Award Number: **W81XWH-17-1-0388**

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

PRINCIPAL INVESTIGATOR: **John Parrish**

CONTRACTING ORGANIZATION: **Massachusetts General Hospital, Boston, MA**

REPORT DATE: **August 2021**

TYPE OF REPORT: **Annual Report**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2021	Annual	20Jul2020-19Jul2021

4. TITLE AND SUBTITLE
Light-Activated Sealing to Improve Outcomes Following Penetrating Bowel Trauma

5a. CONTRACT NUMBER
W81XWH-17-1-0388

5a. GRANT NUMBER
JW160043

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Robert W. Redmond Ph.D., John A. Parrish, MD

E-Mail: redmond@helix.mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
The Massachusetts General Hospital,
55 Fruit Street, Boston, MA 02114.

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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."

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	12	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

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) **100% 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) **100% complete**
3f. Burst pressure measurement of colon anastomotic repair groups (Months 15-21) **100% complete**
3g. Data analysis, conclusions and consultation with military surgeons (Months 21-24). **100% complete**
3h. Manuscript preparation based on Task 3 Studies (Months 22-24). **100% complete**
3i. Planning with CIMIT for translation to human studies on successful outcomes (Months 22-24). **50% complete**

What was accomplished under these goals?

In the year covered by this report the major accomplishment was completion of ***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.

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. The large animal, MGH IACUC and ACURO approved, traumatic hypotensive swine study in Task 3 was initiated in December 2019 with the first six surgeries completed by March 2020, when the Covid-19 pandemic-induced shutdown of all research at MGH came into effect. Animal experiments were allowed to recommence in June 2020 and the eight remaining surgeries were performed between June and October, 2020.

The operative procedure involved laparotomy and exposure of the small bowel. A complete transection was made in the small bowel and a controlled blood draw was concomitantly initiated to induce a hypotensive state in the animal, which was then held for a period of one hour to simulate a hypotensive wounded warrior with penetrating bowel injury. No attempt was made to prevent leakage of bowel contents into the abdomen during the shock period. Following the shock period the abdominal cavity was cleaned by lavage and the repair performed as the withdrawn blood was resupplied to the animal to reverse the shock. Animals were randomly assigned to standard microsurgical repair with anastomosis performed using interrupted sutures placed ~ 2 mm apart or to standard repair augmented with a circumferential strip of crosslinked human amnion product (xHAM) that was 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 was then closed in layered fashion. Figure 1 shows the experimental procedure for the photosealing process.

