Permanent Vision Correction Via Laser-Induced Collagen Crosslinking

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INTRODUCTION:

Myopia, commonly referred to as near-sightedness, is a common refractive error caused by light being focused anterior to the retina, leading to blurred vision. If left uncorrected, high myopia leaves patients at increased risk of developing other ocular pathologies, including glaucoma, choroidal neovascularization, and cataracts [1,2]. Currently, 28.3% of the global population is affected by myopia, and projections indicate that nearly half of the world's population will be myopic by 2050 [3].

Refractive surgeries, such as laser-assisted in situ keratomileusis (LASIK), are popular options for patients seeking permanent vision correction. While effective in improving visual acuity, these invasive procedures can lead to postoperative complications, including epithelial ingrowth, diffuse lamellar keratitis, and corneal flap dislocation [4]. An alternative treatment involves soaking the cornea in the photosensitizer riboflavin and exposing it to ultraviolet-A (UVA) light to induce the formation of collagen crosslinks and reduce myopic refractive error [5]. However, this procedure has primarily demonstrated effectiveness in patients with low myopia [6]. Combining LASIK with riboflavin and UVA-assisted crosslinking has been shown to significantly reduce myopic regression compared to LASIK alone, demonstrating the potential for collagen crosslinking to provide greater long-term efficacy and stability in the treatment of myopia [7].

We propose a noninvasive method for permanent vision correction which uses femtosecond lasers to generate collagen crosslinks. This technique generates low-density plasma within the stroma, leading to the production of reactive oxygen species, which triggers the formation of covalent bonds between corneal collagen fibrils [8]. The increased crosslinking alters the cornea's biomechanical rigidity and curvature, resulting in a decrease in myopic diopter while minimizing operative trauma and postoperative complications. We hypothesize that implementing this method in ex vivo rabbit eyes will result in a decrease in effective refractive power (ERP).

METHODS:

Rabbit Samples Preparation: Ex vivo experiments were performed on three pairs of fresh rabbit eyes. Each eye that underwent laser treatment was paired with a control eye from the same rabbit. Eyes were harvested approximately 30 minutes after sacrifice, and excess tissue was removed from the enucleated eyes. The eyes were placed on custom-built, 3D printed eye holders. To facilitate later topography exams, a Trypan Blue ophthalmic solution was injected into all albino eyes. Intraocular pressure was maintained throughout the experiment by connecting an intravenous (IV) system with a 0.9% NaCl solution to the eyes.

Pre-Treatment Refractive Power Measurements: Prior to treatment, corneal topography exams were performed using an EyeSys Vision clinical eye topographer (EyeSys System 2000, EyeSys Vision) to determine the ERP of all the eyes. During these exams, the corneal surface was hydrated with phosphate-buffered saline (PBS).

Sample Set-Up and Laser Treatment: A 1 mm thick microscope cover glass was placed on the corneal surface to ensure uniform irradiation of the cornea during treatment. A melanin pigment was applied to the surface of the cover glass in contact with the eye. The treatment samples were mounted on a motorized stage, and the focal point was positioned at the bottom surface of the cover glass by generating a spark at the cover glass-cornea interface. The treatment samples were exposed to a laser power of 185 mW using a Ti: Sapphire Fidelity Mode Locked Fiber Laser. The laser treated a 5 mm diameter circle in the center of the cornea. The motorized stage was programmed to move such that the laser path followed a zigzag pattern in the x-y plane, with a 25 μm distance between parallel paths. Five layers of the cornea were treated. Layers were spaced 20 μm apart along the z-axis, resulting in a total treatment depth of 100 μm from the anterior cornea. Control eyes were subjected to an identical protocol, but they were not exposed to the laser. The microscope cover glass was removed immediately after the treatment.

Post-Treatment Refractive Power Measurements: Corneal topography exams were performed on all eyes hourly for six hours after treatment using the EyeSys Vision clinical eye topographer to assess laser-induced ERP changes. Before each exam, PBS was applied to the corneal surface.

Multiphoton Confocal Microscopy: To investigate the effect of the CxL treatment, the samples underwent second harmonic generation (SHG) imaging roughly 24 hours after treatment. Collagen's non-centrosymmetric structure generates a strong SHG signal, enabling visualization of collagen proteins [9]. The presence of crosslinks increases the

density of collagen fibers, which enhances the intensity of the SHG signal. This increased signal intensity allows for the assessment of CxL presence between collagen fibers following laser irradiation.

