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TITLE: Light-Activated Sealing to Improve Outcomes Following Penetrating Bowel Trauma

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14. ABSTRACT

The overarching hypothesis of this proposal is that a rapid, simple, light-activated sealing technology can provide a more secure wound closure and reduce complications leading to improved outcomes for wounded warfighters following traumatic penetrating colon injury. Penetrating bowel wounds can be rapidly sealed and stabilized using biocompatible patches in conjunction with light-activated bonding. Our objective is to determine the optimal implementation strategy for this technology in a large animal model that recapitulates the military trauma scenario and to address a priority research area in the Combat Casualty Care Research Program "to identify and develop medical techniques and materiel for early intervention in life-threatening battle injuries".

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1. Introduction.

Penetrating colon injury occurs in around 5% of all military trauma in current conflicts. A penetrating injury, such as a puncture or a complete severance, is a highly dangerous injury as the waste materials in the colon contain high levels of bacteria that can leak into the abdominal cavity and induce a series of events that can lead to infection, inflammation, sepsis and shock and if unchecked will be lethal to the patient. Penetrating injuries will generally be closed by one of various suture approaches but even in the best civilian trauma centers with top-end surgeons and equipment there is a 1-3% rate of failure that can lead to considerable complications, morbidity and even mortality. However, this rate is far higher (~20-30%) in wounded warfighters for a number of reasons. The patient will generally have other extensive injuries, especially those associated with hemorrhage and excessive blood loss, that impact on the physiological status of the patient during and after surgery. In addition, the wound is likely to be "dirty" with respect to elective surgery wounds with contamination leading to infection-related complications. The background, expertise and experience of the military surgeons performing the repair are typically more diverse than the specialist in a civilian trauma center and the resources available to the military surgeon will also be limited with respect to the civilian environment. In order to close this gap and improve outcomes of wounded warfighters that suffer penetrating bowel injury, we have developed a light-activated method for bowel wound closure that produces a stronger wound closure, involves considerably less specialized technical skill and is faster than current suture closure techniques. Wound surfaces are painted with a red dye and placed in close contact. Green light illumination causes chemical reactions to occur at the wound surfaces that form innumerable chemical bonds that hold the wound securely closed in a water-tight, leak-free fashion. In this proposal we aim to optimize the procedure and the materials used for clinical efficacy in a military-relevant wound model and validate its potential for rapid adoption for use in humans, with particular emphasis on improving outcomes for wounded warfighters. It should also be noted that the same technology is equally applicable for bowel repair in civilian medicine, including trauma surgery and rejoining the bowel after removal of diseased tissue such as cancer.

2. Keywords: trauma, penetrating bowel injury, colon repair, wound closure, human amniotic membrane, PTB, photosealing, Rose Bengal, swine intestinal submucosa, sutureless repair, crosslinking, photochemistry.

3. Accomplishments:

What were the major goals of the project?

The overarching goal of this JWMRP proposal "Light-Activated Sealing to Improve Outcomes Following Penetrating Bowel Trauma" is to develop a rapid, simple, light-activated sealing technology can provide a more secure wound closure and reduce complications leading to improved outcomes for wounded warfighters following traumatic penetrating colon injury. Our objective is to determine the optimal implementation strategy for this technology in a large animal model that recapitulates the military trauma scenario and to address a priority research area in the Combat Casualty Care Research Program "*to identify and develop medical techniques and materiel for early intervention in life-threatening battle injuries*".

Milestones for this award are listed below, along with percentage completion to date (in bold) where appropriate.

Task 1– *Determine the immediate seal strength of candidate photosealing materials to identify a lead material for use in colon wound closure*.

