REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
08-04-2016	Final Report	1-Aug-2014 - 31-Jul-2015

4. TITLE AND SUBTITLE
Final Report: Tensiometer for bandage-wound adhesion studies

5a. CONTRACT NUMBER
W911NF-14-1-0430

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611103

6. AUTHORS
Mark A Carlson

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Nebraska Medical Center
Sponsored Programs Administrat
987835 Nebraska Medical Center
Omaha, NE 68198 -7835

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
65184-LS-RIP.1

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
This 2013 DURIP proposal is for the purchase of an Instron model 5943 tensiometer (system price = $58,020). This instrument will support research in an active DoD project, “Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound” (award number: W81XWH-11-1-0836; active 2011-2015). The intent of this project is to study hemostasis of severe exsanguinating injuries. The data obtained with the tensiometer will facilitate the design of a fibrin sealant bandage to treat hemorrhage from solid organ injury in a coagulopathic subject, thus allowing the PI to fulfill the Statement of Work for the funded DoD project. In addition, the PI has some planned work on

15. SUBJECT TERMS
hemostasis, hemorrhage, bandage, liver, adhesion

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Mark Carlson
UU	UU	UU	UU		19b. TELEPHONE NUMBER 402-995-5371

Report Title

Final Report: Tensiometer for bandage-wound adhesion studies

ABSTRACT

This 2013 DURIP proposal is for the purchase of an Instron model 5943 tensiometer (system price = $58,020). This instrument will support research in an active DoD project, “Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound” (award number: W81XWH-11-1-0836; active 2011-2015). The intent of this project is to study hemostasis of severe exsanguinating injuries. The data obtained with the tensiometer will facilitate the design of a fibrin sealant bandage to treat hemorrhage from solid organ injury in a coagulopathic subject, thus allowing the PI to fulfill the Statement of Work for the funded DoD project. In addition, the PI has some planned work on resorbable hernia mesh which will be the basis of a future DoD submission. In addition, the work enabled as a result of this DURIP would promote education of trainees in research areas (biomaterials, biologic-synthetic surface integration) that are of interest to the DoD.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

see attachment

Technology Transfer

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 Dec 2015	Final	1 Aug 2014 – 31 Jul 2015

4. TITLE AND SUBTITLE

Tensiometer for bandage-wound adhesion studies

5a. CONTRACT NUMBER
W911NF-14-1-0430

5b. GRANT NUMBER
65184-LS-RIP

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Carlson, Mark A.

E-Mail: macarlso@unmc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Nebraska
98735 Nebraska Medical Center
Omaha, NE 68198-6810

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This 2013 DURIP proposal is for the purchase of an Instron model 5943 tensiometer. This instrument will support research in an active DoD project, "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound" (award number: W81XWH-11-1-0836; active 2011-2015). The intent of this project is to study hemostasis of severe exsanguinating injuries. The data obtained with the tensiometer will facilitate the design of a fibrin sealant bandage to treat hemorrhage from solid organ injury in a coagulopathic subject, thus allowing the PI to fulfill the Statement of Work for the funded DoD project. In addition, the PI has some planned work on resorbable hernia mesh which will be the basis of a future DoD submission. In addition, the work enabled as a result of this DURIP would promote education of trainees in research areas (biomaterials, biologic-synthetic surface integration) that are of interest to the DoD.

15. SUBJECT TERMS
Trauma, hemorrhage, hemostasis, exsanguination, coagulopathy, hemodilution, liver injury, biologics, recombinant, clotting factors, fibrinogen, biomaterial, bandage, wound, adhesion

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE		44	19b. TELEPHONE NUMBER *(include area code)*

SF298 Continuation Page

Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

Reportable Outcomes

1. Papers published in peer-reviewed journals: none related to this award.

2. Papers published in non-peer-reviewed journals: none related to this award.

3. Presentations:

   (i) Presentations at meetings, but not published in Conference Proceedings: one; see Appendix A.

   (ii) Non-Peer-Reviewed Conference Proceeding publications (other than abstracts): none related to this award.

   (iii) Peer-Reviewed Conference Proceeding publications (other than abstracts): none related to this award.

4. Manuscripts: none related to this award.

5. Books: none related to this award.

6. Honor and Awards: none related to this award.

7. Title of Patents Disclosed during the reporting period: none related to this award.

8. Patents Awarded during the reporting period: none related to this award.

Final Report
Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

Table of Contents

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

Form Approved
OMB No. 9000-0095
Expires Jan 31, 2008

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE ABOVE ORGANIZATION. RETURN COMPLETED FORM TO THE CONTRACTING OFFICER.

1.a. NAME OF CONTRACTOR/SUBCONTRACTOR	c. CONTRACT NUMBER	2.a. NAME OF GOVERNMENT PRIME CONTRACTOR	c. CONTRACT NUMBER	3. TYPE OF REPORT *(X one)*
University of Nebraska Medical Center	W911NF-14-1-0430	same	same	a. INTERIM [ ] b. FINAL [X]
b. ADDRESS *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**b. ADDRESS** *(Include ZIP Code)*	**d. AWARD DATE** *(YYYYMMDD)*	**4. REPORTING PERIOD** *(YYYYMMDD)*
987835 Nebraska Medical Center Omaha NE68198-7835	20140801	same	20140801	a. FROM 20140801 b. TO 20150731

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME(S) OF INVENTOR(S) *(Last, First, Middle Initial)* a.	TITLE OF INVENTION(S) b.	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER c.	ELECTION TO FILE PATENT APPLICATIONS *(X)* d.				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER *(X)* e.	
			(1) UNITED STATES		(2) FOREIGN			
			(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
Mark Carlson	None							

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(2) (a) NAME OF INVENTOR *(Last, First, Middle Initial)*	(1) TITLE OF INVENTION	(2) FOREIGN COUNTRIES OF PATENT APPLICATION
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER		
(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*	(c) ADDRESS OF EMPLOYER *(Include ZIP Code)*		

SECTION II - SUBCONTRACTS *(Containing a "Patent Rights" clause)*

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR *(If "None," so state)*

NAME OF SUBCONTRACTOR(S) a.	ADDRESS *(Include ZIP Code)* b.	SUBCONTRACT NUMBER(S) c.	FAR "PATENT RIGHTS" d.		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S) e.	SUBCONTRACT DATES *(YYYYMMDD)* f.	
			(1) CLAUSE NUMBER	(2) DATE *(YYYYMM)*		(1) AWARD	(2) ESTIMATED COMPLETION
None							

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR *(Not required if: (X as appropriate))* [ ] **SMALL BUSINESS or** [ ] **NONPROFIT ORGANIZATION**

I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL *(Last, First, Middle Initial)*	b. TITLE	c. SIGNATURE	d. DATE SIGNED
Vetter, Deborah K	Director, Sponsored Programs Administration	Deborah K. Vetter	10/07/15

