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8 DEC 2016

MEMORANDUM FOR SGCEE
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FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **Sealing of Corneal Lacerations Using Photo-Activated Rose Bengal Dye and Amniotic Membrane** presented at/published to **Military Refractive Surgery Safety and Standards Symposium, 10-12 January 2017** in accordance with MDWI 41-108, has been approved and assigned local file #**17011.**

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PAUL T. BARNICOTT, GS-15-DAF
Deputy Director, Clinical Research Division

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Clinical Research Division/SGVU (59 MDW/SGVU)	Timothy A. Soeken, Capt, O-3, Ophthalmology	A-2009-03

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Sealing of Corneal Lacerations Using Photo-Activated Rose Bengal Dye and Amniotic Membrane

1. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED
Sealing of Corneal Lacerations Using Photo-Activated Rose Bengal Dye and Amniotic Membrane

2. FUNDING RECEIVED FOR THIS STUDY? ☒ YES ☐ NO **FUNDING SOURCE:** US Department of Defense contracts W81XWH-09-2-00[illegible]

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7. WHO IS THE PRIMARY 59 MDW POINT OF CONTACT? (Last, First, MI.) (Include email)	DUTY PHONE/PAGER No.
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I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401_IP AND 59 MDWI 41-108. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.

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Sealing of Corneal Lacerations Using Photo-Activated Rose Bengal Dye and Amniotic Membrane

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Conflict of Interest: None of the authors have a conflict of interest to declare.

Keywords: Amniotic Membrane, Photo-Activated Rose Bengal Dye, Corneal Laceration

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Purpose: Watertight closure of perforating corneoscleral lacerations is necessary to prevent epithelial ingrowth, infection, and potential loss of the eye. Complex lacerations can be difficult to treat, and repair with sutures alone is often inadequate. In this study we evaluated a sutureless technology for sealing complex corneal and scleral lacerations that bonds amniotic membrane (AM) to the wound using only green light and the dye, rose bengal (RB).

Methods: AM was impregnated with RB, then sealed over lacerations using green light to bond the AM to the de-epithelialized cornea surface. This process was compared to suture repair of three laceration configurations in New Zealand White (NZW) rabbits in three arms of the study. A fourth study arm assessed the side effect profile including viability of cells in the iris, damage to the blood-retinal barrier, the retinal photoreceptors, retinal pigment epithelium (RPE) and choriocapillaris in Dutch Belted (DB) rabbits.

Results: Analyses of the first three arms revealed no significant difference between the groups regarding induced edema to the corneal stroma, induced stroma thickening, endothelial necrosis, and inflammation. In the fourth arm, iris cells appeared unaffected and no evidence of breakdown of the blood retina barrier was detected. Retina from greenlight laser treated eyes showed normal RPE, intact outer segments and normal outer nuclear layer (ONL) thickness.

Conclusions: The results of these studies established that a light-activated method to crosslink AM to the cornea can be used for sealing complex penetrating wounds in the cornea and sclera with minimal inflammation, or secondary effects.

The views expressed are those of the authors and do not reflect the official views or policy of the Department of Defense or its Components.

The experiments reported herein were conducted according to the priniciples set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Welfare Act of 1966, as amended.

Introduction

Penetrating injury to the eye is a frequent ophthalmic emergency at trauma centers worldwide. From the standpoint of the military ophthalmologist, fragments and debris propelled at high velocity by improvised explosive devices (IEDs) have increased the incidence of penetrating eye injuries compared to conflicts prior to the development of IEDs. At the height of the recent conflict in Iraq, 29% of all evacuations from Iraq were due to ocular injuries (Joint Theater Trauma Report, 2012)

When an eye sustains a penetrating or perforating injury, rapid closure with the formation of a water-tight seal is critical to preventing infection and preventing surface epithelium from gaining entry into the eye. This stabilizes the eye until further reconstructive surgery can occur. Despite the small surface area of the eye, repair of complex injuries can be labor and time intensive. Effective repair of these injuries requires the use of specialized instruments and advanced surgical training.