1a. Regulatory approval of use of discarded human tissue (human amniotic membrane, HAM). (Months 1-3) **100% complete**
1b. Regulatory (MGH IACUC and ACURO) approval of non-survival rodent colotomy model. (Months 1-3) **100% complete**
1c. Purchase and receipt of supplies for Task 1. (Months 1-2) **100% complete**
1d. Crosslinking of HAM and SIS with EDC/NHS to make xHAM and xSIS. (Months 2-4) **100% complete**
1e. Rodent non-survival surgeries and burst pressure measurements (Months 3-6) **100% complete**
1f. Determine resistance of colon patch materials wraps to enzymatic digestion in vivo. (Months 3-6). **100% complete**
1g. Data analysis, conclusions and consultation with military surgeon partners to determine next steps. (Months 4-6). **100% complete**
1h. Establish lead colon repair material for photosealing with PTB or consider alternative repair materials, if required (Month 6) **100% complete**

Task 2 - *Determine the resistance of lead candidate photosealing materials to degradation in a rodent model of penetrating bowel injury and repair*.

2a. Regulatory (MGH IACUC and ACURO) approval of survival rodent high-risk colon anastomosis model. (Months 1-3). **100% complete**
2b. Rodent penetrating bowel survival surgeries. (Months 7-9) **50% complete**
2c. Burst pressure measurement of colon repair groups (Months 7-10) **50% complete**
2d. Blinded adhesion scoring at euthanasia of colon repair groups. (Months 7-10) **50% complete**
2i. Data analysis, conclusions and consultation with military surgeon partners to determine next steps. (Months 10-11)
2j. Establish which crosslinked materials can best persist in presence of enzymatic degradation in penetrating colon injury models and consider alternative wrap materials, if required (Months 11-12).
2k. Manuscript preparation based on Task 1-2 studies (Months 14-16).

Task 3 – Explore efficacy of PTB approach vs. standard repair in a hypotensive swine model that recapitulates the military trauma scenario.

3a. Regulatory (MGH IACUC and ACURO) approval of model for penetrating colon injury in a hypotensive swine at risk for infection. (Months 10-14)
3b. Ex vivo testing of PTB sealing of large anatomical scale defects with LED-based illuminator (Months 10-12)
3c. Swine survival surgeries and colon anastomotic repair (Months 14-20)
3d. Consultation meeting with military surgeons regarding modifications to light-activated repair technique for clinical use, if required (Months 14-16).
3e. Blinded adhesion scoring at euthanasia of colon anastomotic repair groups. (Months 15-21)
3f. Burst pressure measurement of colon anastomotic repair groups (Months 15-21)
3g. Data analysis, conclusions and consultation with military surgeons (Months 21-24).
3h. Manuscript preparation based on Task 3 Studies (Months 22-24).
3i. Planning with CIMIT for translation to human studies on successful outcomes (Months 22-24).

What was accomplished under these goals?

Task 1– *Determine the immediate seal strength of candidate photosealing materials to identify a lead material for use in colon wound closure*.

The institutional animal use protocol for this study (MGH #2017N000177) was submitted on 10/26/2017 and approved by the MGH IACUC on 12/29/2017. A subsequent submission to ACURO (1/10/2018) led to approval of the protocol by ACURO 3/19/2018.

Non-survival studies using Sprague Dawley rats commenced in April 2018. Four experimental groups were employed to determine acute bond strength of different patch materials. Five-mm full thickness incisonal wounds were created on the anti-mesenteric surface of the descending colon and 10x10 mm sections of the patch materials were photosealed over the wound using 100J/cm^2 of 532 nm light, delivered at an irradiance of 0.5 W/cm^2. Burst strengths of the repairs were then measured be harvesting a 6 cm long section of the colon, clamping both ends and increasing the pressure within the colon by infusion of saline until leakage of fluid was observed at the repair site. This maximum pressure was recorded as the Burst Pressure (BP) and notes were taken as to the nature of the leak.

Two different base materials were used for potential sealant patches (1) Human amniotic membrane (HAM) and (2) Single layer swine intestinal submucosa (SIS). Both were used in their natural forms and also crosslinked using the biocompatible crosslinker EDC/NHS to give xHAM and xSIS. Figure 1 shows the results of these acute studies. Although the SIS and xSIS appeared to provide a stronger seal than HAM or xHAM, ANOVA analysis showed that no statistically significant differences in burst pressure were observed between any of the experimental groups.

