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

PRINCIPAL INVESTIGATOR: **John A. Parrish, MD**

CONTRACTING ORGANIZATION: **Massachusetts General Hospital**

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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) **100% complete**
2c. Burst pressure measurement of colon repair groups (Months 7-10) **100% complete**
2d. Blinded adhesion scoring at euthanasia of colon repair groups. (Months 7-10) **100% complete**
2i. Data analysis, conclusions and consultation with military surgeon partners to determine next steps. (Months 10-11) **100% complete**
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). **100% complete**
2k. Manuscript preparation based on Task 1-2 studies (Months 14-16). **100% complete**

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) **100% complete**
3b. Ex vivo testing of PTB sealing of large anatomical scale defects with LED-based illuminator (Months 10-12) **100% complete**
3c. Swine survival surgeries and colon anastomotic repair (Months 14-20) **75% complete**
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) **75% complete**
3f. Burst pressure measurement of colon anastomotic repair groups (Months 15-21) **75% complete**
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 3*– Explore efficacy of PTB approach vs. standard repair in a hypotensive swine model that recapitulates the military trauma scenario.*

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.

The major accomplishment in this annual period was commencing the large animal, MGH IACUC and ACURO approved, traumatic hypotensive swine study in Task 3. To date, 6 of the 16 scheduled surgeries have been performed and the animals followed to euthanasia on day 28. As a result of rodent outcomes in Task 2 and in ex vivo testing in Task 3 the control microsurgical repair is compared to the experimental group of photosealing of bowel wounds sealed with chemically-crosslinked human amniotic membrane (xHAM) stained with the photoactive dye, rose Bengal (RB, 0.1% w/v) with 532 nm from a KTP laser delivered at an irradiance of 0.5 W/cm^2 and a cumulative fluence of 90 J/cm^2.

Table 1: Planned Operative Schedule and Results to Date

Swine	Scheduled Case Date (Pre-Covid)	Actual Case Date (Post-Covid)	Euthanasia Date	Group	BP (mmHg)	Adhesion score	Rupture site
1	12/4/19	12/4/19	1/2/20	control	278	1	anastomosis
2	2/7/20	2/7/20	3/6/20	control	237	2	anastomosis
3	2/14/20	2/14/20	3/13/20	control	159	0	anastomosis
4	2/25/20	2/25/20	3/24/20	PTB	282	1	adj. bowel
5	2/27/20	2/27/20	3/26/20	PTB	279	2	adj. bowel
6	3/9/20	3/9/20	4/6/20	control	202	1	anastomosis
7	3/19/20	6/16/20	7/15/20	PTB	270	2	adj. bowel
8	4/2/20	6/18/20	7/16/20	PTB	317	3	adj. bowel
9	4/3/20	7/7/20	8/4/20	control	220	1	anastomosis
10	4/21/20	7/21/20	8/18/20	PTB			
11	5/4/20	7/27/20	8/24/20	control			
12	5/7/20	8/11/20	9/8/20	PTB			
13	5/12/20	8/20/20	9/17/20	PTB			
14	5/14/20	9/1/20	9/29/20	control			

Table 1 shows the planned operative schedule for the hypotensive swine study in Task 3. Unfortunately, due to the COVID-19 pandemic the MGH CCM facility stopped receiving animal orders on March 13, 2020. Thus, the surgeries planned for swine 7-16 were postponed. Swine 4-6 had already undergone surgery and the PI gained MGH approval for the swine to continue to date of euthanasia with staff receiving exemption to attend MGH and perform daily care until euthanasia. These animals therefore remained fully invested in the study. Animal experiments were allowed to recommence in June 2020 and rescheduled dates reflect the rescheduled time-frame. Highlighted dates denote procedures that are yet to be completed.

