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Electrochemical Clearing of Rabbit Cornea Post-Acidic/Alkaline Injury

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ABSTRACT

Chemical injuries to the cornea account for 11 to 22% of all ocular injuries. Acidic injuries are commonly due to sulfuric, hydrochloric, hydrofluoric, and battery acids, while basic injuries are commonly due to sodium hydroxide, chlorine bleach, and ammonia products. We have previously studied potential-driven electrochemical clearing (P-ECC) for alkaline injuries. In this study, we investigated the use of P-ECC on both acidic and alkaline injuries to determine its effect on restoring corneal transparency. Optical coherence tomography (OCT) was performed before and after P-ECC to determine adequate corneal clearing. Severity of chemical injury was measured through second harmonic generation (SHG) imaging. HCl or NaOH was applied to the corneas of New Zealand white rabbit globes. P-ECC was performed on opacified cornea while OCT imaging was simultaneously performed to evaluate depth resolved clarity. SHG imaging evaluated the structure of collagen before HCl or NaOH application and after P-ECC. Irrigation with water served as positive control. Native rabbit corneas were used as a negative control group. P-ECC induced clearing in the rabbit cornea, shown through OCT. Clearing occurred in regions where the working electrode made contact with the cornea. SHG imaging showed restoration of collagen fibril signal in P-ECC treated corneas compared to control. P-ECC is a potentially effective therapy for clearing acidic and alkaline corneal injuries. However, more ex-vivo experiments are required to determine the specific parameter for optimal clearing. In-vivo experiments are necessary to determine its potential for clinical use.

Keywords: corneal clearing, corneal injury, electrochemical therapy, optical coherence tomography, second harmonic generation

1. INTRODUCTION

The corneal surface provides a physical and immunological barrier to the ocular system. This specialized layer is composed of several cellular components including keratocytes, endothelial cells, collagen, and the outermost epithelial cells. Remarkably, the cornea maintains its barrier function while also preserving the ability to transmit light and thus enable clear vision. Trauma, autoimmune disorders, genetic diseases, and notably chemical injury have all been previously described as leading causes of corneal injury.[1,2] In fact, chemical injury, commonly occurring via work or occupational exposure, has been reported to account for up to one-fifth of all ocular injuries.[3]

Corneal chemical injuries (CCI) can be classified as either acidic or alkaline with the latter being more severe. This is largely because corneal proteins act as a barrier against acidic injury, whereas alkali substances are lipophilic and penetrate the eye further resulting in permanent visual impairment.[4,5] The immediate phase of treatment involves thorough irrigation of the eye with further treatment focusing on the reduction of inflammation and re-epithelialization.[6] In the chronic phase, treatment promotes the repurposing of corneal anatomy and restoration of visual acuity. Studies have examined the therapeutic potential for *in situ* electrochemical therapy in these injuries. Our group has previously described the suitability of a potential-driven electrochemical corneal clearing (P-ECC) system.[7] This system promotes the neutralization of chemical injury by producing a complementary acid-base reaction.

Using optical coherence tomography (OCT) and second-harmonic generation imaging (SHG), this study examines the P-ECC driven restoration of optical clarity in the cornea following HCl and NaOH injury.

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2. METHODS

2.1 Specimen Collection

Whole, intact globes of post-mortem New Zealand White rabbits were extracted. Care was taken to not damage the cornea or rupture the globe at this time. Globes were immediately placed in phosphate-buffered saline (PBS) to maintain hydration.

2.2 Optical coherence tomography

Globes were placed in a machined silicone jig with a custom-fit holder and were placed in a secondary dish on the viewing stage of the OCT system. Hydration was maintained through irrigation with PBS. Globes were imaged prior to introduction of HCl or NaOH and after P-ECC clearing.

2.3 Induced acidic or alkaline injury

1 mL of 5M HCl or NaOH was introduced to the globes (Sigma-Aldrich, St. Louis, USA). After one minute of injury, globes were irrigated with copious amounts of PBS to prevent continued injury.

2.4 Potential-driven electrochemical clearing

A series of three platinum electrodes was then placed over the globe as per previously described P-ECC protocol.[7] The working electrode is laid horizontally over the center of the cornea, the reference electrode rests over the outer edge of the cornea, and the counter electrode is placed near the bottom of the globe (Fig. 1). Our P-ECC parameters were as follows: no treatment (control), 2V for 8 minutes, or 2V for 20 minutes for base-damaged globes and no treatment (control), -1.5V for 10 minutes, or -2V for 7 minutes for acid-damaged globes. OCT was performed prior to treatment and through the entirety of treatment.