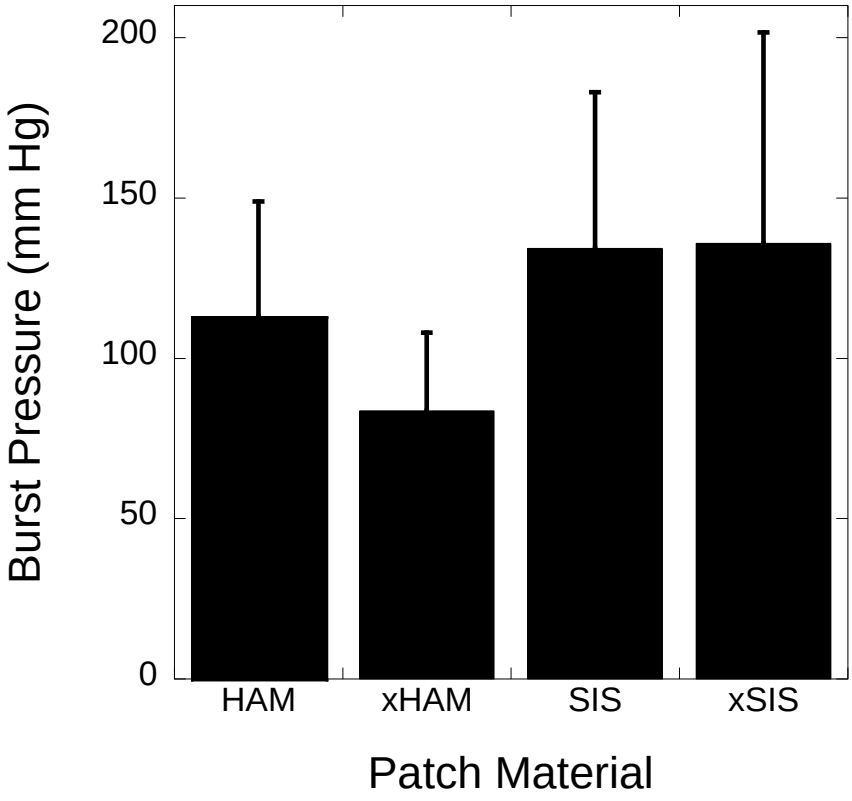

Figure 1: Acute burst pressures following photobonding of HAM and SIS and their chemically crosslinked forms (xHAM and xSIS) over full thickness incisional wounds in the descending colon of Sprague Dawley rats. No statistically significant differences were observed between treatment groups.

These results mirror that seen when these materials were considered as nerve wraps in peripheral nerve repair, as shown in Figure 2, with SIS again showing a slightly increased bond strength over HAM. Of note was that prior chemical crosslinking of either HAM or SIS with EDC/NHS had no effect on the

ability of the patch material to *photobond* to the colon, evidence that different substituent groups on the proteins are involved in chemical and photochemical crosslinking and are independent of each other.

In addition to burst pressure measurements the effect of EDC/NHS crosslinking on the resistance of patch materials to collagenase degradation was investigated. Figure 3 shows for the example of HAM that pre-treatment with EDC/NHS solution has a dramatic effect on reducing enzymatic degradation by collagenase. Thus, Task 1 has shown that chemical crosslinking of patch materials is likely to increase the persistence of the patch material under clinical conditions of exposure to catabolic enzymes, at the same time having little effect on the photosealing chemistry to provide a strong wound closure.