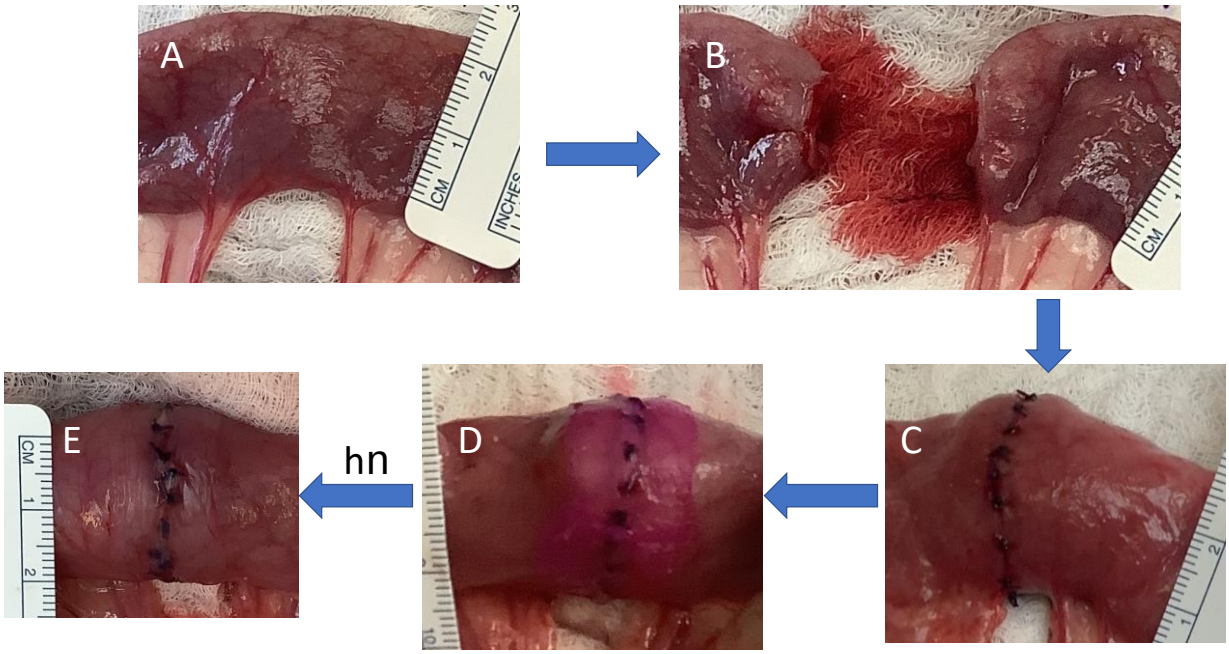

Figure 1: Photosealing (PTB) procedure. A. Intact small bowel. B. Small bowel after transection. C. Small bowel after suture anastomosis (SR). D. Circumferential application of RB-stained (pink color) xHAM wrap around anastomosis. E. Photosealed anastomosis accompanied by photobleaching of RB after illumination at 532 nm.

Immediately prior to euthanasia at 28 days post-operatively, 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 cyanoacrylate glue 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 procedure was videotaped and pressure in the bowel was recorded using a hydrodynamic transducer. Burst pressure and site of leakage was recorded for each specimen. Additionally, an adjacent section of naïve small bowel was also harvested and subjected to burst pressure testing in the same manner to establish the baseline strength of native small bowel.

All animals (n = 14) survived the procedure and the 28-day study duration. The mean volume of blood drawn off to reach MAP 30 – 35 mmHg (shock level) in control animals (n = 7) and PTB animals (n = 7) was 979 ± 216 mL and 973 ± 103 mL, respectively. Control animals were found to have a mean increase in lactate of 259 ± 92% from baseline after hemorrhage, while PTB animals exhibited a mean increase of 309 ± 72%, validating the hemorrhagic shock model. 93% of animals (13/14) were found to have an acute kidney injury (AKI) on the day of surgery or POD1, and all resolved by POD7. Additionally, 64% (9/14) animals were found to have acute pancreatitis based on physical exam and serum lipase levels greater than 400% from the upper limit of normal. All nine animals with pancreatitis had elevated serum lipase on POD1 and/or POD3 and all resolved by POD7. 36% of animals (5/14) displayed elevated gamma-glutamyl transferase (GGT) on serum liver function tests (LFTs). LFTs were normal for all animals by POD7. (Figure 3)

Mean length of time required for photosealing in the PTB group was 15 ± 5 minutes. No animals displayed clinical signs of anastomotic leak. One animal was found to have an infected CVL site upon removal on POD14 and returned to the OR for washout of abscess and received a ten-day course of antibiotics.

At post-euthanasia exploration of the abdomen on POD28 no evidence of anastomotic leak was found in any animal. The mean burst pressure for native tissue (n=9) was 282 ± 47 mmHg. The mean burst pressure for SR (n=7) was 229 ± 40 mmHg and for PTB (n=7) was 282 ± 20 mm Hg. Statistical analysis via ANOVA showed a significant difference ($p = 0.02$) in burst pressure strength between the SR group and the PTB and native tissue groups. (Figure 2) Tukey post-hoc comparison demonstrated statistically significant differences in burst pressure strength between the SR and PTB groups ($p=0.04$) and the SR and native tissue groups ($p=0.03$). Notably, all leaks occurred at the anastomotic site in the SR group, while all leaks in the PTB group occurred at least 2.5 cm away from the anastomotic site. (Figure 3). Thus, PTB treatment generates an increased repair strength of ~25% that is unlikely to fail and induce sepsis.