Final Report
Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

List of Appendices

A. Figure of liver peel test.

B. Description of ex vivo adhesion assay (liver peel test).

C. Abstract accepted for presentation at the 2016 Academic Surgical Congress (Jacksonville, FL; 2-4 Feb 2016)

D. Manuscript in preparation for *PLOS ONE*

Final Report
Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

Statement of Problem Studied

Acquisition of this instrument described in this proposal allowed the project's PI (Mark A. Carlson) to study adhesion phenomena at the bandage-wound interface. The acquired data will facilitate the design of a fibrin sealant bandage to treat hemorrhage from solid organ injury in a coagulopathic warfighter, which is one of the objectives in the PI's ongoing project with the DoD ("Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound;" contract no. W81XWH-11-1-0836). In addition, the PI is developing a resorbable hernia mesh which will be the basis of a future DoD submission; this type of hernia mesh is intended for the treatment of the difficult open abdomen, such as can occur in the severely injured warfighter. The tensiometer described in this proposal is facilitating the design and iteration of this hernia mesh. In addition, the work enabled as a result of this DURIP has been and will continue to foster the education of trainees in research areas that are of interest to the DoD.

Final Report
Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

Result Summary

I. Bandage Adhesion

The study of adhesion at the bandage-wound interface first required the development of an *ex vivo* assay to measure this adhesion. The organ of interest was the porcine liver. Initial studies were directed at documenting the tensile strength of *ex vivo* strips of porcine liver tissue using the tensiometer acquired with the DURIP award. These liver strips would be of the type to be used in the *ex vivo* adhesion assay. The resultant data are shown in Table 1.

Table 1. Tensile strength of fresh liver strips (5 x 1 x 0.5 cm) harvested from 35 kg swine.

Liver Region	Orientation	Number of Specimens	Average of Tensile Stress at Max Load (N/mm^2)	StdDev of Tensile Stress at Max Load (N/mm^2)
Left lateral lobe	X-axis	16	0.0951	0.0419
Left lateral lobe	Y-axis	16	0.1243	0.0519
Left medial lobe	X-axis	17	0.0657	0.0174
Left medial lobe	Y-axis	16	0.0870	0.0453
Right lateral lobe	X-axis	15	0.0748	0.0352
Right lateral lobe	Y-axis	15	0.0778	0.0305
Right medial lobe	X-axis	16	0.0812	0.0194
Right medial lobe	Y-axis	16	0.0973	0.0383

After the inherent tensile strength of the porcine liver was documented, a "peel-test" of synthetic bandage adhesion to porcine liver (using fibrin glue at the bandage-tissue interface) then was evolved. The basic schema of this test is shown in Appendix A; the final assay details that derived from extensive preliminary testing are described in Appendix B.

With the ex vivo assay of bandage adhesion to liver well established, some preliminary data was generated on the optimization of fibrin glue used at the interface of the synthetic bandage and the liver. It was determined that addition of Factor XIII to the fibrin glue augmented adhesion strength (see Appendix C). Studies in this area are ongoing in the PI's lab.

II. Hernia Mesh

In order to place design of a novel synthetic resorbable hernia mesh into context with currently commercially-available hernia meshes, several mesh products were purchased and underwent tensile testing with the tensiometer acquired with the DURIP award. These data are shown in Table 2.

Table 2. Tensile testing of select commercial hernia meshes.

Material	Alignment	Average Tensile Stress (N/mm^2)	StdDev of Tensile Stress (N/mm^2)
Phasix	Long rect hori	1.50	0.21
Phasix	Long rect vert	7.24	0.91
ProLite Ultra	Long rect hori	8.17	1.01
ProLite Ultra	Long rect vert	9.22	1.08
UltraPro	Blue line hori	0.81	0.21
UltraPro	Blue line vert	6.95	0.65

The data obtained in Table 2 are being utilized in the design and development of a synthetic resorbable hernia mesh for use in the difficult abdomen. Studies are ongoing in the PI's laboratory, and future extramural funding proposals for this work are in the planning stage.

III. Synthetic Resorbable Bandage For Minor Hemorrhage

In addition to the above studies, the tensiometer acquired with the DURIP award also has been used in the design and development of a synthetic resorbable bandage intended for the treatment of minor hemorrhage incurred during elective surgical procedures. These studies are completed, and a manuscript describing the results is under preparation (Appendix D); the target journal is *PLOS ONE*.

IV. Reportable Outcomes

1. Papers published in peer-reviewed journals: none related to this award.

2. Papers published in non-peer-reviewed journals: none related to this award.

3. Presentations:

   (i) Presentations at meetings, but not published in Conference Proceedings: one; see Appendix A.

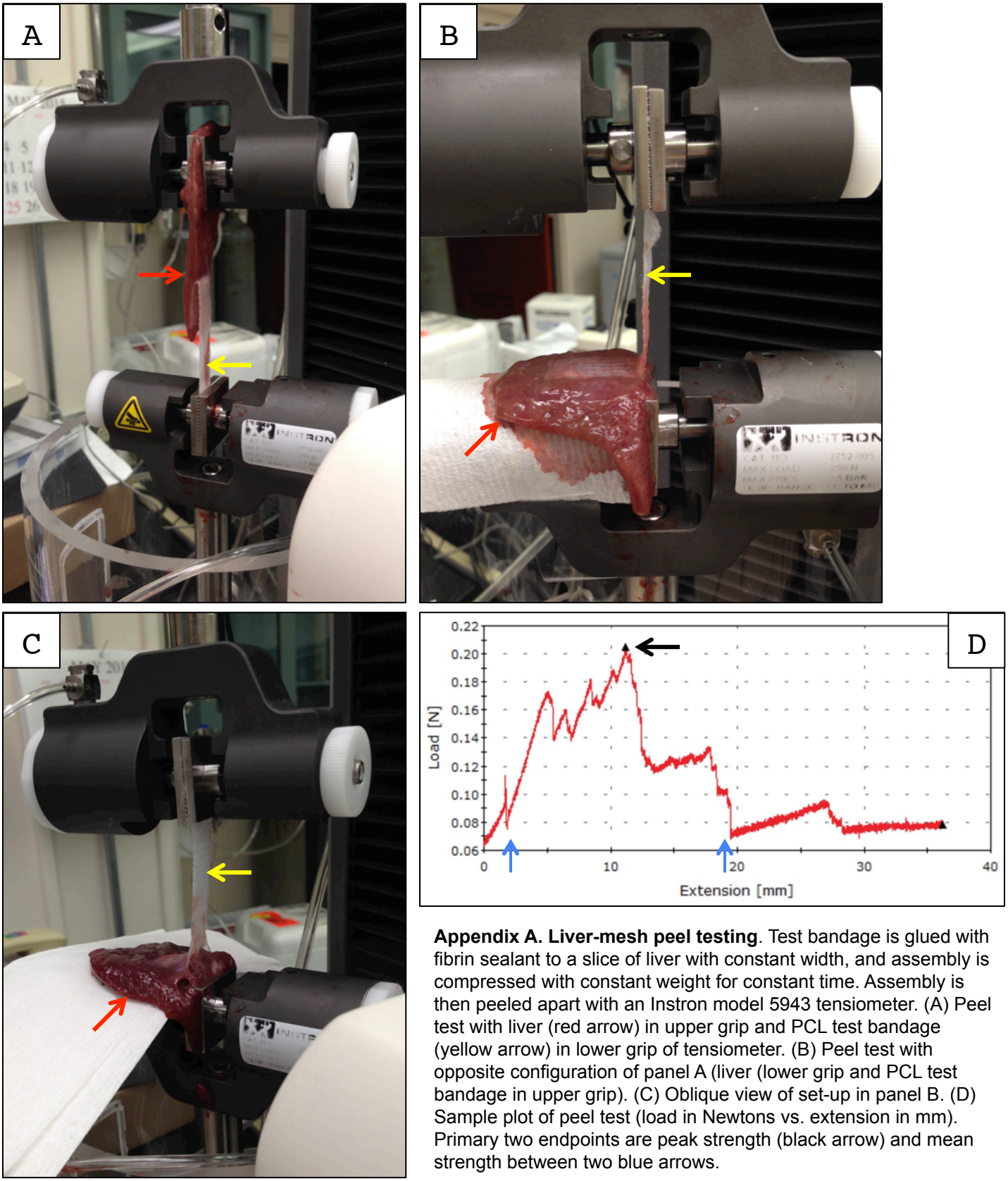
   (ii) Non-Peer-Reviewed Conference Proceeding publications (other than abstracts): none related to this award.