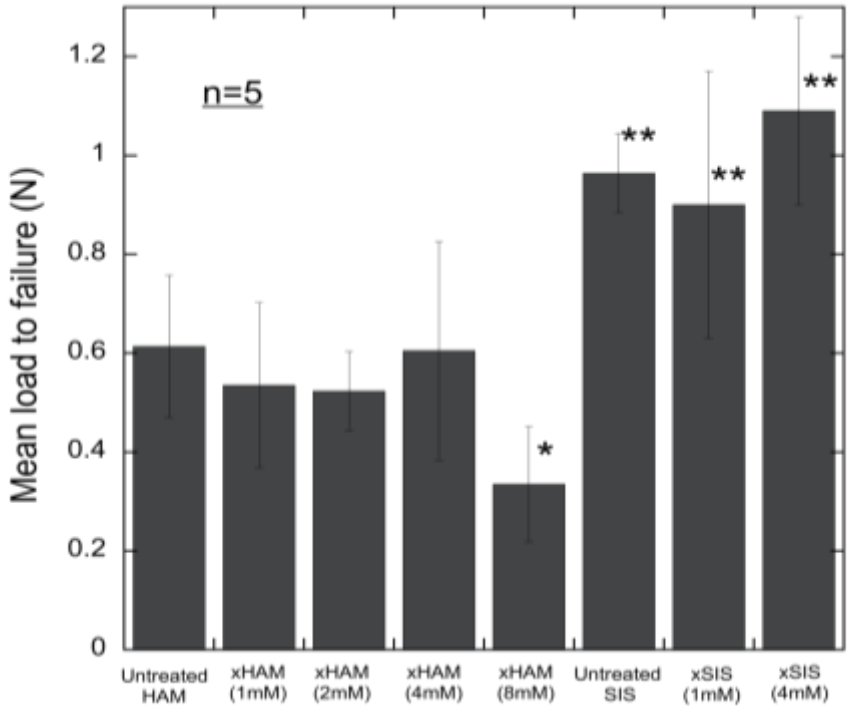

Figure 2: Effect of chemical crosslinking by EDC/NHS on bond strength of HAM and SIS to rat sciatic nerve. Molarity in parentheses refers to EDC concentration with a 4:1 molar ratio of EDC to NHS used in each case.