The operative procedure involves laparotomy and exposure of the small bowel. A complete transection is made in the small bowel and a controlled blood draw is concomitantly initiated to induce a hypotensive state in the animal, which is then held for a period of one hour to simulate a hypotensive wounded warrior with penetrating bowel injury. No attempt is made to prevent leakage of bowel contents into the abdomen during the shock period. Following the shock period the abdominal cavity is

cleaned by lavage and the repair performed as the withdrawn blood is resupplied to the animal to reverse the shock. Animals are randomly assigned to standard microsurgical repair with anastomosis performed using interrupted sutures placed ~ 3 mm apart or to standard repair augmented with a circumferential strip of crosslinked human amnion product (xHAM) that is coated with 0.1% rose Bengal solution and illuminated at 532 nm using a KTP laser (60J/cm^2) to photoseal the xHAM over the anastomotic line. The abdomen is then closed in layered fashion.

At this time 9 animals have completed the study term of 28 days post-surgery with n=5 animals undergoing the control suture anastomosis and n=4 animals undergoing photosealing. Immediately prior to euthanasia, the repair site was re-exposed surgically and the extent of post-surgical adhesions was scored by two independent clinicians blinded to the nature of the repair using the following scale.

Score	**Gross Observation**
0	No adhesions
1	Minimal adhesions, mainly between omentum and anastomosis
2	Moderate adhesions, between omentum and anastomosis and between anastomosis and small bowel or abdominal wall
3	Severe, extensive adhesions, opaque with capillaries present

Following adhesion scoring each animal was then euthanized and a 10 cm long section of the small bowel with the anastomosis centrally-located was then harvested for burst pressure testing as follows. The ends of the bowel section were securely closed with silk ties and a needle inserted into the bowel lumen with the insertion site sealed with superglue to prevent leakage at the insertion. Saline containing blue dye was then injected at a controlled rate into the lumen and the pressure inside the lumen recorded using a transducer. The pressure was recorded until leakage occurred and the site and burst pressure were recorded, as shown in Table 1.

The primary aim of this technology is to strengthen the anastomotic repair of penetrating bowel injury using photosealing with a biocompatible "bandage". Figure 1 shows the average burst pressure recorded for control and photosealed groups at 28 days post-operatively.

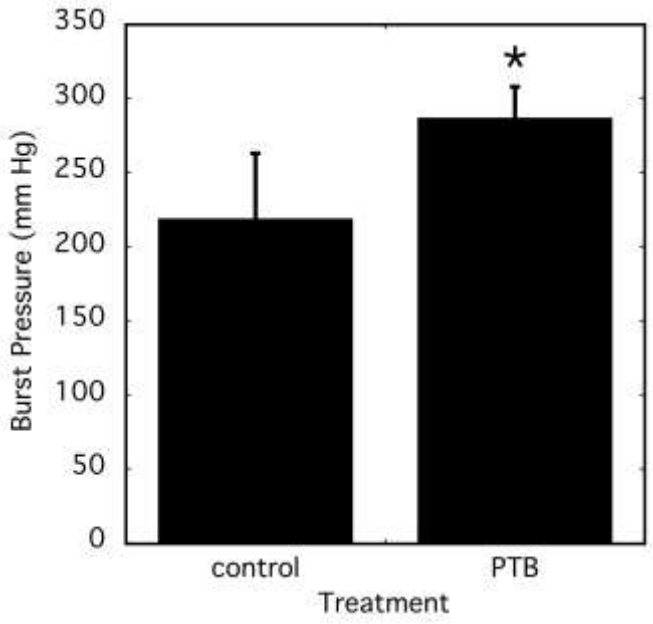

Figure 1 – Average burst pressure measured for control and photosealed (PTB) repair of anastomotic repair of small bowel transection injury in sheep at 28 days post-operatively.

The mean burst pressure value for the photosealed group (n=4) was 287+/- 21 mm Hg, compared to 219+/- 44 mm Hg for the control group (n=5). This 30% increase in burst pressure was already statistically significant (P value=0.027) with the incomplete cohorts so far. A significant difference was observed in the site of bowel rupture during the burst pressure test with all control repairs failing at the anastomotic line while all PTB repairs leaked away from the anastomotic line, suggesting that the repair site is even stronger than the burst pressure measured.