   (iii) Peer-Reviewed Conference Proceeding publications (other than abstracts): none related to this award.

4. Manuscripts: none related to this award.
5. Books: none related to this award.
6. Honor and Awards: none related to this award.
7. Title of Patents Disclosed during the reporting period: none related to this award.
8. Patents Awarded during the reporting period: none related to this award.

Final Report
Contract no. W911NF-14-1-0430
Proposal no. 65184-LS-RIP
Proposal title: Tensiometer for bandage-wound adhesion studies

Bibliography: see individual Appendices



Appendix A. Liver-mesh peel testing. Test bandage is glued with fibrin sealant to a slice of liver with constant width, and assembly is compressed with constant weight for constant time. Assembly is then peeled apart with an Instron model 5943 tensiometer. (A) Peel test with liver (red arrow) in upper grip and PCL test bandage (yellow arrow) in lower grip of tensiometer. (B) Peel test with opposite configuration of panel A (liver (lower grip and PCL test bandage in upper grip). (C) Oblique view of set-up in panel B. (D) Sample plot of peel test (load in Newtons vs. extension in mm). Primary two endpoints are peak strength (black arrow) and mean strength between two blue arrows.

Appendix B: Details of Peel Test (*ex vivo* Adhesion Assay)

Constant parameters:

- 2 x 1 cm overlap of PCL and porcine liver cross section
- All PCL samples will be folded in three to produce 3 layers of PCL
- All liver cross sections will be cut in the "x-axis", as shown in the attached liver orientation image
- 0.5 mL tissue sealant applied to overlap for each test (equivalent to ~2.5 mm sealant layer, soaked into the bandage and attached to liver)
- Prior to testing, compression (180 grams) for 5 minutes using a warm (37°C) surface
- Porcine liver placed on upper Instron grip and PCL bandage placed on lower grip
- Instron pneumatic grips set to 40 psi, extending at 8 mm/min

Optimal composition of sealant, according to thromboelastography:

- Plasma derived fibrinogen (pdFI) = 9 mg/ml
- Recombinant factor FXIIIa (rFXIIIa) = 0.36 mg/ml
- Recombinant thrombin (rFIIa) = 105.6 U/ml
- $CaCl_2$ = 12 mM
- Ringers solution (155 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4)

Measurements:

- Final length and width of overlap on grips just prior to testing
- Average force will be defined from initial peak (tagged automatically by Instron) to end of test
- Reporting will be in N/cm, based on average force divided by measured width of each sample

Variables

	Left Medial	Left Lateral	Right Medial	Right Lateral
Optimal solution	n=2	n=2	n=2	n=2
1.41x pdFI, 1x other	n=2	n=2	n=2	n=2
1.11x rFXIIIa, 1x other	n=2	n=2	n=2	n=2
1.34x rFIIa, 1x other	n=2	n=2	n=2	n=2

Subject: 2016 ASC Abstract Submitted:ASC20161426

Date: Monday, August 10, 2015 at 3:48:28 PM Central Daylight Time

From: abstracts@abstractdashboard.com <abstracts@abstractdashboard.com>

To: Zhou, Daniel J <daniel.zhou@unmc.edu>, rspretz@yahoo.com <rspretz@yahoo.com>, Larsen, Gustavo <glarsen1@unl.edu>, Velander, William H <wvelander2@unl.edu>, Carlson, Mark A <macarlso@unmc.edu>

Academic Surgical Congress
11th Annual
Abstract Submission

Abstract Submission Confirmation

Abstract ID: ASC20161426

Effect of Factor XIII in an ex vivo assay of hemostatic bandage adhesion

D. J. Zhou[1,2] , R. Spretz[1,3] , G. Larsen[3,4] , W. H. Velander[4] , M. A. Carlson[1,2] ; [1]University Of Nebraska Medical Center, Department Of Surgery, Omaha,NE,USA ; [2]VA Nebraska-Western Iowa Healthcare System, Omaha,NE,USA ; [3]LNK Chemsolutions, LLC, Lincoln,NE,USA ; [4]University Of Nebraska-Lincoln, Department Of Chemical & Biomolecular Engineering, Lincoln,NE,USA

Introduction:
Bandage adhesion to bleeding tissue in the setting of traumatic coagulopathy can be improved with fibrin glue (FG). The objective of this study was first to establish an ex vivo assay of bandage adhesion to liver and then to test our hypothesis that use of FG containing recombinant Factor XIII (rFXIII) would improve the adhesion strength (AS) of bandage glued to liver compared to FG without rFXIII.

Methods:
Customized FG (0.2 mL; 9 mg/mL plasma-derived fibrinogen, pdFI, + 106 U/mL r-thrombin + 0.36 mg/mL rFXIII) or commercial FG (0.2 mL Tisseel; Baxter), an FG that contains ~75 mg/mL pdFI and only trace pdFXIII, was applied to a 1×2 cm interface between custom electrospun polycaprolactone (PCL) mesh and a fresh porcine liver strip, and the interface was compressed with a 170 g weight for 5 min at 37°C (default setup). A T-peel adhesion test was performed with an Instron 5943 tensiometer with a 10 N load cell. Force vs. displacement data were used to calculate AS (N/cm), defined as average force during the peel divided by the interface width. AS data were compared with ANOVA ($\alpha<0.05$) and unpaired t-tests ($p<0.05$).

