft-tissue matrix, which ultimately may lead to an inexpensive scar treatment or skin rejuvenation therapy.

LEVEL OF EVIDENCE NA.
car formation is the natural response to tissue injury. In the skin, scars result from the biological process of wound repair in which the excess deposition of collagen forms to counteract the developed incident forces across the damaged tissue matrix.\(^1\) Trauma, burns, and surgery often result in the formation of complex and disfiguring scars, and currently, there are many solutions (eg, chemical peeling, triamcinolone acetonide injections, and wound modification through pressure) that attempt to control or modulate this type of aberrant wound healing but come at a significant cost.

Previously, our group developed a technique termed electromechanical reshaping (EMR), a needle-based technique that uses in situ reduction-oxidation (redox) chemical therapy to reshape the structural framework of cartilage and other proteoglycan-rich tissues. The insertion of platinum electrodes into the cartilage combined with the application of an electrical potential generates hydrogen ions at the anode site and hydroxide ions at the cathode site, creating steep pH gradients in the vicinity of the electrodes that lead to localized stress relaxation within the cartilage matrix.\(^2,3\) Electromechanical reshaping is a low-cost treatment, requiring only platinum-plated electrodes and a DC battery.

In previous EMR studies,\(^4-6\) changes in skin turbidity and texture were incidentally observed surrounding the needle insertion sites during cartilage reshaping. Because the platinum-plated needles were not insulated, we hypothesized that voltage-driven pH gradients also propagated into the adjacent skin. In addition, softening of the skin was qualitatively appreciated via palpation, which is consistent with observations of post-EMR effects on the elastic modulus of the cartilage,\(^7\) although a long-term study\(^8\) of 5 months demonstrated the return of normal collagen architecture. This finding suggests that the pH perturbations generated by redox chemical therapy have the potential to alter the local mechanical behavior of the soft-tissue matrix in skin and potentially trigger a remodeling response similar to laser or radiofrequency (RF) but via a chemical mechanism.

Adapting the principles of EMR, we found that it is possible that skin may also undergo changes in response to electrochemical modification. With proper and precise selection of electrode configuration, electrical potential, and application duration, spatially localized regions of steep pH gradients can be generated in skin near the electrodes. It has been well established that chemical peels induce pH gradients in skin for therapeutic effects; however, chemical peels are difficult to control because the penetration depth depends on local tissue properties, which are inhomogeneous. The risks of chemical peeling include loss of epidermal tissue, injury to the dermis and subcutaneous soft tissue, and postinflammatory hyperpigmentation. As such, we posit electrochemical therapy (ECT) as a means to locally alter skin mechanics and potentially be a low-cost means to treat scars and rejuvenate the skin.

Results of our initial attempts of characterizing mechanical changes in porcine skin immediately after ECT via classic mechanical testing (ie, needle puncture and indentation) were limited by several factors, including tissue hydration, sample geometry, and limitation in our mechanical testing apparatus. These limitations led us to investigate the use of optical and ultrasonic methods to study the effects of ECT in skin.

In this pilot study, mesoscopic imaging modalities in the form of optical coherence tomography (OCT), optical coherence elastography (OCE), and high-frequency ultrasonography were used to image the optical and mechanical changes in ex vivo porcine skin treated before and after ECT. The objective of the current study was to examine the effects of electrochemical modification on ex vivo porcine skin by using the 3 mesoscopic imaging modalities.

### Methods

#### Tissue Preparation and Dosimetry

This preclinical study, performed from August 1, 2015, to November 1, 2016, investigated the effects of localized pH-driven ECT of ex vivo porcine skin by using 24 skin samples. Porcine skin was obtained from a local abattoir and was sectioned into 50 × 50-mm squares for ultrasonography and 25 × 25-mm squares for the OCT and OCE experiments. The samples were maintained in phosphate-buffered saline (PBS) at 4°C. Before the application of ECT, the samples were removed from PBS and air dried for 10 minutes. Controls include native unmodified tissue and samples immersed in 1 × acetic acid (pH 2.4; Fisher Science Education) and 1 × sodium hydroxide (pH 8.6; Fisher Scientific) to investigate the effect of pH change alone. These acid- and base-immersed samples were placed into their respective solutions for 30 minutes, and imaging measurements were subsequently taken with ultrasonography and OCT. For the experimental group, the samples were subjected to ECT using different dosimetry variables, and imaging was performed using ultrasonography, OCT, and OCE (Figure 1). The OCE images were performed with specimens submerged in PBS during imaging, which was performed immediately after ECT treatment. The University of California Irvine Animal Care and Use Committee waived the need for institutional review board approval.