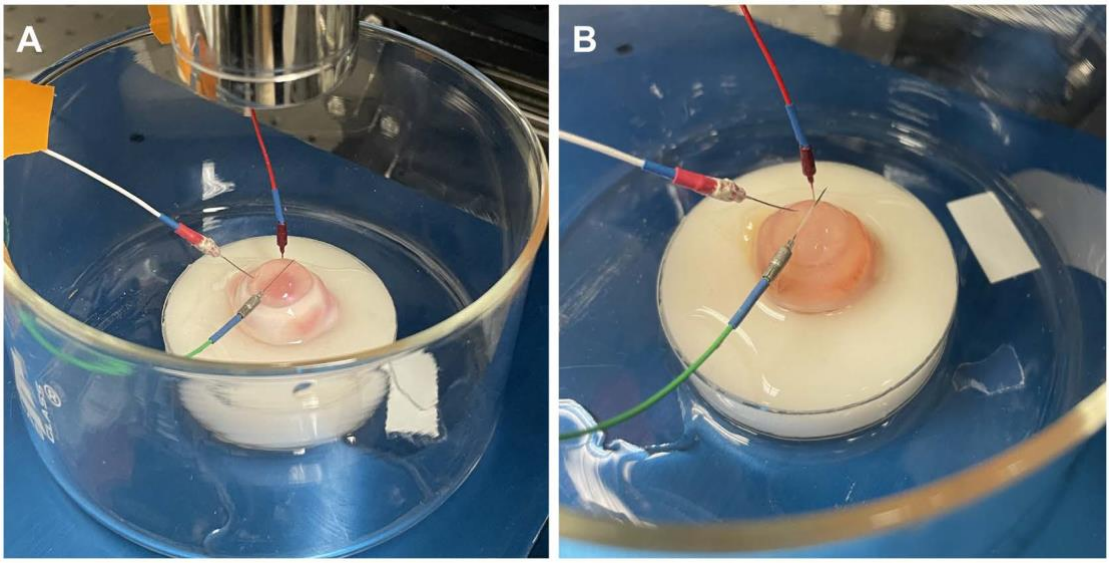

Figure 1. Digital image of (A) experimental setup for potential-driven electrochemical clearing, including electrode placement: reference electrode (white), working electrode (green), and counter electrode (red). Proper placement of electrodes (B) can be confirmed by the presence of hydrogen bubbles at the working electrode when P-ECC has begun.

2.5 Second harmonic generation imaging

In addition to OCT imaging, pre- and post-treatment globes were imaged using SHG on a Leica TCS SP8 MP microscope (Leica Microsystems, Wetzlar, Germany) set to a detection range of 395 nm - 415 nm and a Spectra-Physics Insight Tunable Ultrafast laser (MKS Instruments, Milpitas, CA, USA) with an excitation wavelength of 810 nm. Globes were placed over PBS-soaked gauze in a 3D printed jig for imaging. A coverslip is placed over the opening of the jig, contacting the cornea to produce a flat surface for imaging. Tile scans were taken at a depth of ~150 µm. Directionality analysis, using ImageJ, was performed on SHG images and data was expressed as orientation analysis histograms (ImageJ 2.9.0, NIH, New York, USA).

2.6 Digital photography

In addition to OCT and SHG imaging, digital images were taken using a smartphone (iPhone 12 Pro, Apple, Cupertino) before and after P-ECC treatment.

3. RESULTS

3.1 Digital photography of the cornea

An array of digital images of the cornea shows the results of respective times and voltages of P-ECC treatment after alkaline and acidic chemical injury. Before treatment, the cornea showed optical clarity and no visible deformities (Figs. 2A, 2C, 2E, 2G, 2I, 2K). The control cornea shows opacification during both NaOH (Fig. 2B) and HCl (Fig. 2H) chemical injury. P-ECC treated cornea show restored optical clarity in the area surrounding the anode electrode (Figs. 2D,2F, 2J, 2L).