In complex lacerations in which flat objects are propelled through the cornea, the lamella of the cornea can shred, making closure by suture impossible. In these cases surgical adjuncts are used to close the wound, none of which are currently approved by the FDA for this purpose. Cyanoacrylate glue is the most commonly used adjunct, effectively binding to the cornea and sealing the wound. While effective, there are a number of disadvantages to the use of this glue. These include difficulty in removal, adhesion to the sutures, and the opacity it creates. Thus, the overall goal of this research was to find a more efficient method of sealing complex corneal lacerations.

To achieve this goal, we utilized a light-activated technology in which amniotic membrane (AM)

69 impregnated with rose bengal (RB) dye is cross-linked to the surface of the cornea. RB is a
70 common FDA-approved photoactive vital dye used as a diagnostic tool for staining ocular
71 surface abnormalities. Since it uses currently FDA approved materials/devices (clinical laser,
72 RB, AM) this method may be integrated rapidly to the modern civilian and deployed
73 environment with materials already in the ophthalmic surgical set.
74

Methods

This study utilized two separate groups of rabbits. For the treatment arms, 98 New Zealand White (NZW) rabbits were acquired. These rabbits were used to perform the surgical procedures and to record the clinical and histopathological data, all of which were completed at the United States Army Institute of Surgical Research (USAISR), San Antonio, TX. To evaluate the side effect profile of the procedure, 17 young female Dutch Belted (DB) rabbits were acquired. These rabbits were supplied by Millbrook Labs (Amherst, MA), and all procedures were performed at Massachusetts General Hospital, Boston, MA. All experiments were humanely performed in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. The study protocols were approved by the USAISR Institutional Animal Care and Use Committee (IACUC) and the Subcommittee on Research Animal Care at Massachusetts General Hospital.

Methods, Treatment Arms

A summary of the methods can be found in Table 1. The 98 NZW rabbits of the treatment arms were separated into three treatment arms. For every animal, only the right eye was operated on in accordance with IACUC guidelines. Each treatment arm was separated into two groups—one control group treated with sutures only and one study group treated with the AM technique. In the first arm, a 4 mm linear central corneal laceration in the visual axis was made. In the second arm, a V shaped laceration in the central cornea with two 3 mm legs in an equatorial triangle fashion was made. In the third arm, a 4 mm corneal-scleral laceration centered in the limbus was made. The RB and the human AM used in this study were prepared and attained as previously described (Verter et al, 2011). A prototype light delivery system was constructed to deliver diffuse retina-safe light levels while providing sufficient energy for sealing corneal wounds

98 (Verter et al, 2011). All studies employed a clinical laser that emits green light at 532 nm. The
99 laser instrument is an IRIDEX OcLightTX Ophthalmic 532 nm Laser Diode. The set power was
100 400 mW, and the beam diameter was 13 mm with a beam area of 1.33 cm^2. Due to losses in the
101 delivery system, the measured power was 360 mW. The irradiance at 2 cm from the exit point of
102 the lens was 271 mW/cm^2. Laser power was measured with a Scientech model 365, SN: 6277
103 (head model 380101).
104 The first arm included a total of 40 live NZW rabbits. The right eye of all rabbits received a
105 simple 4 mm linear central corneal laceration. The rabbit was placed under anesthesia, then
106 prepped and draped in the normal sterile fashion for eye surgery. A lid speculum was placed in
107 the eye and centered under the operating microscope. A surgical ruler was used to measure the
108 central 4 mm of the cornea and 2 dots were placed. A diamond knife was then used to make a
109 central laceration between the dots, which resulted in a gaping wound and collapse of the
110 anterior chamber. In the 19 rabbits that served as the control group, repair of their corneal wound
111 was completed with interrupted 10-0 nylon sutures. In the 17 rabbits of the treatment group the
112 epithelium in the center of the cornea was debrided, and viscoelastic was injected into the wound
113 to protect the lens and deepen the chamber. One central 10-0 nylon suture was placed. Pre-
114 prepared AM impregnated with RB was then centered on the wound. The amnion was gently
115 stroked with a 27 gauge cannula until it dried sufficiently to adhere to the cornea. The
116 microscope was moved, the laser hand piece was centered over the wound and the AM was
117 irradiated. The laser was set to a 13 mm beam diameter and 271 $mW/cm^2$was delivered to the
118 AM surface for 250 seconds for a total fluence of 68 J/cm^2. Afterwards the wound was tested for
119 water-tightness with fluorescein staining, and a tonopen was used to ensure the eye was at
120 physiologic pressure.