Results:
Using the default setup, AS of custom FG was ~2-fold greater by gluing the PCL mesh to the capsular surface of the liver vs. raw parenchyma (Fig. 1A), so use of the liver capsule was incorporated into the default setup. Neither capsular surface wetness (patted dry vs. pre-wet with PBS) nor prolonged compression time (5 vs. 10 min) affected AS (Fig. 1B). There appeared to be decreased AS with lower temperature during compression (25 vs. 37˚C), but this was not significant (Fig. 1B). Decreasing FG volume by 50% (0.05 vs. 0.1 mL/cm^2) resulted in a lower AS (Fig. 1B). Increasing FG volume beyond 0.1 mL/cm^2 was ineffective secondary to glue spillage during compression. Removing rFXIII from the default setup decreased AS by ~50%, but doubling the [rFXIII] did not increase AS (Fig. 1C). AS of customized FG vs. commercial FG was not different (Fig. 1C).

Conclusion:
An ex vivo adhesion test of synthetic resorbable mesh applied to porcine liver with customized FG was optimized with respect to liver surface qualities, adhesion compression time and temperature, and FG quantity. AS was augmented by rFXIII in the FG. The customized FG produced AS similar to that of commercial FG, despite the former having only ~1/8 the pdFI. The AS equivalence between these two FGs likely was a result of the added rFXIII to the customized FG, suggesting that efficacy testing of rFXIII addition to biologic hemostatic devices may be warranted.

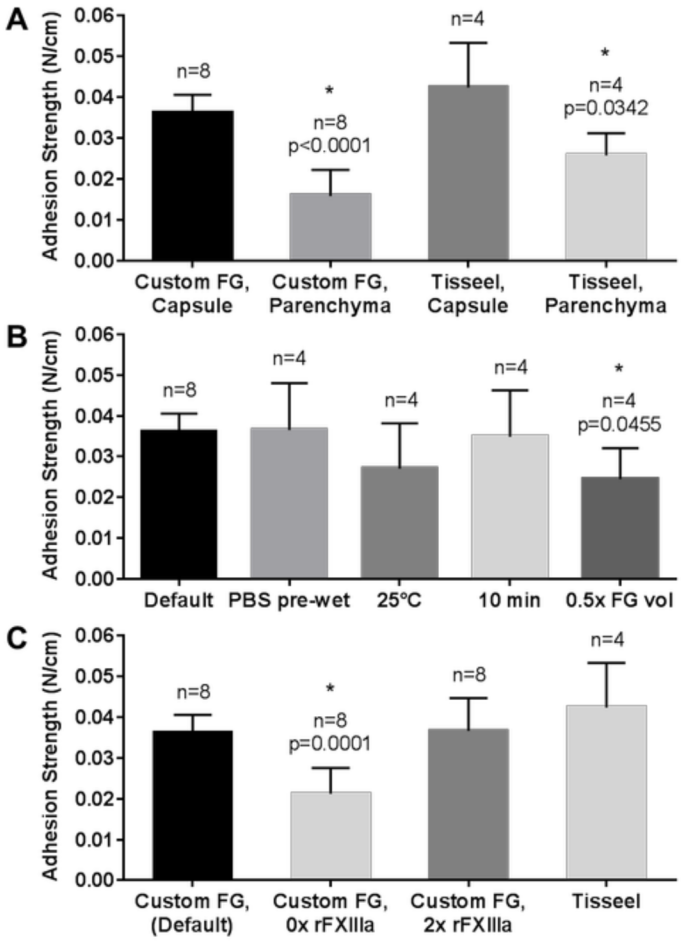


Fig. 1. AS data. *p < 0.05. Customized FG and Tisseel groups in A paired separately; groups in B compared pair-wise with Default; groups in C compared together via ANOVA.

Title

[250 character limit for title; current = 142, with spaces]

Comparison of a Synthetic Resorbable Bandage vs. Oxidized Regenerated Cellulose for Treatment of Minor Surgical Hemorrhage in a Porcine Model

Short Title

[50 character limit for short title; current = 40, with spaces]

Bandage Comparison for Minor Hemorrhage

Ujwal R. Yanala[1,2], Sandra Noriega[3], Ruben Spretz[3], Jorge Ragusa[3], Yuri M. Sheinin[4], Daniel J. Zhou[1,2], Luiz Nuñez[3], Gustavo Larsen[3,5], Mark A. Carlson[1,2,6*]

[1]Department of Surgery, University of Nebraska Medical Center, Omaha, Nebraska, United States of America; [2]Department of Surgery, VA Nebraska–Western Iowa Health Care System, Omaha, Nebraska, United States of America; [3]LNK Chemsolutions LLC, Lincoln, Nebraska, United States of America; [4]Department of Pathology, University of Nebraska Medical Center, Omaha, Nebraska, United States of America; [5]Department of Chemical and Biomolecular Engineering, University of Nebraska–Lincoln, Lincoln, Nebraska, United States of America; [6]Department of Genetics, Cell Biology and Anatomy, University of Nebraska Medical Center, Omaha, Nebraska, United States of America.

[*]Corresponding author

Email: macarlso@unmc.edu (MAC)

Abstract [Unstructured, 300 word limit; current = 314]

Commercially-available topical hemostats for minor hemorrhage incurred during elective surgical procedures are relatively expensive. We believe that more economical synthetic hemostats could be produced. Our objective here was to compare the efficacy and toxicity of a synthetic resorbable hemostatic bandage *vs*. an analogous commercial product in a porcine model of minor hemorrhage. For the nonsurvival efficacy study, anesthetized domestic swine (boars, 3 months, 29-40 kg) underwent arterial/venous line placement and splenectomy. A 1 x 8 cm section of liver was resected from the edge of the left lateral lobe, and test bandage (macroporous polycaprolactone mesh, PCL; N = 10) or oxidized regenerated cellulose (ORC; Surgicel®, Ethicon®; N = 10) was applied with manual pressure for 5 minutes. Resuscitation then was performed with warm LR (target MAP = 80% of preinjury), and blood loss was measured 60 min after injury. For the survival toxicity study, a similar resection technique was employed (N = 6 for each material), and necropsy was performed at 30 days to evaluate for bandage toxicity (subject growth, serum chemistry, histology). Pre-injury weight, VS, and laboratory testing did not differ among groups. Resection mortality was zero. In the efficacy study, there were no differences between the PCL vs. ORC groups in blood loss or other post-injury variables (Table), except that the resuscitation fluid volume in the ORC group was greater. Other results from the efficacy study not shown in the Table include platelet counts and coagulation testing (no significant differences). Other than minor granuloma formation at the implantation site with both PCL and ORC, the survival study did not reveal any measurable toxicity. The efficacy and toxicity of the PCL test bandage vs. the ORC comparator were not different in a porcine model of minor hepatic hemorrhage. Based on projected costs of production (not shown), the PCL bandage could represent a lower-cost alternative to ORC for the treatment of minor surgical bleeding.