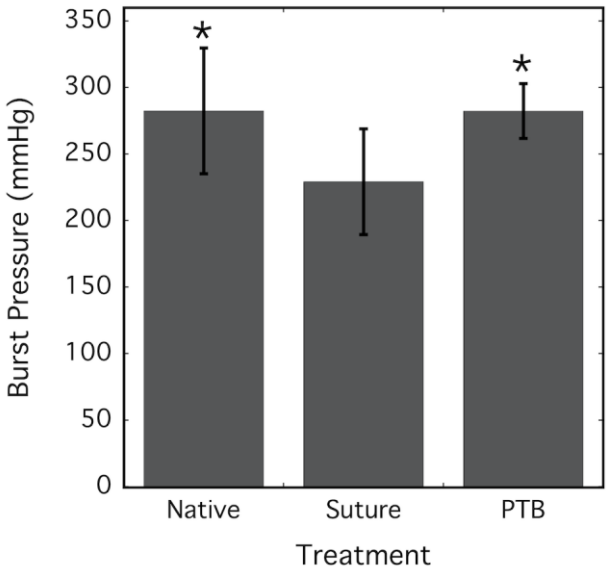

Figure 2: Anastomotic burst pressure (mean +/- SD) at 28 days post-operatively with respect to treatment group. Native, native tissue; Suture, suture repair only; PTB, suture repair with xHAM wrap sealed with photochemical bonding. Asterisks denote statistically significant increase in burst pressure from the suture repair group.

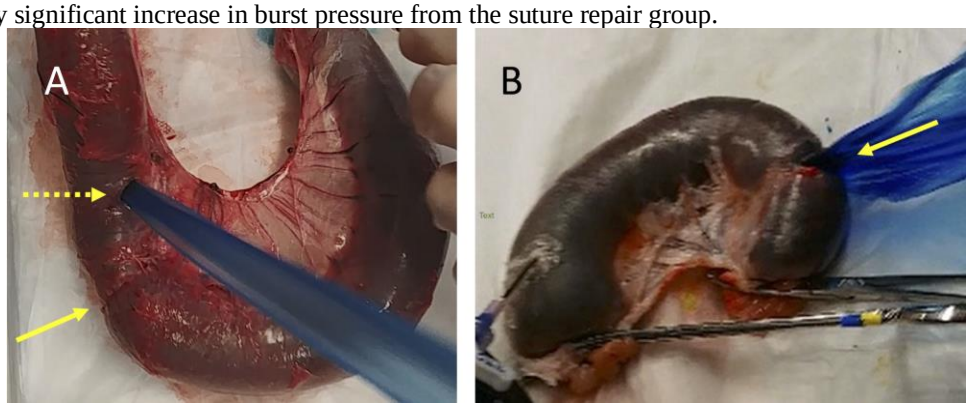

Figure 3: Location of burst failure in anastomoses repaired using (A) PTB (burst (dashed arrow) occurs several centimeters away from the anastomotic line (solid arrow)) and (B) suture repair where burst occurs at the anastomotic line.

Adhesion Scoring

Figure 4 shows the summed results if adhesion scoring for the two clinical evaluators. Excellent agreement was seen between scores between evaluators. Adhesion scoring shows a median value of 1 for suture repair compared to a score of 3 for photosealing, 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. Adhesions and more extensive scar at the anastomosis is potentially strengthening the repair site for the photosealed repair.