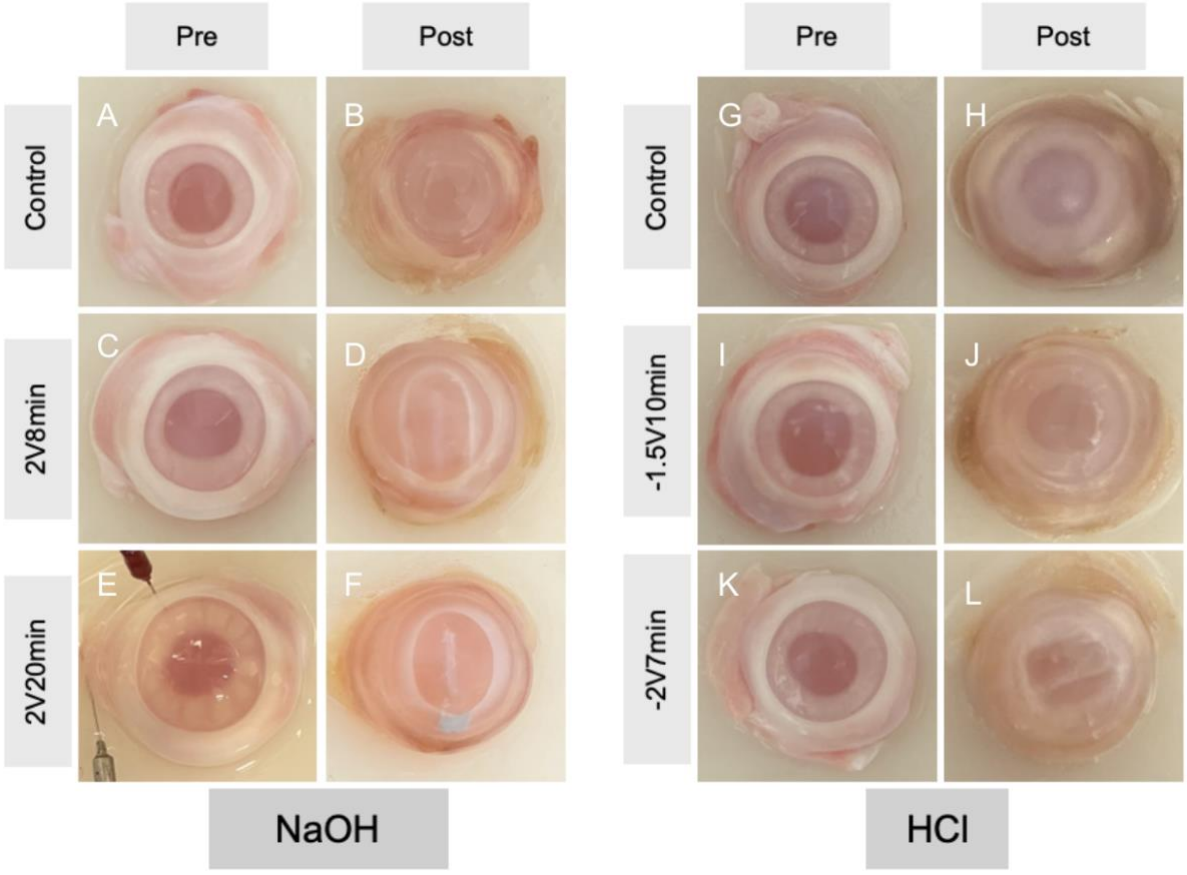

Figure 2. Digital images of the rabbit cornea that display varying degrees of corneal opacity before chemical injury, after chemical injury, and after P-ECC.

3.2 OCT Imaging of P-ECC treatment

Cross-sectional OCT images of cornea pre-injury and post-treatment showed a thin, curved structure (Figs. 3A, 4A). OCT images after alkaline (Fig. 3B) and acidic injury (Fig. 4B) showed a thickening and deformation of the corneal curve. Images near the beginning of treatment (Figs. 3C, 4C) and later in treatment (Figs. 3D, 4D) to show the results of P-ECC at various times during treatment. At the onset of P-ECC treatment, swelling is immediately reduced and optical clarity restored (Figs. 3C, 4D). After continuous P-ECC treatment, the reduction of swelling and restoration of clarity spreads along the cornea.