Introduction

Materials and Methods

2.1. Animal Welfare

This animal research study was carried out in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (8th ed.) from the National Research Council and the National Institutes of Health {Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011 #38}, and also in accordance with the Animal Welfare Act of the United States (U.S. Code 7, Sections 2131 – 2159) {United States Department of Agriculture, 2013 #39}. The animal protocol was approved by the Institutional Animal Care and Use Committee of the VA Nebraska-Western Iowa Health Care System (protocol number 00760), by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center (protocol number 11-064-07-ET), and by the Animal Care and Use Review Office of the United States Army Medical Research and Materiel Command (award number W81XWH-11-1-0836). All procedures were performed in animal facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC; www.aaalac.org) and by the Office of Laboratory Animal Welfare of the Public Health Service (http://grants.nih.gov/grants/olaw/olaw.htm). All surgical procedures were performed under isoflurane anesthesia, and all efforts were made to minimize suffering. Euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals {American Veterinary Medical Association Panel on Euthanasia, 2013 #40}.

2.2. Determination of Subject Numbers

The minimum number of swine (n = 12) utilized in each group was determined with a statistical power analysis {Neter, 1990 #41} using Δ/σ (Cohen's *d*, in which Δ is the desired difference in means of

numerical data set by the observer, and σ is the estimated standard deviation) = 2.0, false positive rate (α) = 0.05, false negative rate (β) = 0.2, and power (1 – β) = 0.8. The endpoints targeted for the power analysis were blood loss and final hemoglobin level.

2.3. Animal Preparation

Domestic swine (castrated males, 3 months) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska–Lincoln and acclimatized for 3-4 days under veterinary supervision. Subjects were fed ad lib with corn-soybean meal and had access to water. Prior to surgery, subjects were fasted for 12 hours, but with no water restriction. On the day of procedure, animals were premedicated {Carlson, 2014 #42} with a single 3 mL IM injection containing 150 mg Telazol® (tiletamine hydrochloride and zolazepam hydrochloride, 1:1 by weight; Fort Dodge Animal Health, New York, NY), 90 mg ketamine, and 90 mg xylazine.

Sedated subjects then were weighed, intravenous access was established via an ear vein, endotracheal intubation was performed, and anesthesia was maintained with 0.5-1.5% isoflurane throughout the procedure using a Matrx™ Model 3000 Veterinary Anesthesia Ventilator (Midmark Corp., Versailles, OH). Central arterial and venous lines were placed through a cutdown in the right neck for pressure monitoring, blood sampling and fluid resuscitation. MAP (mean arterial pressure), end-tidal pCO_2, rectal temperature, cardiac electrical activity, and pulse oximetry were continuously recorded. Mechanical ventilation was maintained at 12-15 breaths per minute, with a tidal volume of 10-15 mL/kg, in order to keep the end-tidal pCO_2 at 30-40 mm Hg. A heating pad was placed under each subject to support body temperature.

Subject preparation followed a previous descriptions {Yanala, 2014 #35}. A ventral midline laparotomy incision then was made, splenectomy was performed, a transabdominal cystostomy tube was

placed. The excised spleen was weighed, and then a volume of warm Lactated Ringers (LR; 37˚C) solution equivalent to three-fold the splenic weight was administered through the jugular line, using a rapid infusion pump (Cole-Palmer Masterflex L/S; Vernon Hills, IL) set at 150 mL/min. Prior to injury, any blood loss incurred during the preparation was quantified by weighing tared surgical sponges that were used to absorb lost blood, and then a volume of LR equivalent to three-fold the pre-injury blood loss (typically <50 mL) was given using the infusion pump.

2.4. Injury Mechanism and Resuscitation

Pre-injury vital signs were recorded, the lower half of the midline incision was closed with towel clips, and then the injury mechanism (hepatic left lower lobe hemitransection) was applied, as previously described {Yanala, 2014 #35}. In brief, liver injury was performed by making a 4 cm cut across the base of the left lateral lobe of the liver. Immediately after injury, the laparotomy incision was closed with towel clips. All the subjects were allowed to bleed without any efforts for local hemostasis (compression, bandage, vessel clamping, etc.). All injuries were intended to have no intraabdominal treatment models in order to depict the course of hemorrhage in a battlefield from an uncontrolled noncompressible truncal injury.

At 60 s after injury, Lactated Ringers (LR) solution (stored at 37˚C) was begun at either 150 or 20 mL/min IV (rapid and slow group, respectively, N = 12 per group) using the infusion pump. The maximum volume post-injury LR resuscitation volume was capped at 100 mL/kg; this limit (equivalent to 7 L fluid in a 70 kg person) was empirically chosen, based on the assumption that most patients in hemorrhagic shock eventually will receive blood products, as opposed to unlimited crystalloid resuscitation. The resuscitation goal (i.e., target MAP) was defined as 80% of the pre-injury MAP; as

long as the MAP was below this target, resuscitation continued until the animal expired or the 100 mL/kg fluid maximum was reached.

The rapid and slow groups were monitored for 60 or 180 min, respectively, while under general anesthesia, with continuous monitoring of vital signs and periodic blood draws for laboratory testing (see Discussion for explanation of the different post-injury observation periods). If a subject was still alive at the end of observation period, then it was euthanized by transection of the inferior vena cava and intentional exsanguination, while under deep general anesthesia (method approved by the AMVA {American Veterinary Medical Association Panel on Euthanasia, 2013 #40}).

2.5. Endpoints

Heart rate, MAP, pulse oximetry, end-tidal pCO_2, and rectal temperature were continuously recorded, as described above. Blood samples were drawn pre-injury, at 15 min post-injury, and then at 60 min post-injury or death ("final" time point), whichever came first. Death was defined as MAP $\leq$20 mm Hg with no identifiable pressure wave on the monitor's arterial tracing, end-tidal pCO_2 <5 mm Hg, and absent corneal reflex.

Immediately after the planned observation period or after the animal expired (whichever came first), the laparotomy incision was re-opened, and all clots and blood were rapidly evacuated into tared buckets with a combination of tared laparotomy pads, suction, and manual extraction. The buckets were weighed in order to calculate blood loss. Necropsy was performed immediately after expiration; the liver was explanted for inspection, dissection, photography, and documentation of the injury anatomy.

2.6. Laboratory Testing and Statistical Analysis

The CBC, PT/PTT, INR, fibrinogen, and ABG testing were contracted to the Clinical Laboratory of the VA Nebraska-Western Iowa Health Care System. This laboratory used the quantitative fibrinogen assay based on the von Clauss method {Lowe, 2004 #37}. Numerical data was reported as the mean ± standard deviation (SD). Groups of unpaired numerical data were compared with the nonparametric Kruskal-Wallis analysis of variance. Groups of categorical data were compared with the Fisher exact test. Significance was defined as $p < 0.05$. If a data for a given subject at a given time point was empty secondary to the subject's expiration prior to the time point, then that data cell was filled with the lowest value (for MAP, hemoglobin, platelets, and fibrinogen) or highest value (for PT, INR, APTT) recorded among the surviving subjects at that time point.

