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14. ABSTRACT Purpose: To develop a light-activated technology (called PTB) with the potential to decrease vision loss and ocular complications in warfighters sustaining penetrating eye injuries. Scope: In year 2, the scope was to establish the treatment for direct photo-sealing of corneal lacerations, to identify the best treatment for sealing eyelid skin lacerations, and to optimize and build a prototype light delivery system that is safe for the retina. Major findings: Demonstrated that fibrin glue was not competitive with PTB for sealing is amnion over penetrating cornea injuries, determined that two potential adverse effects (inhibition of epithelial cell migration and keratocyte phototoxicity) are not significant problems, demonstrated that PTB can be used to seal lacerations in thin (e.g., eyelid or periorbital) skin without deep sutures and that this repair requires less time than suturing and stimulates less inflammation than sutures, built and tested a prototype retina-safe optical delivery system that effectively seals amnion to cornea and substantially reduces the treatment time compared to the laboratory optical fiber system.					
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INTRODUCTION

The overall goal of this research is to develop a light-activated technology with the potential to decrease vision loss and ocular complications in warfighters sustaining penetrating eye injuries. Fragments and debris propelled at high velocity by improvised explosive devices (IEDs) have increased the incidence of penetrating eye injuries in the current conflicts compared to earlier wars. Rapid closure of penetrating eye wounds with formation of a water tight seal is critical to preventing infection and stabilizing the eye for further surgery, thus improving vision outcomes. Suturing the cornea, sclera and eyelid skin requires specialized training to precisely place hair-fine sutures and requires long surgery time. Cyanoacrylate glues can complicate further surgery by sticking to sutures and possibly causing additional damage when removed. Our sutureless, glueless method is rapid and uses currently FDA-allowed devices (clinical laser, light-activated dye, amniotic membrane) and thus may move rapidly to the deployment environment. The scope of the research includes evaluating two light-activated approaches to closing penetrating injuries in the cornea and sclera of rabbit eyes. In one method, amniotic membrane is stained with the dye, placed over the wound and treated with green light; in the other, the dye is applied to the wound walls and activated by green light to directly close the wound. The scope also includes developing a light-activated method for rapid closure of eyelid lacerations using hairless mouse skin as a model. Finally, the scope includes designing, constructing and evaluating a green laser light delivery system that meets ANSI standards for retina and iris safety. Major tasks for Year 2 at Brooke Army Medical Center were to determine healing after PTB treatment and to establish best treatment parameters for PTB in conjunction with sutures.

BODY

This research project is a collaboration with Dr. Irene Kochevar at the Wellman Center, Massachusetts General Hospital. The Statement of Work includes tasks to be carried at both the Massachusetts General Hospital (MGH) and the BAMC. Dr. Kochevar and Dr. Johnson have discussed results and plans for this project frequently by phone. Dr. Kochevar 's lab assembled and sent to Dr. Johnson the light delivery system used by his group for in vivo studies. Dr. Kochevar visited Dr. Johnson at BAMC in December 2010 to help set up the irradiation system and advise on the first ex vivo studies. These co-PI's also had extensive discussions at the ATACCC meeting in Fort Lauderdale in August 2011 where this work was presented as an oral communication.

Delay: Due to unforeseen construction delay at Brooke Army Medical Center due to the BRAC initiative our project is currently 1 year behind schedule.

#2 Due to my PCS move from Brooke Army Medical Center to the US Army Institute of Surgical Research, Ocular Trauma Division, LTC(P) Raymond Cho has been named the temporary Principal Investigator, pending rewriting of the protocol and moving the protocol to USAISR.

Task 1. Determine Healing after PTB and suture treatments.