#### Electrochemical Modification

For sham control and experimental groups, a single positive electrode (anode) was inserted into the center of the tissue and a single negative electrode (cathode) was inserted 2 mm to the right of the positive electrode (Figure 2). Platinum-plated needle electrodes (0.3-mm diameter; Grass Technologies) were chosen for...
their high standard potential and minimal risk of electrode oxidation and for having been used in previous studies.\textsuperscript{1,5,8-14} The electrodes were connected to a DC power supply (model E3646A; Agilent Technologies). Voltage differences of 4 and 5 V were applied to the experimental samples for 3 minutes. No voltage was applied to controls. Voltage was controlled and current was monitored using a MATLAB program and PC workstation.\textsuperscript{5} In addition to single paired electrodes, a 3 × 4 needle array (2-mm spacing between electrodes) was used to perform ECT at 4 V for 3 minutes; however, this setup was imaged only with ultrasonography because changes occur in a large region of interest that exceeds the scanning capabilities of the present OCT and OCE systems. After voltage termination, the needle electrodes were removed, and the specimens were immediately imaged with OCT, OCE, or ultrasonography.

**OCT, OCE, and Ultrasonography System Hardware, Imaging, and Data Analysis**

Optical coherence tomography is an interferometric imaging modality analogous to ultrasonography in its ability to provide cross-sectional images of tissue substructure based on the tissue optical scattering properties and has been previously described in the literature.\textsuperscript{9} Acoustic radiation force OCE was used to characterize subtle changes in tissue mechanical properties and has been previously described in the literature.\textsuperscript{10,11}

A clinical high-frequency dermatologic ultrasonography system (Episcan I-200; Longport Inc) was used to image the tissue and has been previously described.\textsuperscript{12} After ECT, the surface of the skin specimens was covered with ultrasound gel and the ultrasound probe was placed directly on top relative to the needle placement orientation (Figure 2). Measurements were taken at the needle insertion site for the experimental group specimens and the central region between needle insertion sites in each of the control specimens.

**Results**

**OCT Image Analysis**

A total of 24 ex vivo porcine skin samples were used for this pilot study. En face OCT images of controls revealed normal tissue architecture (Figure 3A). The epidermis and dermis had relatively uniform texture and signal intensity, indicating that the sample was largely homogeneous. The structure of skin under OCT has been characterized by several studies and well described elsewhere.\textsuperscript{13-15} In ECT specimens (4 and 5 V for 3 minutes) (Figure 3B and C), each image revealed a region of lower signal intensity centered at each electrode insertion point, which overlapped with the region between the insertion points. Normal tissue is characterized by a high-intensity signal for OCT. Normal tissue is heterogeneous, indicating changes in the index of refraction. The low-intensity OCT signal indicates minimal changes in the index of refraction, suggestive of tissue homogeneity after ECT was performed. This effect was observed at increasing depths in the tissue and radially from the electrode sites, orienting conically into the tissue. The size of the affected regions in the tissue strongly correlates with the duration of ECT. At 4 V for 3 minutes, the calculated volumetric region of interest that underwent change was 0.47 mm\textsuperscript{3} at the anode site and 0.51 mm\textsuperscript{3} at the cathode site. At 5 V for 3 minutes, the calculated volumetric effects were 0.85 mm\textsuperscript{3} at the anode site and 1.08 mm\textsuperscript{3} at the cathode site. Note that the imaging depth of OCT in skin is limited to 500 μm.

**OCE Image Analysis**

Optical coherence elastography generates a 2-dimensional image (elastograph), which plots the spatial distribution of vibration amplitudes in a specimen that is actuated by a piezo.
High-intensity pixel values indicate large relative displacements, whereas low-intensity pixel values indicate small relative displacement. Higher displacements correlate with more compliant tissue properties. The relative displacement inversely correlates with the elastic modulus. Local mean displacement was 50 nm for the control (homogenous) (Figure 4A), 100 nm for 5 V for 3 minutes (Figure 4B), and 150 nm for 5 V for 3 minutes (Figure 4C), located centrally at each electrode site.