The second arm of the study included a total of 30 NZW rabbits. For this arm, a complex V-shaped laceration was made in the center of each cornea. Each leg of the "V" was approximately 3 mm in length. The control group consisted of 15 rabbits, each of which received closure of their corneal wound with 5 interrupted 10-0 nylon sutures. The treatment group also consisted of 15 rabbits. After the epithelium was debrided, a single 10-0 nylon suture was placed at the wound apex. The AM was then applied and irradiated as previously described. All wounds were tested for water-tightness with fluorescein staining, and a tonopen was used to ensure the eye was at physiologic pressure.

The third arm of the study included 28 NZW rabbits. For this arm, a 3 clock hour peritomy was placed from 10 0'clock until 1 o'clock. A 4mm laceration centered on the limbus in the 11:30 meridian was made. The control group consisted of 15 rabbits, each of which received closure of their corneoscleral laceration with four interrupted nylon sutures (10-0 on the cornea, 9-0 at the limbus, and 8-0 on the sclera). The treatment group consisted of 13 rabbits. After the epithelium was debrided, a single 10-0 nylon suture was placed at the limbus. The AM was then applied and irradiated as previously described. All wounds were tested for water-tightness with fluorescein staining, and a tonopen was used to ensure the eye was at physiologic pressure.

In the post-operative period, each animal in all treatment groups was further subdivided into necropsy times of 3, 7 and 28 days. This allowed for clinical and histopathologic evaluation at various postoperative stages. Following euthanasia, the treated globes were isolated and the AM was removed. The globes were fixed in Modified Davidson's solution for 24-48 hours and then transferred to 10% buffered neutral formalin. After fixation, each globe was transected sagitally, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Corneal sections were analyzed for mononuclear and polymorphonuclear inflammation,

144 vascularization of the stroma, stromal edema, endothelial necrosis, and regeneration of the
145 corneal epithelium.
146 Histopathological analysis was performed using a five point grading system (0-4) for each of the
147 above variables. A score of zero was assigned for no observable characteristics, one was scored
148 for minimal observable characteristics, two for mild, three for moderate, and four for severe
149 characteristics. Inflammation was quantified by counting inflammatory cells per high powered
150 field.
151 The results were then analyzed utilizing a repeated measures mixed model ANOVA for time
152 dependent analysis. If the effect of time or the time-treatment interaction were not significant, the
153 data was pooled together and a one-way ANOVA or Wilcoxon's Test were performed. All p-
154 values < 0.05 were deemed statistically significant. All statistical analysis was performed using
155 JMP v10.0.
156 **Methods Side Effect Arm**
157 The fourth arm of the study included 17 DB rabbits. The right eye of each rabbit was subjected
158 to a simple 3 mm linear central corneal laceration. The epithelium was mechanical scratched
159 after adding 30% ethanol for 15 sec on the cornea, and then a 3 mm-long linear incision was
160 made in the central cornea. Human AM in diameter previously stained with RB was then placed
161 over the incision as previously described (Verter et al, 2011). The cornea was then exposed to
162 green light at an irradiance of 0.25 W/cm^2 for a total of 6.6 min (100 J/cm^2). Half way through
163 the 6.6 min, an additional 30 sec addition of RB was re-applied, then irradiated for the second
164 half of the 6.6 min. This is done because the first 3.3 min bleaches the pink color of the dye and
165 sufficient dye present to absorb the green light for crosslinking.