- 1. Establish model for carrying out the PTB treatments.** The forme fruste of our efforts to date have been to develop a suitable model to evaluate the PTB treatments and then to evaluated the healing process after PTB and Suture treatments. Our initial approach mirrored the approached used by Dr. Kochevar at the Wellman Center to bond the amnion to the surface of the cornea. This approach involved placing 50% alchocol on the surface of the eye, scraping the epithelium off of the eye,

then preparing the amniotic membrane in the prescribed fashion, (washing the amniotic membrane, drying the membrane, staining it with rose Bengal, placing it stromal side down on the surface of the rabbits cornea, then applying the laser treatment.) Although this was quite effective in achieving bonding, in the in-vivo model in which we evaluated the rabbits for one month after the treatment we noted that after approximately one-two weeks this led to neovascularization of the cornea. (Of note minimal neovascularization was noted at the wound site pathologically, indicating that the actual bonding did not result in surface inflammation, or appeared to the source of the neovascularization.)

Due to the corneal neovascularization we first modified our application of alcohol, using a Epilasik well to contain the alcohol. This method was effective but not consistent. 2 rabbits had excellent results while 2 others developed neovascularization at week 1-2. The method was again modified. We reduced the concentration of alcohol by 50% to 25% and then soaked a pupil blocking sponge with alcohol and placed the sponge on the cornea to limit the extravasation of the alcohol. This improved the outcome however some neovascularization is still seen on a few of the rabbits. We were able to achieve better results by reducing the irradiance of the laser however the source of the neovascularization is still elusive. We have thus modified our post op regiment to include more anti-inflammatory treatment (dexamethasone 2mg/ml, 0.2cc injection subconjunctivally at the end of the procedure. (The next step will be to 1) place a physical barrier to protect the limbal stem cells of the rabbit from the alcohol (viscoelastic). Additionally a laser physicist, Peter Edsall is modifying the laser device provide to us by Dr. Kochever to collimate the beam more to limit limbal stem cell exposure with the treatment.

Additional evaluation was made to directly close the wounds using Rose Bengal to the walls of the laceration. In simple lacerations this worked well, however when a small wound gap was present we noted leakage at one week post op. In that experiment we hydrated the stroma by injecting Rose Bengal into the stroma on both sides of a V shaped laceration. When they applied the laser in the standard fashion. We achieved a water tight closure despite noting a small wound gap at the ocular surface, which began to leak 1 week post-op. (likely we only had a small crosslinking bridge which leaked with elevated IOP involved in transferring the rabbits to and from their cages. We then took the rabbit back to the OR. The wound was cleaned of epithelium, 2 sutures were placed and then a small amniotic membrane patch soaked in rose Bengal was placed over the laceration repair. It was then sealed in the standard fashion, resulted in a water tight closure. (at one week post op the rabbit is doing fine)

Task 2. Establish the treatment parameters and determine healing after PTB and suture treatments. A V shaped laceration was placed in the central cornea of the rabbit. Due to the tendency for the anterior chamber of the rabbits eye to collapse after the first aspect of the laceration was placed, viscoelastic was injected through the wound to stabilize the anterior chamber to facilitate creating the second arm of the laceration. In our first attempt we made an outstanding wound however the increased aqueous formation of the rabbit pushed the viscoelastic out of the eye during the laser phase of the treatment. This interfered with the bonding of the amnion, requiring a second intervention (the aqueous formation in the rabbit appears faster than humans so we had to modify the technique). Our subsequent modification was as follows: The first arm of the laceration was placed. A small amount of viscoelastic was injected in the AC through the wound. The second leg of the wound was created. Then the viscoelastic was flushed from the anterior chamber from the wound created using a 30 gauge needle on a 3cc syringe injected through the peripheral cornea. 2 11-0 nylon sutures were used to approximate the wounds (using a operating microscope). Then a fresh amniotic membrane was placed (prepared as above). The laser was applied with excellent results- using the collimated beam with the assistance of Peter Edsall. The needle wound was closed via wound hydration. We will repeat this procedure to ensure consistent results. Then we will begin the 50 rabbits of the formal protocol.