**Ultrasonography Analysis**

Ultrasound devices use sound waves for diagnostic imaging or medical treatment. Higher ultrasound frequencies lead to higher resolution with limited penetration depth, whereas lower frequencies are able to penetrate tissue much deeper at lower resolution.

The commercial device used in this study generates exceptional images of tissue in depth extended to the deep subcutaneous layers, albeit with significantly lower resolution than OCT. The control (Figure 5A) had key layers of native porcine skin. The stratum corneum, epidermis, dermis, and subcutaneous fat were clearly demarcated and labeled. The epidermis was represented by a higher-intensity reflected signal, whereas the dermis had a homogeneous pattern consistent with moderately dense collagen. The intensity is a function of the acoustic impedance in the tissue and is visualized by contrast in pixel intensities. As such, the images are at a lower resolution than OCT or OCE but cover a larger area laterally and in depth.

In Figure 5D-F, a general trend is observed in that signal intensity increases and broadens within the dermis as voltage or application time increases (along with total charge trans-

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**Figure 3. Optical Coherence Tomography (OCT) of the Skin**

(A) Control, (B) 4 V, (C) 5 V

En face OCT images of no electrochemical therapy (ECT) (control) (A), ECT of 4 V for 3 minutes (B), and ECT of 5 V for 3 minutes (C). Scale bar indicates 1 mm. Low signal intensity indicates change in optical properties consistent with effects of ECT. AN indicates anode; CT, cathode.

**Figure 4. Optical Coherence Elastography (OCE) of the Skin**

(A) Control, (B) Anode, (C) Cathode

The OCE images of no electrochemical therapy (ECT; control) (A), ECT at the anode of 5 V for 3 minutes (B), and ECT at the cathode of 5 V for 3 minutes (C). Scale bar indicates 250 μm. Higher-intensity values are represented by brighter colors and equate to regions of larger displacement, indicating tissue softening. Local mean displacement was 50 nm for the control (A), 100 nm for the anode site (B), and 150 nm for the cathode site (C), located centrally at each electrode site. The 0 to π scale is based on the phase shift of the Doppler optical coherence tomography signal.
fer). In Figure 5B and C, tissue samples were submersed in 1 × acetic acid and 1 × sodium hydroxide. The contrast in pixel intensity of the ultrasound images demarcates layers within the tissue (Figure 5B and C) and is similar to the control (Figure 5A). In stark contrast to Figure 5D and E, regions of interest are centrally located from the surface of the stratum corneum to at least a depth of 3 mm into the tissue; this effect is observed throughout the tissue (Figure 5F). Figure 5D and E illustrates a tissue effect that has a conical geometry, in which the effect spreads radially outward with depth. On the periphery, the tissue structures are well defined, but within the center region, a large acoustic impedance mismatch clearly shows tissue that has been altered. In Figure 5F, use of a dense needle array ECT demonstrates a widespread effect on the tissue, with several conically shaped regions of tissue overlapping one another. The relative uniformity of the higher signal intensities indicates regions where acoustic impedance mismatch is homogenous compared with unaffected regions of tissue. A previous study observed that increasing the voltage correlates with an increase in charge transfer.

**Discussion**

Contemporary treatment of scars and other injuries to skin relies on proper surgical technique in tandem with numerous adjuvantive methods, including corticosteroid injections, silicone sheet placement, laser, RF, and microneedling. However, microneedling produces variable success rates, whereas laser and RF are expensive and generate nonspecific thermal damage. Although the concept of ECT is derived from previous studies on electrochemical effects in cartilage, the present study characterizes the redox-induced alteration in the optical and mechanical behavior in skin. A previous study suggests that the electrochemical reactions that are triggered are related to the hydrolysis of water, which produces a localized pH perturbation that drives hydrogen and hydroxide ions into the tissue. More than half a century of detailed clinical experience has already demonstrated how topical application of acid to the skin (ie, chemical peels) alters appearance and texture for beneficial effect. Although these methods are similar, the clinician may be offered the potential of more control of the spatial distribution of pH changes with our technique. With ECT, the active agent (hydrogen and hydroxide ions) is generated in situ at the tissue-electrode interface, propagating into tissue down an electrical potential and concentration gradient. For ECT, the question remains whether the changes in tissue properties caused by these electrochemically generated pH changes can be exploited for therapeutic effect, particularly in dense scars. This study represents a starting point for future investigations aimed at this long-term objective.