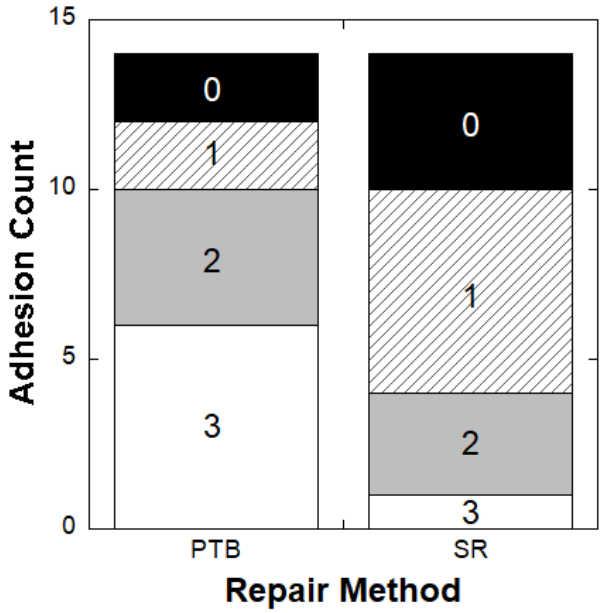

Figure 4: Distribution of adhesion scores at post-operative day 28. SR, suture repair; PTB, suture repair with xHAM wrap sealed with photochemical bonding.

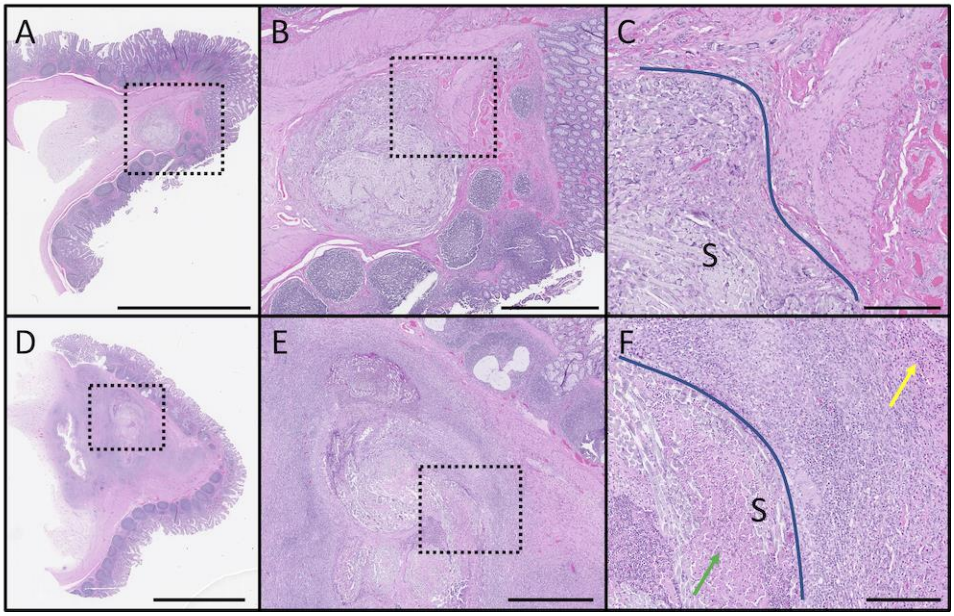

Figure 5: Suture repair vs. PTB histology demonstrating increased inflammation for PTB. A-C. Progressive magnifications of histology of SR case showing a modest rim of foreign body reaction and granulation tissue around the suture (S). Note, there is little inflammation to the right of the suture boundary (blue line in C). D-E. Progressive magnifications of histology of PTB case showing extensive rim of diffuse inflammatory reaction surrounding the suture, showing distinctive features including caseous necrosis (green arrow) and an eosinophilic infiltrate (yellow arrow). The inflammatory reaction extends far beyond the suture boundary (blue line in F) in the PTB case. Scale bars A, D – 5 mm; B, E – 1 mm, C, F – 250 µm.

Histological Analysis

Sections were prepared and analyzed by Dr. Gary Tearney, an expert pathologist at MGH and the Wellman Center for Photomedicine. The small bowel was opened up longitudinally and then sections taken through the anastomotic line and stained with H&E or trichrome. Slides in Figure 5 are oriented with the luminal surface to the right and the external surface to the left in each slide. Comparison of sutured repair (A,B,C) and PTB-wrapped repair (D,E,F) slides is quite striking. Suture material can be seen in both repairs but the major difference is a thicker band of inflammatory tissue seen in the PTB repair. This corresponds to the thicker band of tissue that is seen on gross observation of the PTB

repairs that acts as a bolster to the anastomosis and significantly increases the burst pressure compared to sutured repair. We are currently investigating the cause for the inflammatory response to the crosslinked amnion as this was not observed in prior experiments in rodents using uncrosslinked amnion. Potentially, the crosslinking generates a material that elicits a foreign body response to the amnion. Irrespective of the cause, the ultimate effect is to bolster the anastomotic line.