Results

Discussion

Conclusions

Acknowledgements

This work also was supported in part with resources and the use of facilities at the VA Nebraska-Western Iowa Health Care System. The authors would like to acknowledge the anesthesia expertise of John Cavanaugh, and the technical assistance Chris Hansen, Dean Heimann, and Gerri Siford. Portions of this work were presented at the 8th Annual Academic Surgical Congress (February, 2015, Las Vegas, Nevada, USA).

References

Supporting Information Captions

Figure Legends

Fig. 1. Dissection demonstrating the anatomy of the porcine intrahepatic portal venous system. *Ex vivo* porcine liver, inferior aspect (scale in cm). The soft tissues overlying the portal venous system have been dissected and retracted with silk stay sutures. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; Gb = gallbladder; 1 = cut orifice of main portal vein; 2 = intrahepatic portal vein; 3 = RM lobe portal vein branch; 4 = 1st LL lobe portal vein branch; 5 = cut orifice of 2nd LL lobe portal vein branch (proximal end); 6 = distal end of structure

Table tt01.

Table tt02.

Table TT01. Hemoglobin and platelet count (acute efficacy study).

Material	Hemoglobin (g/dL)		Platelet (1,000/μL)	
	Pre-injury	60 min	Pre-injury	60 min
ORC	12.3 ± 0.8	12.9 ± 0.9	324 ± 88	327 ± 94
PCL	12.2 ± 0.7	12.8 ± 1.2	289 ± 72	266 ± 56
p, unpaired t-test	0.661	0.854	0.363	0.115
p, paired t-test (ORC)	0.099		0.276	
p, paired t-test (PCL)	0.082		0.136	

Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury.

Table TT02. Arterial blood gas data (acute efficacy study).

Material	Term	pH		pCO2 (mm Hg)		HCO3 (mEq/L)		BE (mEq/L)		pO2 (mm Hg)	
		Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	Mean	7.53	7.59	31.6	27.1	26.5	25.5	4.4	4.5	446	429
	SD	0.05	0.06	2.5	4.5	2.2	1.4	2.5	1.6	37	92
PCL	Mean	7.50	7.51	35.4	28.6	26.9	25.7	4.1	4.1	423	448
	SD	0.05	0.18	5.7	4.3	1.3	1.6	1.2	0.8	28	54
p, unpaired t-test		0.1255	0.2117	0.0753	0.4468	0.6076	0.7562	0.7465	0.5361	0.1392	0.5808
p, paired t-test (ORC)		**0.0257**		**0.0129**		0.0846		0.8448		0.4557	
p, paired t-test (PCL)		0.8928		**0.0013**		0.1181		0.9282		0.1347	

BE = Base Excess; SD = standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT03. Protime, INR, APTT, and fibrinogen (acute efficacy study).

	Protime (s)		INR		APTT (s)		QFA (mg/dL)	
Material	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	10.7 ± 0.9	10.9 ± 0.4	1.0 ± 0.1	1 ± 0.0	21.6 ± 4.8	18.7 ± 3.1	113 ± 20	88 ± 22
PCL	10.7 ± 0.5	10.6 ± 0.7	1.0 ± 0.0	1.0 ± 0.1	20.1 ± 2.3	18.2 ± 1.9	112 ± 13	88 ± 15
p, unpaired t-test	0.880	0.292	0.514	0.143	0.419	0.732	0.968	0.972
p, paired t-test (ORC)	0.963		0.681		**0.026**		**<0.001**	
p, paired t-test (PCL)	0.456		0.193		**0.012**		**<0.001**	

INR = International Normalized Ratio; APTT = Activated Partial Thromboplastin Time; QFA = Quantitative Fibrinogen Assay. Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT04. Blood pressure, heart rate, and temperature (acute efficacy study).

	MAP (mm Hg)		HR		Temp (˚C)	
Material	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	116 ± 18	93 ± 11	108 ± 12	101 ± 6	37.6 ± 0.8	36.3 ± 0.5
PCL	115 ± 16	89 ± 8	102 ± 12	102 ± 15	37.5 ± 0.9	36.3 ± 1.3
p, unpaired t-test	0.885	0.298	0.304	0.890	0.834	0.932
p, paired t-test (ORC)	**<0.001**		0.106		**<0.001**	
p, paired t-test (PCL)	**<0.001**		1.000		**0.003**	

MAP = mean arterial pressure; HR = heart rate. Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT05. Weight of subject, liver, spleen, and resection specimen (acute efficacy study).

Material	Subject (kg)	Liver (g)	Spleen (g)	Resection specimen (g)
ORC	36.1 ± 2.5 (33.0 – 40.4)	934 ± 161 (699 – 1,242)	319 ± 55 (235 – 402)	6.7 ± 2.0 (2.9 – 9.5)
PCL	33.9 ± 2.3 (29.4 – 37.0)	939 ± 120 (726 – 1,143)	299 ± 42 (219 – 360)	7.6 ± 1.9 (4.8 – 11.6)
*p**	0.057	0.943	0.387	0.317

Data are mean ± standard deviation, with range in parentheses; *Unpaired t-test (two tailed), comparing ORC *vs*. PCL.

Table TT06. Intravenous fluid and blood loss (acute efficacy study).

Material	IV fluid (mL)		Blood loss (mL)	
	Pre-injury	Post-injury	Pre-injury	Post-injury
ORC	1,229 ± 342 (860 – 1,900)	1,952 ± 1,363 (220 – 3,715)	380 ± 61 (265 – 475)	111 ± 55 (58 – 237)
PCL	1,061 ± 219 (800 – 1,540)	594 ± 425 (110 – 1,180)	374 ± 78 (252 – 532)	93 ± 27 (56 – 140)
**p*, unpaired t-test	0.209	**0.012**	0.870	0.381
p, Kruskal-Wallis		**0.008**		

Data are mean ± standard deviation; *two tailed, comparing ORC *vs*. PCL; **p < 0.05 are bolded**.

Table TT07. Thromboelastography data (acute efficacy study).

Material	R (min)		K (min)		α (degree)		MA (mm)	
	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	3.74 ± 0.91	3.25 ± 0.50	1.99 ± 1.52	1.35 ± 0.48	64.7 ± 15.2	71.2 ± 5.5	60.3 ± 18.0	61.7 ± 11.1
PCL	4.31 ± 0.52	3.85 ± 0.65	1.17 ± 0.18	1.17 ± 0.15	73.2 ± 3.1	72.8 ± 2.2	68.7 ± 4.6	65.2 ± 3.3
p, unpaired t-test								
p, paired t-test (ORC)								
p, paired t-test (PCL)								

R = Reaction time (first evidence of clot formation); K = Achievement of 20 mm clot strength (amplitude); α = alpha angle, a measure of clot formation or kinetics of clot development; MA = Maximal amplitude (maximum clot strength). Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury.