In cartilage, there has been extensive analysis of the association between the electrochemical modification of tissue and...
changes in tissue mechanics, and these studies\textsuperscript{1,6,7,12,19,20,23-26} correlated dosimetry with shape retention, altered tissue biomechanics, stress relaxation, molecular mechanisms, and long-term tissue viability. Previous studies\textsuperscript{1,17} have specifically examined the effects that surround the electrodes, including pH mapping in cartilage. Furthermore, other studies\textsuperscript{4,8,20,28,29} have used EMR to mimic procedures that emulate the classic cut and suture operation ex vivo and in vivo. Unfortunately, unlike EMR studies\textsuperscript{3,6,7,12,19,20,23-26} in cartilage, quantifying the mechanical effects of ECT on skin is not tractable.

Classic mechanical analysis of ECT-treated samples is difficult to perform because the regions of interest created by ECT (or, for that matter, laser or RF) are one order of magnitude smaller than the dimensions evaluated using conventional mechanical testing platforms. Common methods to measure mechanical properties focus on classic tensile testing; however, although tensile strain-generating devices require large and homogenous samples, skin consists of a heterogeneous, multilayered structure. Although needle penetration and indentation tests are alternative methods to study skin behavior, hydration and geometry of the sample as well as testing conditions affect the consistency of results. Properly controlling these variables is an arduous task because the inconsistency is evidenced by a diverse body of literature.\textsuperscript{18-20} One commercial device, the Cutometer (MPA 580; Courage & Khazaka Electronic GmbH), aims to measure skin mechanical behavior based on optical displacement measurement but provides only relative measures and does not provide a means to calculate classic mechanical measurements, such as the elastic modulus or Poisson ratio.\textsuperscript{21} Furthermore, it has a limited spatial resolution of 2 to 3 mm at best. The ECT-treated samples, however subjective, demonstrated palpable softening. Although we are currently developing a customized microindentation system, it is beyond the scope of the present study.

Hindered by classic mechanical tests, we sought to evaluate optical imaging modalities, including optical (OCT and OCE) and ultrasonic (high-frequency ultrasonography) methods, all of which can indirectly estimate tissue mechanical properties but are not direct measurements of mechanical properties.

Optical coherence tomography provides micrometer scale resolution images based on the optical properties in the tissue, which are sensitive to changes in the refractive index. The OCT result demonstrates a dose-response relationship between index of refraction mismatch and voltage applied (Figure 4); however, an increased sample size is needed to make a substantive claim. In the OCT images, the diameter of the low-intensity region increased as the voltage increased. It has been established that the hydrolysis of water by EMR reduces the pH in cartilage, inducing structural changes. This reduced intensity in the OCT images was likely attributable to a structural change in skin that leads to less optical scattering.\textsuperscript{1,21} Although fibrils in skin are mainly formed by type I collagen and fibrils in cartilage by type II collagen, both form similar structures; thus, we postulate that skin could have a similar structural change as post-ECT cartilage.\textsuperscript{22,23} We understand that there are limitations to this assumption because the fibrous elements of skin are thicker and more spatially organized compared with the collagenous components in fibrous cartilage. Other changes we are unable to account for include differences between composition of noncollagenous extracellular matrix in cartilage and skin, tissue hydration levels, and the state of bounded water. In addition, visualizing thermal damage in skin via OCT has been studied by Lo et al\textsuperscript{24} and Pierce et al.\textsuperscript{25} Using OCT, Lo et al\textsuperscript{24} visualized a thermal coagulation signature (60 W/cm\textsuperscript{2}) in ex vivo porcine skin, whereas Pierce et al\textsuperscript{25} quantified burned human skin. Both studies\textsuperscript{24,25} found distinct patterns of thermal damage in skin, and based on their results, our OCT images of ECT-treated samples indicated no thermal injuries. We also did not observe a temperature elevation using an infrared measurement device in previously unpublished studies.