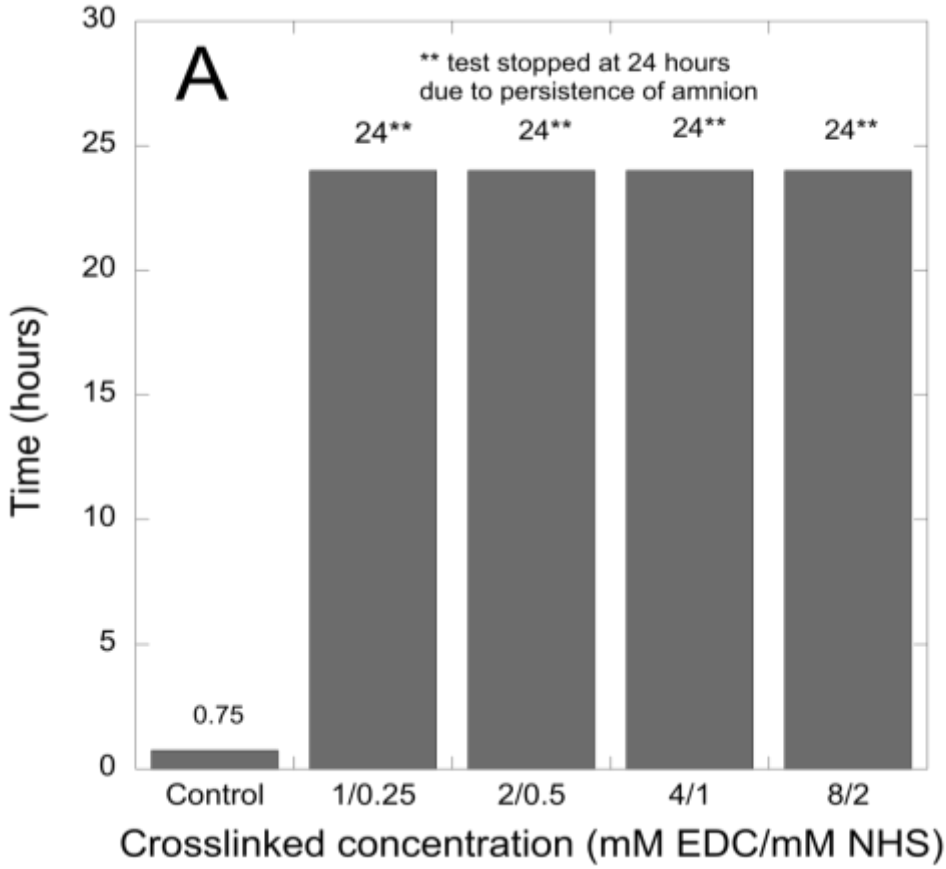

Figure 3: Effect of EDC/NHS crosslinking on gross degradation time on incubation of control and crosslinked HAM samples with 0.1% collagenase in PBS at 37ºC.

Thus, both crosslinked patch materials were viable candidates for Task 2 survival studies to identify the best patch material that would subsequently be used in the military-trauma relevant swine model in Task 3.

Task 2 - *Determine the resistance of lead candidate photosealing materials to degradation in a rodent model of penetrating bowel injury and repair*.

Regulatory approval for the rodent survival studies in Task 2 were obtained under the same MGH IACUC and ACURO submissions referred to above in Task 1. In Task 1 the acute photoseal strength of the different patch materials was determined and are sufficient in each case to provide a strong seal. However, the seal strength and patch integrity could be affected by the wound healing process and exposure of the patch to gut contents leading to inflammation and degradation of the patch itself that could cause leak and sepsis. Thus, Task 2 looked at 5 treatment groups over a survival period of 14 days where complications due to loss of patch integrity would manifest. A positive control of repair by interrupted suture was compared to photosealing using HAM, xHAM, SIS and xSIS with 0.1% RB applied to the patch surface and photosealed using 532 nm light (100 J/cm^2, 0.5 W/cm^2).

At the 14-day end point the animals are euthanized and the colon exposed. The repair site is graded for signs of abscess, inflammation, scarring and degree of bowel adhesions. In a subset of animals the colon repair site is excised and submitted for histological examination and in another subset a 6 cm section of the colon is excised for burst pressure measurement. Figure 4 shows the 14-day outcome of the positive control suture group with obvious presence of bowel adhesions from the colon to the surrounding viscera. All treatment groups are expected to have reduced adhesions due to the barrier function of the patch and lack of inflammatory suture material. These survival studies are underway at this point and complete results will be forthcoming in the next few weeks.