Dr. Kochever has provided us with the laser treatment parameters we are using for our in-vivo experiments. Additionally she was provided us with the laser device to perform the treatments. Our difficulties to date have involved the effects of neovascularization of the cornea 2 weeks after treatment. Our efforts to date have been dedicated to developing a consistent approach that will minimize neovascularization.

Additionally, Major Hanson, Veterinary Pathologist has been extremely helpful in allowing us to evaluate the corneas pathologically to assess possible sources of neovascularization. Her direct observations of treatments and also evaluating the pathological specimens has been very helpful in determining which aspects of our procedure to modify.

Figure 1. Prototype of the light delivery system for photobonding on the cornea that reduces the laser power at the retina to below the threshold for damage according to ANSI 136.1 standards.



KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated that excellent results can be obtained crosslinking the amionic membrane to the ocular surface in the in-vivo model with evaluation for 30 days post treatment. This has been verified for simple lacerations and complex lacerations. The laser irradiance in conjunction with the laceration was particularly promising. (Modification of the technique is still needed due to post op neovascularization seen at weeks 2-3 in some rabbits).

Demonstrated that hydration of the wound with rose Bengal and irradiating the cornea can obtain a water tight closure, however any gap in the wound can lead to leakage. (alternatives to enhance this technique are 1) suture + hydration with rose Bengal 2) rose Bengal mixed with collagen to make the rose Bengal more viscous, and thus bridge the wound gap better.

Determined the laser irradiance was not sufficient to break the 11-0 nylon suture under the amnion (laser suture lysis is common procedure and potential complication of employing laser closure in conjunction with sutures) . This substantiates the initial premise that the crosslinking can be a useful adjunct to laser suture repair. In simple well approximated lacerations it appears to be the equivalent with less inflammation, in complex laceration it will be a useful adjunct speeding closure time, and permitting early secondary repairs.

REPORTABLE OUTCOMES

Photoactivated technology for repair of penetrating eye injuries. E. Verter, A.J. Johnson, M. Yao, R.W. Redmond and I.E. Kochevar. Poster presented at the 4th Military Vision Symposium, Schepens Eye Research Institute, Boston, MA, September 27-29, 2010,

Light-initiated bonding of amniotic membrane to cornea. E. Verter, A.J. Johnson, T. Gisell, P. Yang, R.W. Redmond and I.E. Kochevar. Presented at the 2011 ATACCC Conference, Fort Lauderdale FL, August 15-18, 2011.

CONCLUSIONS

We extended the development of a simple and rapid light-initiated tissue bonding technology to decrease vision loss and ocular complications after penetrating eye injuries. Rapid closure of these wounds is critical to preventing infection and stabilizing the eye for further surgery. Current methods have substantial drawbacks: suturing is tedious, time-consuming and can damage the tissue; fibrin glues are not strong enough; and cyanoacrylate glues can cause damage upon removal and interfere with subsequent surgery.

In Year 2 (Kochever) we established that lacerations in thin eyelid and periorbital skin are effectively sealed using our sutureless, glueless technology in an animal model system. The light-activated sealing procedure was more rapid than suturing, generated less inflammation and provided an immediate water-tight seal. This method eliminates painful suture removal while not using stiff repair materials that inhibit blinking during recovery.

We established that lacerations of the cornea are effectively sealed using a combination of sutures and glueless technology in an animal model system. The light sealing technology is rapid, however the requirement to remove the epithelium poses technical issues that leads to neovascularization in rabbits.

We characterized practical aspects of our method for sealing a biological membrane over penetrating corneal wounds by examining storage and preparation aspects of the biological membrane, by showing that the seal produced was stronger than a fibrin glue seal and by demonstrating that two potential adverse responses are not important.

The prototype light delivery system, designed to be safe for the retina, was shown to be highly efficient for repair of penetrating cornea injuries by sealing a biological membrane, thus shortening the treatment time.

These results indicate that significant problems are not expected in the translation of this light-activated repair technique to clinical use. Of importance for translation are safety studies, which are not included in this project. This should be addressed.

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