Table TT08. Initial subject weight, resected liver specimen, and implanted bandage; bandage hold time; and procedural blood loss, all on day zero of 30-day toxicity study.

Material	Initial wt (kg)	Resection specimen wt (g)	Bandage wt (g)	Hold time (min)	Blood loss (mL)
ORC	37.9 ± 5.3 (30.6 – 44.8)	2.12 ± 0.75 (1.36 – 3.04)	0.64 ± 0.24 (0.34 – 0.87)	6.3 ± 4.7 (1.0 – 13.0)	8.7 ± 8.3 (1.0 – 24.0)
PCL	35.7 ± 2.5 (33.0 – 38.8)	1.79 ± 0.11 (1.62 – 1.89)	0.30 ± 0.08 (0.18 – 0.38)	5.8 ± 5.0 (1.0 – 15.0)	8.3 ± 5.5 (2.0 – 17.0)
None	34.8 ± 2.2 (31.0 – 37.6)	2.05 ± 0.85 (0.90 – 3.52)	NA	NA	12.8 ± 8.0 (4.2 – 25.3)
*p**	0.333	0.668	**0.016**	0.862	0.522

Data are mean ± standard deviation, with range in parentheses; *ANOVA (one way), comparing ORC *vs*. PCL *vs*. None; **p < 0.05 is bolded.**

Table TT09. Hemoglobin, platelet count, and WBC from the 30-day toxicity study.

Material	Hemoglobin (g/dL)		Platelets (10^3/μL)		WBC (10^3/μL)	
	Pre-Injury	Final[1]	Pre-Injury	Final	Pre-Injury	Final
ORC	11.4 ± 1.0 (10.4 – 12.5)	10.2 ± 0.9 (9.1 – 11.2)	374 ± 89 (282 – 494)	336 ± 29 (300 – 364)	18.9 ± 5.2 (13.1 – 23.9)	14.0 ± 2.7 (10.7 – 17.2)
PCL	11.5 ± 1.4 (9.4 – 13.8)	10.6 ± 1.3 (9.0 – 12.1)	340 ± 125 (161 – 502)	344 ± 132 (91 – 459)	14.5 ± 3.0 (11.7 – 20.1)	16.7 ± 3.0 (14.0 – 21.3)
None	11.8 ± 1.3 (9.8 – 13.2)	11.6 ± 1.1 (10.3 – 13.5)	314 ± 119 (215 – 445)	304 ± 51 (243 – 372)	16.7 ± 3.0 (12.9 – 20.3)	15.5 ± 2.9 (11.1 – 19.5)
p, one-way ANOVA[2]	0.880	0.152	0.743	0.722	0.214	0.396
p, paired t-test (ORC)	NA		0.204		**0.005**	
p, paired t-test (PCL)	**0.013**		0.473		0.039	
p, paired t-test (None)	0.463		0.491		0.171	

Data are mean ± standard deviation, with range in parentheses; [1]Final blood specimen drawn just prior to euthanasia on day 30; [2]comparing ORC *vs*. PCL *vs*. None; **p < 0.05 are bolded.**

Table TT10. Protime, APTT, INR, and QFA from the 30-day toxicity study.

Material	Protime (s)		APTT (s)		INR		QFA (mg/dL)	
	Pre-Injury	Final[1]	Pre-Injury	Final	Pre-Injury	Final	Pre-Injury	Final
ORC	10.3 ± 1.1 (9.0 – 11.6)	9.7 ± 0.4 9.0 – 10.1)	18.1 ± 2.1 (16.6 – 19.5)	17.6 ± 1.7 (16.6 – 19.6)	0.9 ± 0.1 (0.8 – 1.0)	0.9 ± 0.0 (0.8 – 0.9)	97 ± 5 (93 – 104)	93 ± 8 (84 – 102)
PCL	10.7 ± 0.8 (9.5 – 11.5)	9.4 ± 0.7 (8.3 – 9.9)	21.5 ± 0.6 (21.0 – 21.9)	17.2 ± 1.0 (16.6 ± 18.4	1.0 ± 0.1 (0.9 – 1.0)	0.8 ± 0.1 (0.7 – 0.9)	99 ± 27 (64 – 125)	91 ± 14 (74 – 113)
None	10.3 ± 1.0 (8.6 – 11.0)	9.9 ± 0.7 (9.2 – 10.6)	17.8 ± 1.0 (16.6 – 18.4)	16.6 ± NA (16.6 – 16.6)	0.9 ± 0.1 (0.7 – 0.9)	0.8 ± 0.1 (0.8 – 0.9)	113 ± 46 (78 – 190)	110 ± 6 (103 – 114)
p, one-way ANOVA[2]	0.777	0.578	0.968	0.598	0.185	0.565	0.716	**0.006**
p, paired t-test (ORC)	0.270		NA[3]		0.333		0.312	
p, paired t-test (PCL)	**0.040**		NA[3]		**0.035**		0.224	
p, paired t-test (None)	0.076		NA[3]		0.092		0.394	

APTT = Activated Partial Thromboplastin Time; INR = International Normalized Ratio; QFA = Quantitative Fibrinogen Assay. Data are mean ± standard deviation, with range in parentheses. [1]Final blood specimen drawn just prior to euthanasia on day 30; [2]comparing ORC *vs*. PCL *vs*. None; [3]Test not available because of missing data; **p < 0.05 are bolded.**

Table TT11. Selected serum tests from a comprehensive metabolic panel obtained during the 30 day toxicity study.