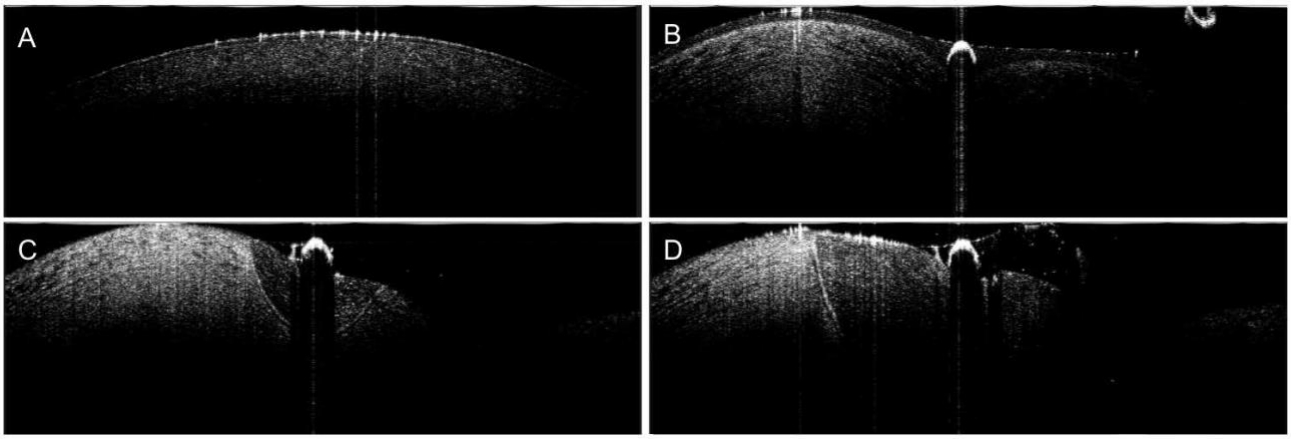

Figure 3. OCT imaging of the cornea (A) before NaOH addition, (B) after NaOH damage, (C) during the beginning of P-ECC, and (D) towards the end of P-ECC. The addition of base causes the cornea to become opaque and swell as seen in (B). A distinct line (C, D) between the damaged and cleared portion can be seen propagating along the cornea bidirectionally during P-ECC.

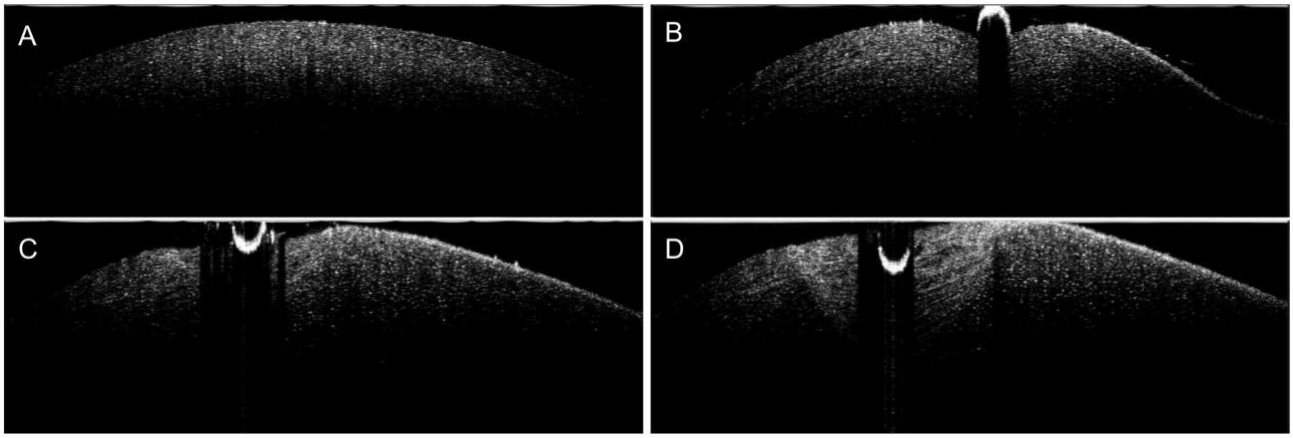

Figure 4. OCT imaging of the cornea (A) before HCl addition, (B) after HCl damage, (C) during the beginning of P-ECC, and (D) towards the end of P-ECC. The addition of acid causes the cornea to become opaque and swell as seen in (B). The (C) formation of hydrogen gas bubbles is seen as P-ECC occurs and (D) its effects moves across the cornea.