166 A LaserScope Aura StarPulse Laser (San Jose, CA) with a 600-μm optical fiber in diameter was
167 used for eye safety evaluation. The power was measured with a power meter (NOVA; Ophir
168 Optronics Ltd., North Andover, MA) before each use. An optical system was designed and built
169 to focus a 12 mm laser beam on the cornea then expand the beam to impinge on a larger area of
170 the retina to minimize potential light-initiated damage as previously described (Zhu et al, 2016).
171 The cornea surface temperature was recorded during treatment using a non-contact precision
172 infrared thermometer (Fluke 572, Fluke Corporation, Everett, WA). The left eye of each rabbit
173 served as a control; it was also de-epithelialized but received no further treatment. For the change
174 in temperature, the measurements were made on each treated eye. The temperature at the
175 beginning of the irradiation (~32 °C) was compared to the mean temperature between 60 and 360
176 minutes irradiation.
177 The lactate dehydrogenase-nitro blue tetrazolium (LDH-NBT) staining method was used to
178 assess the activity of the thermally sensitive enzyme, LDH as an indication of iris cell viability
179 using a previously described method (Zhu et al, 2016). A blue formazan product indicates cell
180 viability. No statistical analysis of the iris damage was done. The response was qualitative,
181 either blue formazan was observed or not.
182 The rabbits were sacrificed on day 1 or 28. Prior to euthanasia, fundus fluorescein angiography
183 (FFA) was performed on day 28 after treatment of DB rabbits (n = 4 / day). After anesthesia the
184 pupil was dilated with 2 drops of 1% tropicamide hydrochloride eye solution. A scanning laser
185 ophthalmoscope (SLO) (HRA2, Heidelberg Engineering, Heidelberg) was used. One milliliter of
186 10% fluorescein sterile solution (Alcon Inc., Fort Worth, TX) was injected into the marginal ear
187 vein, and images were recorded immediately after injection. No statistical analysis of the FFA
188 was done. The vessels were observed for breakdown or leakage from the blood vessels.

Following necropsy, the cornea, iris and retina were separated and fixed in 10% formalin for 24 hours at room temperature, then embedded in paraffin. Five-micron vertical cut sections were stained with H&E and scanned using a digital slide scanner (NanoZoomer Digital Pathology System, Hamamatsu, Photonics, Japan). The morphology of the corneas was observed by light microscopy. Retina H&E sections were used for outer nuclear layer thickness measurements. The thickness of the outer nuclear layer (ONL) from the optic disc was measured at 10 points with 200-μm intervals in each section to obtain the average for each retina. ONL thickness data were tested by a two-tailed unpaired Student t test.

Each histologic evaluation was carried out in a masked fashion.

Results, Treatment Arms

For the first, second and third arms of this study, there was no statistically significant difference between the treatment groups and control groups in terms of induced edema to the corneal stroma, induced stroma thickening, endothelial necrosis, and inflammation. This held true for all post-operative periods. Even after pooling the time points together, differences between these histology values remained non-significant. Figure 1 shows the pooled mean plot demonstrating there is no significant difference comparing crosslinking and suture closure for induced edema to the corneal stroma, induced stroma thickening, endothelial necrosis, nor inflammation (Wilcoxon p-values were 0.11, 0.45, 0.30, and 0.59, respectively).

In order to understand the healing response to the performed interventions, a 2-way repeated measures mixed model ANOVA was utilized to evaluate the effects of using laser versus suture at all the various time points. Additional analysis between laser closure and suture closure demonstrated no difference in neovascularization, endothelial necrosis, inflammation, edema or stromal thickening between the laser and suture techniques.

Between suture repair and laser crosslinking repair for the same time points there were no statistical differences in any of the categories except epithelial hyperplasia. On day 3 there was more epithelial hyperplasia in the control group, but the changes were not persistent as time progressed and none of the animals scored over 1 on the 0-4 scale ($p=0.05$).

On slit lamp evaluations, the sutured corneas remained clearer than the cross-linked corneas at the 3 and 7 day time points. At 28 days there were no significant differences. Of note, by 7 days all the corneas were sufficiently clear to see iris detail, sufficient to allow reconstructive surgery to be performed.

Results, Side Effects Arm

In the fourth arm, the temperature on the surface of cornea during the procedure at 0.25 W/cm^2 in DB rabbits was measured. As shown in Figure 2, the temperature increased 8 degrees.

In order to assess damage to iris cells during the procedure, the LDH-NBT assay was used which shows deposition of a blue formazan product if cells are viable. The positive control was untreated DB rabbit iris, which showed blue deposits in cells near melanin (Figure 3). Iris tissue harvested 1 and 28 days after treatment showed similar deposits of blue formazan product in the cells adjacent to melanin granules as found in the positive control.