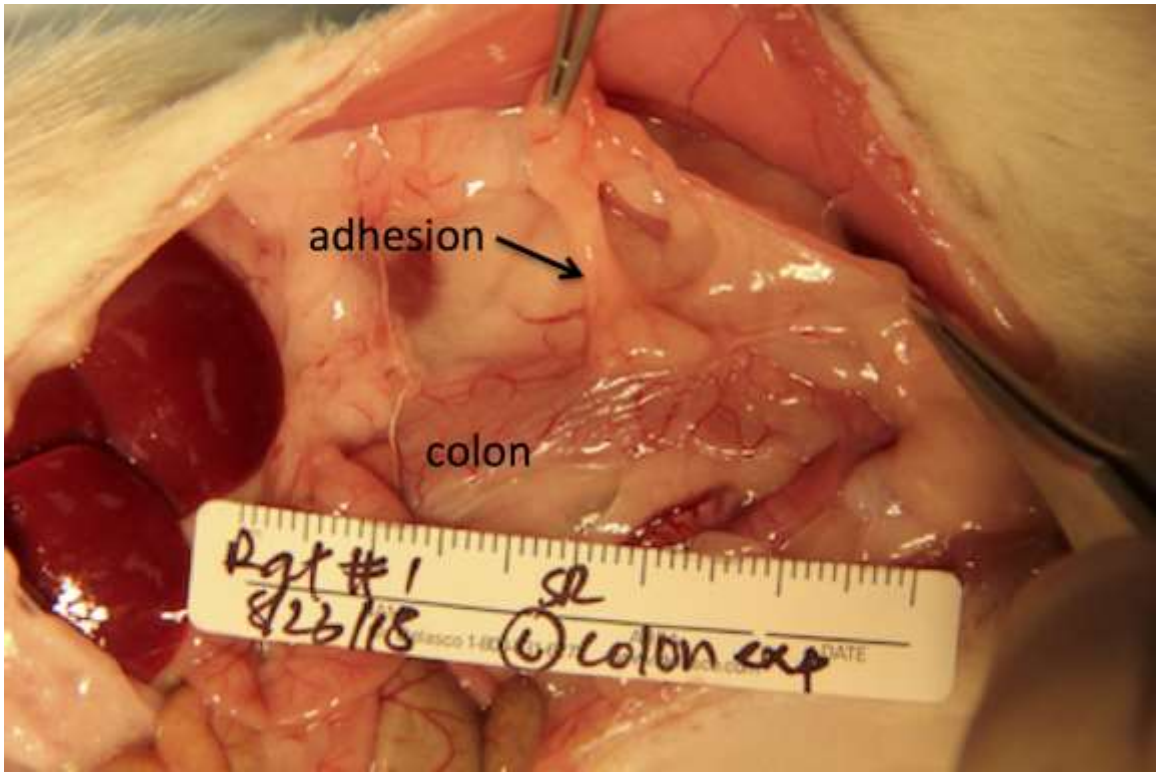

Figure 4: Photograph of suture-repaired colon incisional wound in Sprague Dawley rat at 14 days showing extensive post-surgical bowel adhesions.

What opportunities for training and professional development has the project provided?

Dr. Hansdorfer has received advanced microsurgery training from Dr. Winograd and Dr. Randolph including operating under magnification.

How were the results disseminated to communities of interest?

The results are forthcoming at this time and have been discussed preliminary at an Invited talk to the National Meeting of the American Society for Photobiology in April. We did not have a results package in place on the rodent studies for the MHSRS meeting in August 2018 but will be presenting the complete package at the 2019 MHSRS meeting along with a publication.

What do you plan to do during the next reporting period to accomplish the goals?

In the next annual period we will complete all the rodent studies and also a large animal (swine) model of high-risk injury repair involving hypotension and risk of infection that more accurately reflects military trauma. The outcomes of the rodent survival study will allow us to choose the best material for a photosealed patch between xHAM and xSIS. Should outcomes be equivalent we will proceed with xSIS as the SIS material is easily commercially available and stronger and less expensive than the human-derived amnion products. We will have all the outcomes metrics in hand and analyzed and have a strong grasp on the potential progress towards first in human studies.

4. Impact:

As we are midway through the study it is too early to yet say what the impact will be. We expect that chemically crosslinked patches for photosealing will have sufficient strength and durability through the healing process to prevent any complications form leakage of bowel contents. Additionally, the dreaded

complication of post-surgical adhesions that can lead to pain, bowel stricture and ultimately further surgery can be mitigated through the barrier function of the patch and the removal of inflammatory suture. In addition to potential impact in bowel repair we expect the technology of photosealed wraps and patches to have applications in orthopedics, vascular surgery, gynecology and plastic surgery.

5. Changes/Problems:

The project has gone smoothly to this point and no problems have been encountered or any changes to the overall experimental plan have been necessitated.

6. Products:

Invited Talk

Potential Clinical Applications of Protein Photocrosslinking. Redmond RW. Annual Meeting of the American Society for Photobiology, April 2018, Tampa, Florida.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name:	*Robert W. Redmond PhD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Redmond is responsible for overall coordination of the project*

Name:	*John A. Parrish*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Parrish provides overall guidance to achieve positive outcomes.*

Name:	*Mark A. Randolph MAS*
Project Role:	*Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Mr. Randolph has been instrumental in designing animal protocols and in the behavioral testing design.*

Name:	*Marek Hansdorfer MD*
Project Role:	*Research Fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Hansdorfer has been the lead Fellow on this project and has been involved in all day-to day aspects of regulatory approvals, experimental planning, surgical training and outcomes testing.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report