3.3 SHG Imaging and Collagen Fibril Analysis

SHG imaging of the cornea pre- and post-acidic or basic damage and P-ECC treatment revealed the presence, or lack thereof, of collagen fibrils. Figure 5 displays the results of SHG imaging of globes damaged by NaOH and treated with P-ECC parameters of either 2V for 8 minutes or 2V for 20 minutes. The cornea of the positive control was only damaged by NaOH and did not undergo any P-ECC treatment. In the native cornea, collagen was clearly visible prior to any treatment as its presence was indicated by bright blue signals dispersed across the cornea. After base was added, SHG signals were significantly reduced, suggesting a sharp decrease in viable collagen fibrils. SHG imaging of cornea that underwent P-ECC treatment revealed recovery of collagen fibrils as signified with an increase in collagen fibril signals when compared to globes that underwent no P-ECC treatment.

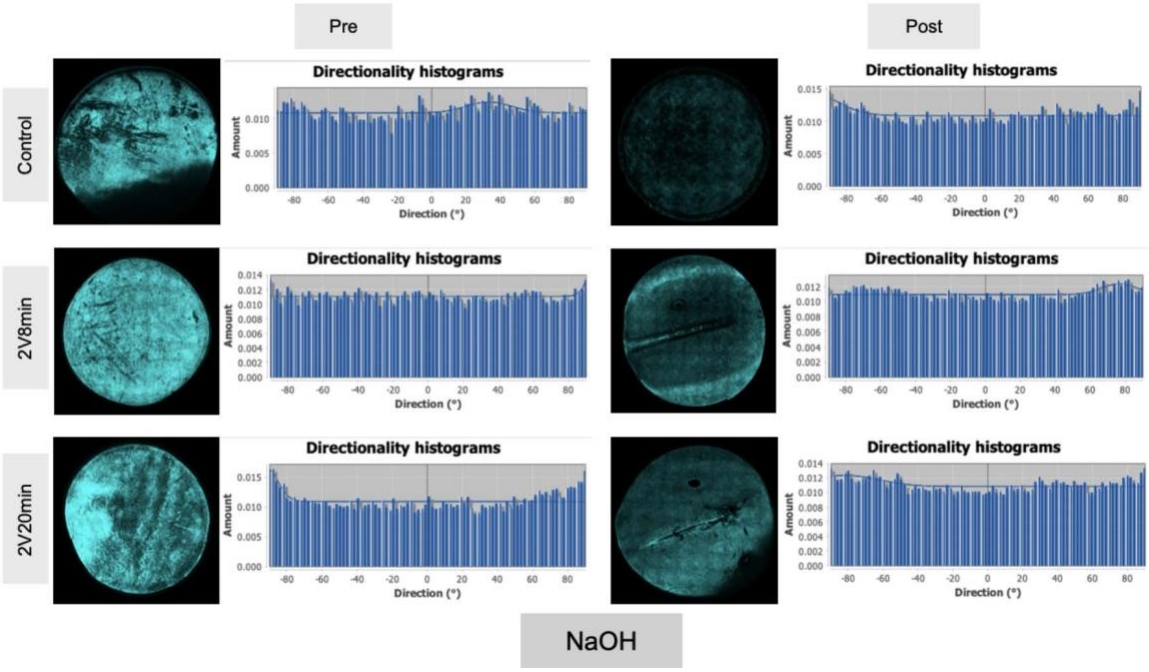

Figure 5. SHG imaging and directionality histograms of native cornea (Pre) and cornea after NaOH damage and treatment (Post) as follows: positive control, 2V8min P-ECC, and 2V20min P-ECC. Limited SHG signal was present in the control group after NaOH was added. Imaging of P-ECC treated globes reveals recovery of SHG signal.

Positive control globes that were damaged with HCl without subsequent P-ECC treatment displayed a lack of collagen signal in comparison to the native pre-acid injured cornea (Fig. 6). However, those treated with P-ECC, with parameters set to -1.5V for 10 minutes or -2V for 7 minutes, exhibited increased blue signal intensity (when compared to no P-ECC treatment) signifying the recovery of healthy collagen fibril. Additionally, on directionality analysis of collagen fibril orientation, it was shown that acidic and alkaline damage caused changes in collagen structure with a preferred directionality, as compared to pretreatment globes. It was also shown that there was some rescue of directionality after P-ECC treatment as compared to globes that did not undergo P-ECC treatment.