FA images of the retina around the optic disc were examined for signs of leakage indicating breakdown of the blood retina barrier, which may result from laser-induced RPE or endothelial cell damage. FFA was performed on Day 28 after treatment in DB rabbit corneas (100 J/cm^2; n = 4 / group). Retinal vessels in the region of the optic disc were visible including small diameter vessels (Figure 4). On Day 28 after treatment, diffuse fluorescein fluorescence in the avascular regions of the fundus was not detected nor was there any appearance of fluorescein leakage from the retinal vessels (Figure 4 B).

Irreversible damage to the photoreceptors could be assessed by measuring the ONLthickness, which decreases after photoreceptor cell death. No significant difference was found in ONL thickness between retinae from treated and untreated eyes on either day 1 or day 28 (n = 6 / group, $p > 0.05$, Figure 4C).

Discussion

On the basis of prior studies, our team designed this study to compare the sealing of a central cornea laceration and a corneoscleral laceration sealed with crosslinked RB impregnated AM to traditional suture closure. Specifically, RB, without the AM, activated with neodymium:YAG laser light at 532 nm has been shown to seal a simple corneal laceration and provide a long duration seal (Proano et al, Invest 2004; Proano et al, J Cataract 2004). Later, it was demonstrated that the technique used in this study sealed incisions in *ex vivo* rabbit eyes to withstand an intraocular pressure of 350 mm Hg, more than 10 times normal pressure (Verter et al, 2011).

To determine the appropriate laser parameters for sealing the AM to the surface of the eye, we carried out pilot *ex vivo* studies. Over 1000 seconds exceeded the calculated safety threshold. Successful cross-linking the AM was attained at 400 seconds, and at 250 seconds there was about 90% strength. Thus, we selected 250 seconds in this protocol, which correlated to a total fluence of 68 J/cm2 when the laser was set to a 13mm beam diameter and 271 mW/cm2. The combination of the 1000 second upper limit and the 250 second treatment would allow up to four tries if the AM did not adhere to the ocular surface satisfactorily.

In the model development stage for this study, we determined that due to gaping of the central lacerations and the larger V shaped laceration, and brisk aqueous outflow, a direct closure with AM was near impossible. This also put the patient at risk because the scar required to fill the gap would decrease visual function, the AM over the gap would tectonically be too weak, and the brisk outflow would also prevent adhesion of the AM to the cornea prior to crosslinking. This was not evident in the abattoir eyes used in pilot studies and the previous study (Verter, 2011) because there was no aqueous outflow and the low pressure did not cause wound gap. Thus the

technique was modified to place one suture in the wound to approximate the corneal stromal surfaces. This accomplished three things. It minimized the scar size, tectonically strengthened the cornea and allowed air or viscoelastic to block the outflow of aqueous sufficient to crosslink AM to the cornea.

Previous studies indicate that RB demonstrated potential toxicity to cultured epithelial cells and that the use of AM in albino rabbits is associated with granulomatous inflammation (Tabery, 1998; Barton et al, 2001). As demonstrated in Figure 1, crosslinking AM to the cornea surface was no more damaging than suturing. This study also demonstrates histopathologically that the AM patched corneas did not cause any adverse reactions in the rabbits. The only statistically significant difference in the histopathological analysis of the three surgical arms was the epithelial hyperplasia noted on day 3. The authors hypothesize that this reflects the healing of the surface after scraping and neovascularization. None of the animals scored over one on the zero to four scale. The neovascularization noted was related to anterior chamber and iris changes that the rabbit developed while the anterior chamber was flat.

On two rabbits the iris was stuck to the underside of the cornea on slit lamp evaluation. This is not an uncommon response and likewise occurs in humans when the iris comes in contact with cornea for a period of time and the iris is irritated or inflamed. This could contribute to gradual loss of endothelial cells, and could prompt a return to the operating room in the future to remove the iris if the adhesion was significant or evidence of endothelial failure was present. There appeared to be no neovascularization responses to the cross-linking procedure in the wounds.

To evaluate potential deleterious effects of laser crosslinking, we analyzed the cornea surface temperature, iris cellular damage, and retinal blood-retinal barrier and outer nuclear layer. None of these measures indicated damage to these ocular structures resulting from the treatment.