Adhesion scoring so far also shows a significant difference between groups with the control goups exhibiting a median adhesion score of 1 and the photosealed groups having a value of 2, signifying more extensive adhesions. This is an unexpected result as prior studies of anastomotic repair in rodents showed the opposite. However, in the current study we have employed chemically crosslinked xHAM compared to the natural, non-crosslinked HAM material used in the rodent study. We hypothesize that crosslinking may have imparted an immune reactivity to the HAM that is responsible for the increased adhesions.

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

The last year of this project has provided an excellent training opportunity for Benjamin Scott MD, who has completed three years of General Surgery residency in the program at Beth Israel Deaconess (BID) Medical Center, currently on a two-year Research Fellowship in the Department of Surgery at MGH. Dr. Scott has had excellent microsurgical training from Dr. Randolph, an expert in large animal surgery, and has had over 75 hours of hands-on surgical experience in the course of these studies to date.

How were the results disseminated to communities of interest?

Results from the in vivo rodent study in Task 2 were presented as a poster at the MHSRS meeting in August 2019 (poster #715, "Light-activated sealing to improve outcomes following penetrating bowel injury" by Hansdorfer *et al*.) The animal model and preliminary results from the hypotensive swine model in Task 3 were accepted for oral presentation at the annual Military Health Systems Research Symposium (Scott *et al*. – abstract # MHSRS-20-02086).

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

In the final quarterly period we will complete all the remaining sub-tasks in Task 3. Everything is now in hand to complete the hypotensive swine studies that are outstanding. At the conclusion, all outcomes metrics will have been obtained and a pathway towards first in human studies will be apparent.

4. Impact:

This project has a large potential impact on both military and civilian medicine. Anastomotic leak following penetrating bowel injury and repair is a dreaded complication with serious outcomes including mortality. We have already shown improved outcomes in rodent and now in a military-relevant large animal model of a hypotensive patient with an at-risk repair. Essential factors for sealing the penetrating colon injury are; 1. An enzyme resistant wrap material, 2. Sufficient pliability to conform to the colon surface anatomy, 3. Ability to photocrosslink securely to the outside surface of the bowel. SIS, while effective in sealing wounds ex vivo, failed in vivo due to the excessive stiffness imparted to the material by chemical crosslinking that led to an incomplete seal. xHAM was the preferred material for Task 3, exhibiting a significantly stronger resistance to anastomotic leak following repair in the hypotensive swine model. 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:

In 2019, MGH experienced a high demand for large animal housing (Swine, sheep etc.) combined with a limited housing availability that affected the ability to perform our studies on the expected time-line. Crisis meetings of the affected PIs with MGH CCM resulted in partial solutions to the problem which allowed Task 3 studies to commence in December of 2019. The first 6 animals had undergone injury and repair before the COVID-19 pandemic necessitated a complete shut-down of animal research activities at MGH in March 2020, which lasted until June 2020. We were subsequently given priority to reschedule our remaining cases such that the study can be completed by the end of September. A no-cost extension was thus requested and awarded until 10/15/2020 to allow the project to be completed.

6. Products:

Invited Talk (plenary)

Preclinical Studies of Photocrosslinking Technologies for Tissue Repair and Regeneration. Redmond RW. 26th Annual Conference of the Society for Free Radicals in Biology and Medicine, Nov. 20-23, 2019 in Las Vegas, NV.

Oral presentation (accepted)

Light-activated reinforced repair of penetrating small bowel injury in a military-relevant hypotensive swine model. Scott BB, Hansdorfer, MA, Wang Y, Nietlespach V, Randolph MA, Redmond RW. Annual Military Health System Research Symposium (MHSRS), August 2020, Kissimmee, Florida. (#MHSRS-20-02086).

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: *Benjamin Scott MD*
Project Role: *Research Fellow*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*
Contribution to Project: *Dr. Scott has been the lead Fellow on Task 3 for 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