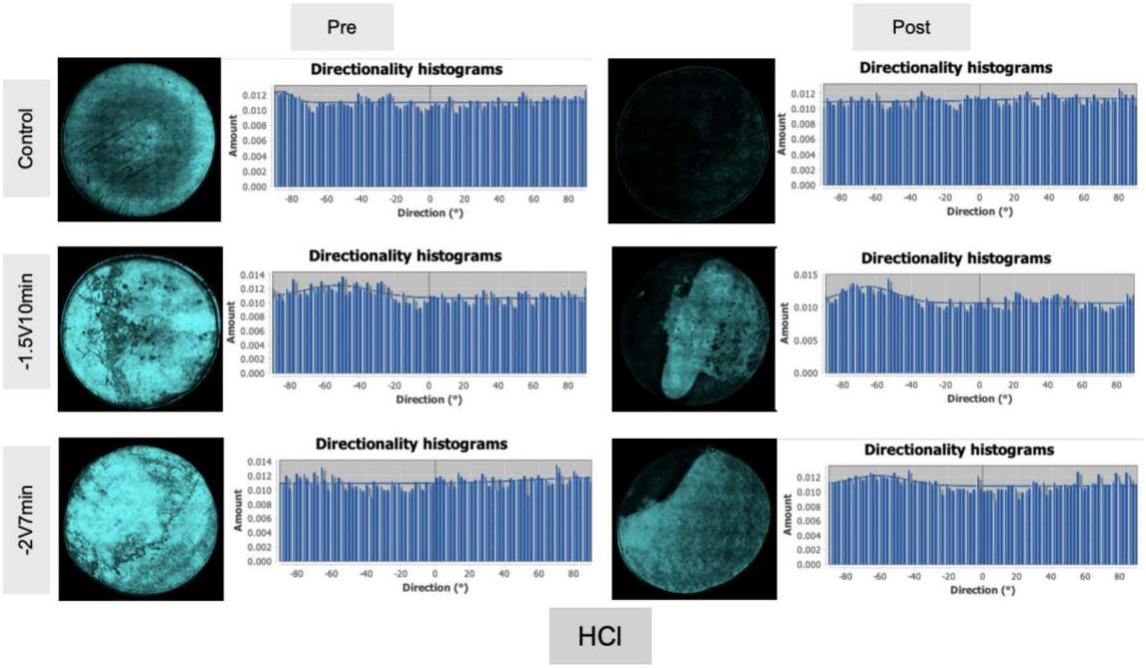

Figure 6. SHG imaging and directionality histograms of native cornea (Pre) and cornea after HCl damage and treatment (Post) as follows: positive control, -1.5V10min P-ECC, and -2V7min P-ECC. Limited SHG signal was present in the control group after HCl was added. Imaging of P-ECC treated globes reveals recovery of SHG signal.

4. DISCUSSION

The results of P-ECC treatment presented in this study suggest that it should be considered as a viable alternative to common treatments of CCIs. Digital images show restoration of optical clarity after alkaline and acidic injury, respectively, in the region immediately surrounding the working electrode of P-ECC treatment (Figs 2D, 2F, 2J, 2L). OCT imaging before and during treatments showed reduced corneal swelling and deformation beginning at the electrode and spreading radially towards the edges of the cornea (Figs. 3, 4). In addition, SHG imaging showed restoration of collagen fibril signal intensity following P-ECC treatment (Fig. 5). Comparing the post images of the untreated control and the globes treated with varying P-ECC parameters, the overall recovery of signal in treated groups suggests that P-ECC was a viable method for clearing acid- or base-damaged corneas.

One limitation of this study includes potential injury sustained by the eye during transport and experiment set-up, but extreme care was taken to prevent any unintentional damage. When imaging, SHG has intrinsic limitations with regards to how data is registered, and this may be due to the swelling and resolution of swelling during P-ECC treatment. Additionally, OCT and SHG imaging modalities can only provide information on structural changes of the cornea without any information on viability of cells and cellular function. To address this issue, further studies will involve the use of live/dead assays to determine the viability of the cells post-injury and post-P-ECC.

In conclusion, P-ECC holds potential in treating base- or acid-induced ocular injuries. This process of treating alkaline or acidic ocular injuries is expeditious, minimally invasive, and relatively inexpensive as treatments only last between 7 to 20 minutes using external, simple, low-cost electrodes. Our study reveals the progression of P-ECC treatment and reversal of damage through SHG and OCT imaging. Further studies will be performed to validate the use of P-ECC to treat chemical ocular injuries in a clinical setting.

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