Since photothermal damage on the cornea might occur due to absorption of the laser light, we measured the temperature on the surface of cornea during the procedure. As shown in Figure 2, the temperature increased less than 42°C, a temperature at which thermal damage is not expected (Landry and Marceau, 1978). In addition to the cornea, we also evaluated the thermal effects on the iris. High irradiances and fluences may heat the melanin particles in the iris and damage adjacent iris cells. Using the LDH-NBT staining method, an accepted method for detecting thermal damage in tissues (Sherwood and Flotte, 2007), our results indicated that iris cells near the melanin particles were still viable (Figure 3). These results demonstrate that the iris cells are not thermally damaged during treatment.

We used two methods to assess potential side effects on the retinas of DB rabbits, namely, FFA and ONL thickness. Fluorescence images of the retina around the optic disc were examined for signs of fluorescein leakage indicating breakdown of the blood retina barrier resulting from laser-induced RPE or endothelial cell damage. FFA performed on Day 28 after treatment showed retinal vessels, including small diameter vessels, without diffuse fluorescein fluorescence in the avascular regions of the fundus or any appearance of fluorescein leakage from the retinal vessels (Figure 4B). These results suggest that the retinal radiant exposure was not sufficient to initiate thermal damage to either the RPE cells or to retinal vessels.

Irreversible damage to the photoreceptors was assessed by measuring the outer nuclear layer thickness, which decreases after photoreceptor cell death. No significant difference was found in outer nuclear layer thickness between retinae from treated and untreated eyes either on Day 1 and Day 28 Figure 4 C).

In conclusion, the results of these studies established that a light-activated method to crosslink AM to the cornea can be used for sealing complex penetrating wounds in the cornea, and the

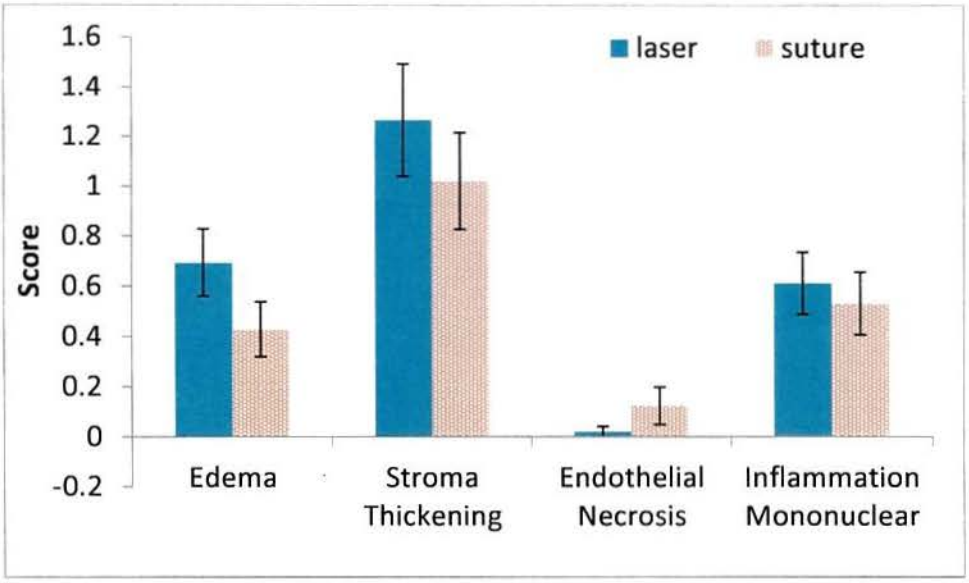
sclera, with minimal inflammation, or secondary effects. We did not identify problems or obstacles that will inhibit the translation of this light-activated repair technique to clinical use. This treatment involves off-label use of three FDA-allowed materials/devices (clinical laser, RB, human AM), which may facilitate translation to the clinic. Rose bengal is approved as diagnostic agent but not as a treatment agent; thus further safety documentation would be beneficial. In all applications a strong seal was produced immediately. The best method for sealing corneal and scleral wounds is to bond AM over the wound using a dye and green laser light, after any significant gaps in the stroma have been reduced with sutures. Thus, for these experiments this technology appears to be best employed as an adjunct to only suturing to reduce operative time or when edges cannot be approximated in cases with brisk aqueous outflow, as occurs in simple lacerations.