Optical coherence elastography uses OCT in conjunction with acoustic radiation force to measure phase changes in the tissue, allowing the detection of microscale mechanical changes in tissue mechanics. With the use of OCE, the mechanical changes in the tissue can be correlated with the magnitude of tissue vibration. In this study, the mean peak displacement increased by 1- to 2-fold after ECT (Figure 4), indicating a decrease in the elastic modulus. The increase in amplitude of the vibrational displacement suggests changes in mechanical properties that are a consequence of tissue softening.\textsuperscript{26}

Ultrasonography uses high-frequency sound waves to detect the acoustic impedance mismatch, serving as a noninvasive mesoscopic imaging method that has become essential for tumor management. The mechanical properties of a tumor differ from those of normal tissue, allowing the visualization of the tumor using ultrasonography; similarly, ECT-treated regions of the skin that have undergone mechanical changes can be interrogated with ultrasonography. Although it has a lower resolution, ultrasonography has a greater imaging depth (up to 5 mm) (Figure 5), demonstrating the effects of ECT into the deeper dermis because needle electrodes can penetrate into the skin deeper than lasers. Because the effect was even more widespread when the needle array was used, ultrasonography provided a greater field of view compared with OCT and OCE, capturing the full effect of the needle array ECT in the skin.

As evidenced by ultrasonographic, optical, and elastographic data, ECT can induce localized changes that alter skin biomechanics without thermal injuries (as seen with laser and RF treatments) or mass loss or ablation (as seen with laser treatment).\textsuperscript{4,27,28} Two potential clinical applications of ECT for therapeutic effects are (1) immediate softening of the tissue matrix for scar treatment and (2) highly localized damage to adjacent soft tissue that triggers reparative processes and remodeling for rejuvenated or sun-damaged skin. The work presented here is the first step toward evaluating the efficacy of ECT for the preceding potential applications.

This study demonstrates that ECT is effective at superficial (<500-μm) and deep (approximately 5-mm) levels, similar to the therapies that result from chemical peels.\textsuperscript{29,30} The beneficial effects of chemical peels on collagen have been well studied, and we hypothesize that by creating localized acid base-type injuries to the skin, ECT can have the same effects...
but in a more controllable fashion and at a reduced cost.\textsuperscript{31-34} Future studies include evaluating this hypothesis and conducting in vivo investigation, ultimately translating this low-cost technology to the clinic. We anticipate the cost of this technology to be approximately US $10 because the simplest version of this device would require only needles and a battery.

**Limitations**

Although classic mechanical testing of tissue samples relies on stress and strain measurements, these imaging techniques assess the optical and acoustic properties, better characterizing physical changes on a mesoscopic scale. One limitation of OCE, however, is that the sample tissue must be immersed in water. In this pilot study, we found that the water content of the sample can alter the optical and mechanical properties of the tissue. The immediate introduction of water can potentially reverse some of the effects of ECT because the primary mechanism of ECT is the localized hydrolysis of water. In addition, OCT and OCE have a limited image depth, resolving only up to 500 μm. Nevertheless, the use of OCT and OCE to interrogate the superficial tissue layers (<1 mm) allows immediate comparison with conventional laser treatments, which only modify superficial tissue layers up to 4 mm in depth.\textsuperscript{34} In this study,\textsuperscript{35} ECT had the same scale effect as RF ablation, which affects up to several millimeters into the skin. Another limitation of the study is the use of porcine skin as a model for its human counterpart. The structure of human scar tissue is more complex than that of native porcine skin; results reported here are meant to illustrate the potential of ECT and the first step in the pathway toward clinical evaluation.

**Conclusions**

Using OCT, OCE, and high-frequency ultrasonography, this study serves as an initial pilot study of the efficacy of ECT to potentially alter collagen biomechanics locally in skin via electrochemically induced pH gradients. We speculate hypothetical mechanisms of type I collagen being modified in such a way that alters its mechanical properties. Additional studies on normal skin and scar tissue in vivo are needed to characterize changes in mechanical properties, optimize treatment variables, and observe changes in vivo.

We envision ECT to be a paradigm-shifting, easily deployable technique that can have a large effect on scar treatments and other cosmetic applications.

**REFERENCES**