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

This project has provided an excellent surgical training opportunity for Benjamin Scott MD, who had completed three years of General Surgery residency in the program at Beth Israel Deaconess (BID) Medical Center, and now 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 100 hours of hands-on surgical experience in the course of these studies to date. In addition, he has been mentored as an academic clinician with full exposure to critical analysis, evaluation and presentation of his work through oral presentations, posters and academic publication. He has received excellent clinical training but, equally importantly, he now has the grounding for a career as an academic clinician.

How were the results disseminated to communities of interest?

Manuscript entitled "Light-Activated Photosealing with Human Amniotic Membrane Strengthens Bowel Anastomosis in a Hypotensive, Trauma-Relevant Swine Model" accepted for publication in *Lasers in Surgery and Medicine*.

"Light-Activated Reinforced Repair of Penetrating Small Bowel Injury in a Military-Relevant Hypotensive Swine Model" (MHSRS-20-02086) accepted for oral presentation at the annual Military Health Systems Research Symposium, Kissimee FL, August 2021.

"Light-Activated Photosealing Strengthens Bowel Anastomosis in a Military-Relevant Hypotensive Swine Model" (MHSRS-21-03087) accepted for poster presentation at the annual Military Health Systems Research Symposium, Kissimee FL, August 2021.

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

The remaining tasks for this project include further data evaluation, looking into more detailed analysis of shock parameters, establishing the basis of increased adhesions using the crosslinked human amnion and planning for initial human studies in targeted patient populations at high risk for anastomotic bowel leak.

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.

Augmentation of intestinal anastomosis with photosealing has a wide range of clinical applications in gastrointestinal surgery. While it was found to strengthen the enteroenterostomy of swine in a trauma setting in this study, trauma is far from the only setting for which this approach has potential applications in surgery. It has the potential to augment the anastomosis and prevent anastomotic leak in other high-risk patients, such as malnourished patients, those with malignancy, and those with inflammatory bowel disease. Additionally, with increased confidence in the strength of the anastomosis the surgeon may feel confident foregoing a diverting ostomy, thus reducing operative time at the initial procedure as well as a potential return to the operating room for ostomy reversal. The simplicity of the technique also makes it attractive as no specialized training is required to learn to wrap the anastomosis with amnion and illuminate.

In conclusion, photosealing of crosslinked human amniotic membrane to augment a mechanical intestinal anastomosis is a quick and simple process that increases the strength of the anastomosis of swine in a trauma hemorrhage model. While efficacious in strengthening intestinal anastomosis, this technology has a wide variety of potential applications that include any tissue susceptible to traumatic injury, such as the esophagus, trachea, vascular structures, and ureters. Photochemical tissue bonding has the potential to become widely used clinically due to the technical ease and variety of applications of use. These promising results in strengthening the intestinal anastomosis of swine in a trauma hemorrhage model validate previous rodent studies and suggest this technology has clinical relevance in humans.

5. Changes/Problems:

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 10/15/2021 to allow the project to be completed.

6. Products:

Publication

Light-Activated Photosealing With Human Amniotic Membrane Strengthens Bowel Anastomosis in a Hypotensive, Trauma-Relevant Swine Model. Scott BB, Wang Y, Wu RC, Randolph MA, Redmond RW. 2021, *Lasers Surg Med*. *In press.*

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).

Poster presentation (accepted)

Light-Activated Photosealing Strengthens Bowel Anastomosis in a Military-Relevant Hypotensive Swine Model. Scott BB, Wang Y, Wu RC, Randolph MA, Redmond RW. Annual Military Health Systems Research Symposium (MHSRS), August 2021, Kissimee Florida, (#MHSRS-21-03087).

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