	Treatment Arms			Side Effect Arm
Location of Experimental Procedure	USAISR, San Antonio, TX			MGH, Boston, MA
Rabbit Type	New Zealand White			Dutch Belted Pigmented
Study Arm	1	2	3	4
Number of Rabbits	40	30	28	17
Experimental Wound	4mm linear central cornea laceration	"V" shaped central cornea laceration	4mm linear corneoscleral laceration	3mm linear central cornea laceration
Number of Experimental Eyes	21	15	13	17
Repair Method	A single central 10-0 nylon suture, and Rose Bengal impregnated amniotic	A single 10-0 nylon suture at the "V" apex, and Rose Bengal impregnated amniotic	A single 10-0 nylon suture a the limbus, and Rose Bengal impregnated amniotic membrane irradiated for	Rose Bengal impregnated amniotic membrane irradiated for 400 seconds of a 12mm diameter 400mW laser

	membrane irradiated for 250 seconds of a 13mm diameter 400mW laser	membrane irradiated for 250 seconds of a 13mm diameter 400mW laser	250 seconds of a 13mm diameter 400mW laser	
Number of Control Eyes	19	15	15	-
Repair Method	interrupted 10-0 nylon sutures	interrupted 10-0 nylon sutures	interrupted nylon sutures: 10-0 on cornea, 9-0 on limbus, 8-0 on sclera	-

320

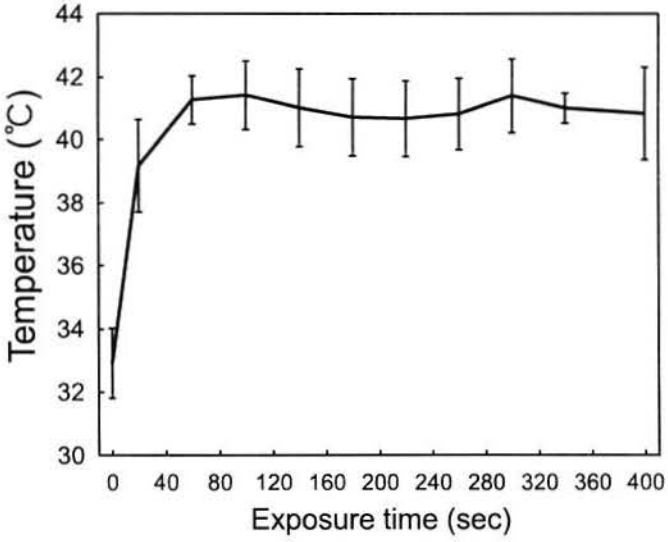
321 Table 1. Summary of Methods.



322

323 Figure 1. Comparison of histopathological results after sealing amniotic membrane over cornea
324 lacerations using laser crosslinking or sutures. The data are the mean values ± SD of the values
325 measured at three time points (3, 7 and 28 days).

326



327

328 Figure 2. Temperature of the cornea surface during irradiation of RB-impregnated amniotic

329 membrane over a central cornea laceration using 0.25 W/cm^2 at 532 nm. (n = 6 / group).