RESULTS:

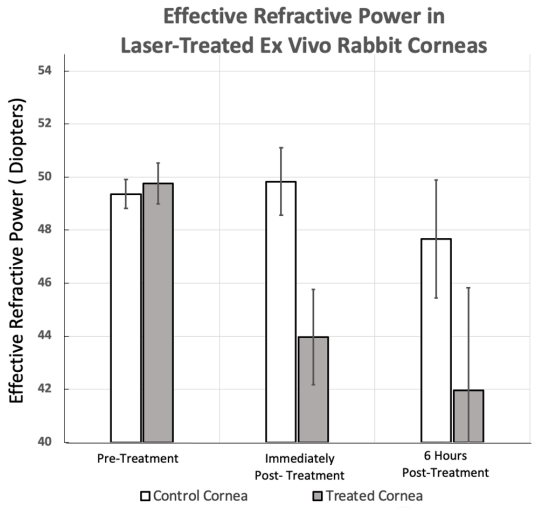

Figure 1. Ex vivo rabbit ERP (n=3). Error bars represent standard deviation.

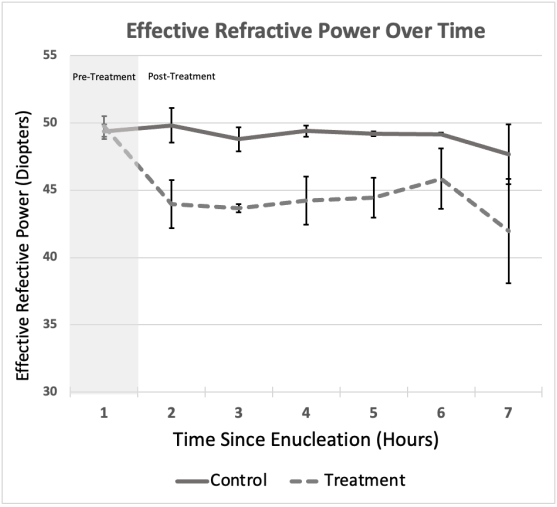

Figure 2. Ex vivo rabbit ERP over 7 hours (n=3). Error bars represent standard deviation.

Figure 1 shows the ERP (mean ± SD) of the control and treatment eyes before, immediately after, and six hours after treatment. Figure 2 depicts the ERP (mean ± SD) of the control and treatment eyes over a period of seven hours after enucleation. There was a decrease in ERP immediately after treatment and six hours after treatment. Comparing the control and treatment ERP immediately post-treatment, there was a decrease of 5.87 diopters. After six hours, the change in ERP was 5.7 diopters.

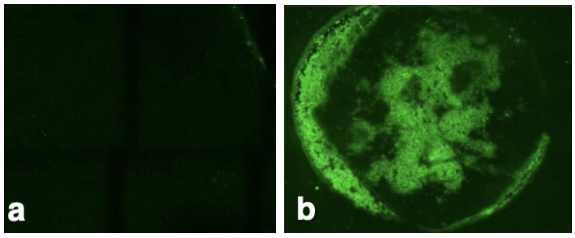

Figure 3. Multiphoton Confocal Microscopy of Laser-Treated Rabbit Cornea. (**a**) untreated portion of cornea (**b**) treated portion of cornea.

Figure 3 shows the change in SHG signal intensity in segments of the cornea which were exposed to the laser treatment. The increased collagen CxL resulted in a greater density of collagen in the treated segment of cornea due to protein aggregation. This collagen aggregation is indicated by the green segments of SHG image.

CONCLUSION:

This study investigated the impact of femtosecond laser treatments on ERP. SHG imaging showed an increase in collagen crosslinks in the segment of the cornea which underwent laser treatment. Corneal topography revealed a decrease in ERP immediately and six hours post-treatment. While our results demonstrate a decrease in ERP, the high standard deviation and small sample size call for further investigation. A possible cause of the large standard deviation was the lack of familiarity with the equipment and experiment protocol.

Future work should investigate the impact of various laser powers and deformation levels on crosslinking and optical breakdown to determine the optimal laser power setting that achieves CxL while minimizing optical damage. These experiments provide a platform for further investigation into mechanical loading of the cornea and treatment power.

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