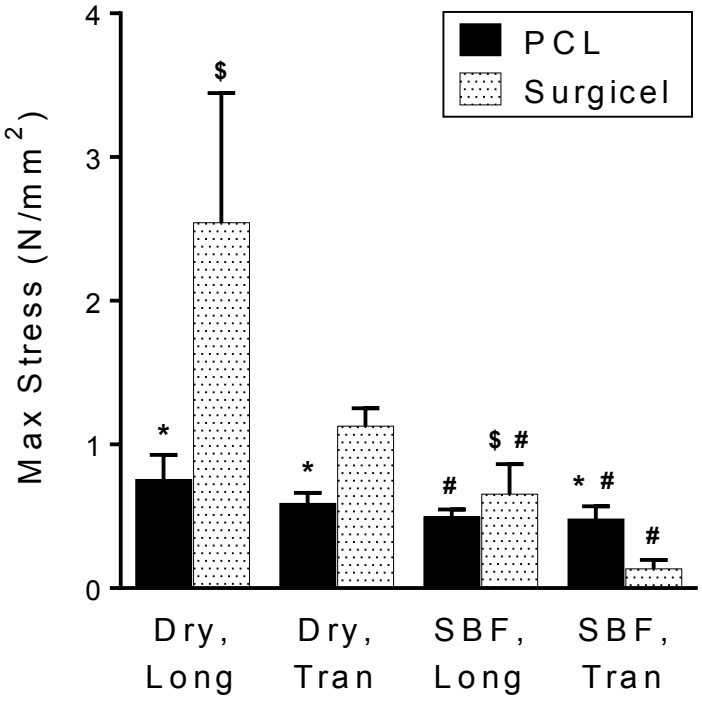
		Gluc (mg/dL)		Creat (mg/dL)		AG (mmol/L)		Ca (mg/dL)		Alb (g/dL)		AlkP (U/L)		AST (U/L)		GGT (U/L)		Amyl (U/L)	
Material	Value	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin
ORC	Mean	112	90	1.2	1.4	11	13	9.7	9.9	3.0	3.0	186	166	32	50	41	48	624	644
ORC	SD	23	15	0.2	0.1	3	2	0.2	0.4	0.3	0.3	40	39	9	14	8	8	120	107
ORC	Min	85	69	0.9	1.3	6	10	9.3	9.2	2.6	2.4	141	122	22	35	30	38	512	543
ORC	Max	152	108	1.5	1.5	14	14	9.8	10.3	3.4	3.3	241	211	47	75	51	59	817	827
ORC	*p*, t-test[1]	**0.003**		**0.034**		0.114		0.095		0.500		**0.005**		**0.020**		0.079		0.109	
PCL	Mean	107	87	1.1	1.3	11	14	10.1	9.9	3.0	3.0	185	161	38	56	24	34	575	696
PCL	SD	18	8	0.2	0.2	2	3	0.7	0.6	0.4	0.3	47	35	17	17	7	15	70	67
PCL	Min	89	72	0.8	1.1	8	9	9.0	9.4	2.4	2.6	134	117	23	41	11	15	482	586
PCL	Max	134	98	1.3	1.6	13	17	10.9	10.9	3.5	3.4	253	195	70	88	30	59	660	774
PCL	*p*, t-test	**0.010**		**0.016**		**0.001**		0.320		0.316		0.068		0.056		0.052		**<0.001**	
None	Mean	109	100	1.2	1.7	13	12	10.3	9.9	2.9	3.1	163	163	37	33	50	46	637	743
None	SD	15	8	0.3	0.4	2	1	0.2	0.2	0.2	0.2	44	44	11	5	23	17	165	166
None	Min	95	90	0.8	1.3	10	10	10.1	9.6	2.6	2.8	134	110	20	28	25	33	444	521
None	Max	127	107	1.5	2.0	15	13	10.5	10.1	3.1	3.3	241	204	48	39	85	74	866	946
None	*p*, t-test	0.122		**0.001**		0.081		**0.016**		0.128		0.414		0.238		0.395		**0.015**	
	p, ANOVA[2]	0.922	0.258	0.708	0.074	0.282	0.300	0.105	0.968	0.808	0.899	0.637	0.971	0.672	0.058	**0.020**	0.212	0.668	0.384

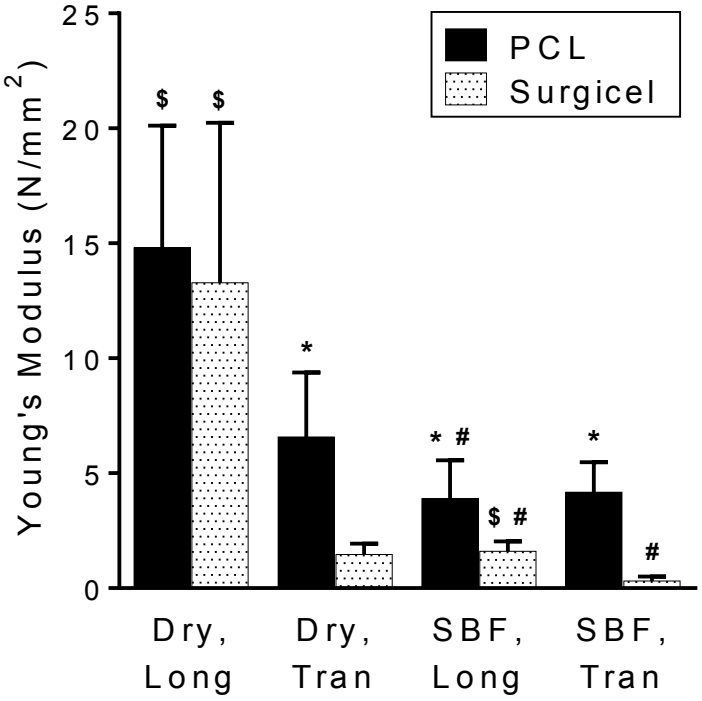
Pre = pre-injury value; Fin = final value (just prior to euthanasia); SD = standard deviation; Min = minimum value; Max = maximum value; Gluc = glucose; creat = creatinine; AG = anion gap; Ca = calcium; Alb = albumin; AlkP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase; Amy = amylase. [1]Paired one tail t-test, comparing pre-injury vs. final; [2]one-way ANOVA, comparing ORC vs. PCL vs. None; **p < 0.05 are bolded**. Note: all total bilirubin values were ≤ 0.2 mg/dL, so these data were not listed here.

Table TT15. Subject weight, 30-day weight gain, and organ weights, all at necropsy from the 30-day toxicity study.

Material	Subject wt (kg)	Wt gain (kg)	Liver (g)	Heart (g)	Lungs (g)	Kidneys (g)	Brain (g)
ORC	50.6 ± 5.6 (44.8 – 59.0)	12.7 ± 2.7 (7.6 – 15.2)	1,178 ± 130 (1,080 – 1,423)	247 ± 21 (219 – 280)	328 ± 22 (295 – 346)	179 ± 21 (158 – 220)	79.3 ± 6.3 (73.0 – 91.0)
PCL	47.8 ± 1.1 (46.0 – 49.0)	12.1 ± 3.0 (7.2 – 15.0)	1,140 ± 70 (1,060 – 1,218)	230 ± 21 (217 – 273)	342 ± 29 (295 – 374)	173 ± 18 (151 – 194)	69.0 ± 11.6 (50.0 – 81.0)
None	43.7 ± 4.0 (38.4 – 47.6)	8.9 ± 3.1 (3.4 – 12.0)	914 ± 117 (776 – 1,067)	204 ± 20 (168 – 223)	297 ± 16 (272 – 313)	129 ± 25 (95 – 161)	69.2 ± 9.1 (57.7 – 81.4)
p, ANOVA (one way)*	**0.029**	0.086	**0.002**	**0.009**	**0.012**	**0.002**	0.121
p, Kruskal-Wallis*	0.051	0.064			**0.034**		0.188

Data are mean ± standard deviation, with range in parentheses; *comparing ORC *vs*. PCL *vs*. None; **p < 0.05 are bolded.**





* p<0.05 for PCL vs. Surgicel.
$ p<0.05 for long vs. tran.
# p<0.05 for SBF vs. dry.