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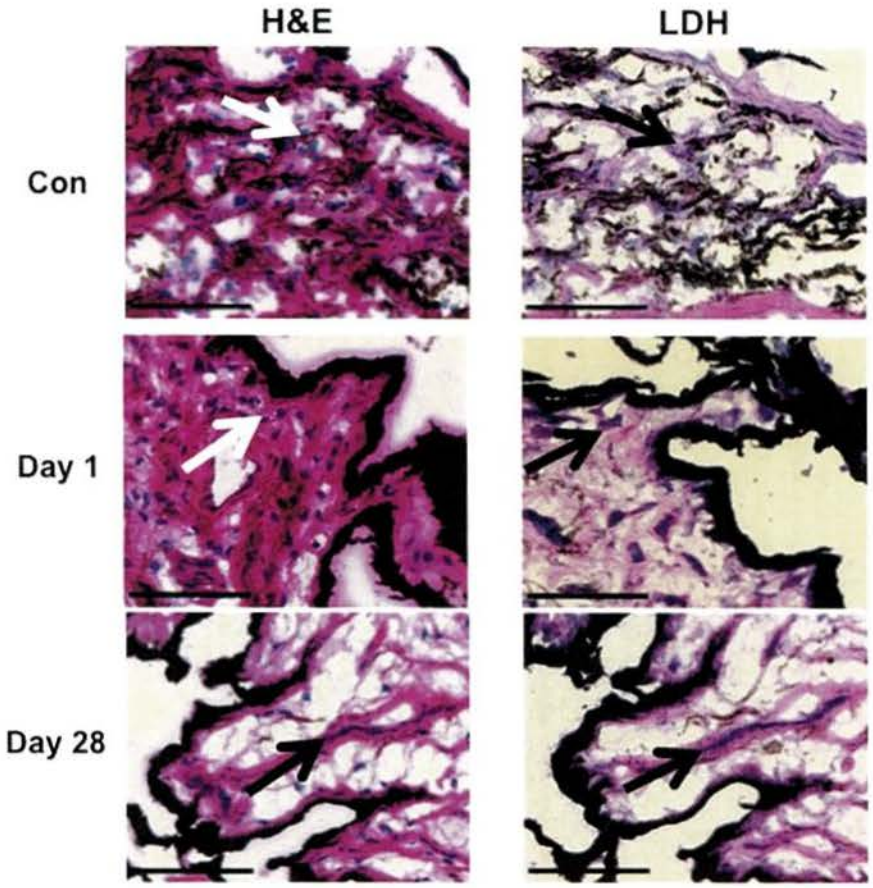

Figure 3. Evaluation of cell viability in iris tissue after sealing amniotic membrane over central cornea lacerations using laser crosslinking. Iris tissue sections were stained with H&E or for LDH activity indicating cell viability by formation of a blue formazan product. Arrows showing the areas of blue formazan.

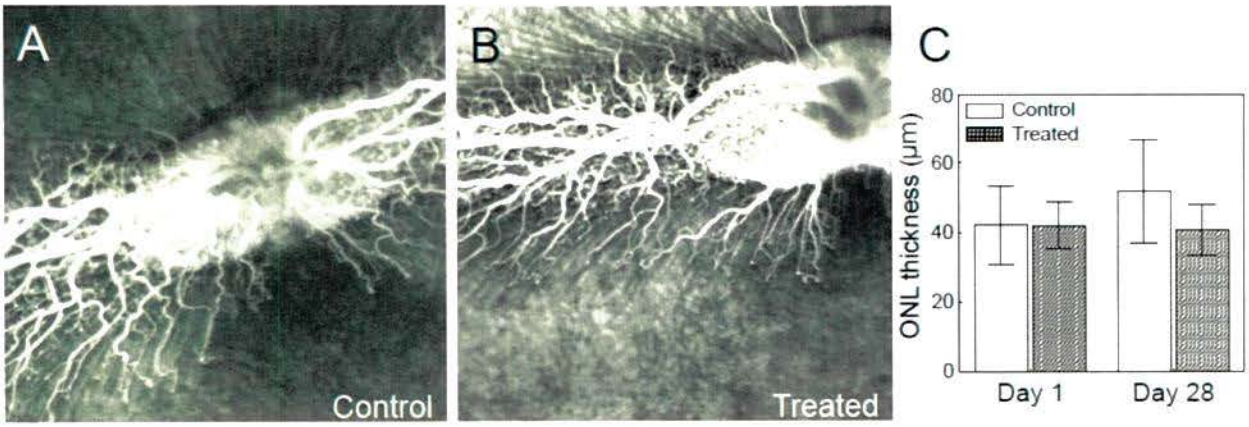

Figure 4. FFA and ONL thickness measurement of retinas from control eyes and from eyes treated with laser crosslinking to seal amniotic membrane over central cornea lacerations (third arm of study). Images were taken on Day 28 post treatment of (A) control and (B) treated eyes. (C) ONL thickness measured on H&E sections of retinas in control and treated eyes on Day 1 and Day 28. (n = 6 / group).

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