A 6-month Evaluation of Novel Antibiotic Bonded Prosthetic Graft Patency in the Setting of a Polymicrobial Infection in Swine (*Sus scrofa*)

Lt Col Brandon W. Propper, USAF, MC
LTC Zachary M. Arthurs, USA, MC
Lt Col Shaun M. Gifford, USA, MC
Capt Kai W. Hata, USAF, MC
Michael Clemens, USA, MC
Mamie Stuff, USAF, MC
Jennifer Cox, CTR

June 10, 2019

59th Medical Wing
Office of the Chief Scientist
1100 Wilford Hall Loop
Bldg. 4554
JBSA Lackland AFB, TX 78236-7

DECLARATION OF INTEREST

The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of Defense or its Components. This work was funded by Project Code Number DS21RES07. The experiments reported herein were conducted according to the principles set forth in the National Research Council's Guide for the Care and Use of Laboratory Animals (8th ed.), and the Animal Welfare Act of 1966, as amended. Authors are military service members, employees, or contractors of the US Government. This work was prepared as part of their official duties. Title 17 USC §105 provides that 'copyright protection under this title is not available for any work of the US Government.' Title 17 USC §101 defines a US Government work as a work prepared by a military service member, employee, or contractor of the US Government as part of that person's official duties

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

HISTOPATHOLOGICAL CHANGES ASSOCIATED WITH VARIOUS ENDOVASCULAR STENT/GRAFTS USING A PORCINE MODEL (SUS SCROFA) HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

Michele F. Tavish

Michele F. Tavish, DAF
Program Analyst
En route Care Research Program
59MDW Office of the Chief Scientist

Robert T. Gerhardt, MD, MPH, FACEP, FAEMS
Director, Trauma & Clinical Care
59MDW Office of the Chief Scientist

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
10 June 2019	Final Report	Jun 2015 – Sep 2016

4. TITLE AND SUBTITLE

A 6-month Evaluation of Novel Antibiotic Bonded Prosthetic Graft Patency in the Setting of a Polymicrobial Infection in Swine (*Sus scrofa*)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Lt Col Brandon W. Propper, USAF, MC
LTC Zachary M. Arthurs, USA, MC
Lt Col Shaun M. Gifford, USA, MC
Capt Kai W. Hata, USAF, MC
Michael Clemens, USA, MC
Mamie Stuff, USAF, MC
Jennifer Cox, CTR

5d. PROJECT NUMBER
AC16EM03

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
59th MDW/ST
1100 Wilford Hall Loop, Bldg. 4554
JBSA Lackland AFB, TX 78236

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Medical Support Agency (AFMSA)

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT-

This study was a unique in-vivo study that examined the long-term ability of novel antibiotic grafts to resist infection and maintain patency. A 6-month survival model with 5 proposed groups that included sham, a positive control group ePTFE, a positive control group for Dacron, experimental group bonded with ePTFE, and an experimental group bonded with Dacron was developed with technical and veterinary staff; the longest survival model our group has used to date. Due to unforeseen technical issues with animal growth, a number of amendments were necessary yielding an overall decreased number of total animals enrolled. A decision was made to remove the positive control Dacron group and experimental group bonded with Dacron. With such low number of test animals, a definitive comparison of groups could not be reached. However, none of the test animals with antibiotic-bonded vascular grafts (ePTFE) revealed infectious changes or showed evidence of rejection.

15. SUBJECT TERMS-

Bonded grafts, infection resistant graft, porcine survival model, vascular injury, ePTFE, Dacron

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Lt Col Brandon Propper, USAF, MC
U	U	U	UU	8	**19b. TELEPHONE NUMBER** *(include area code)* 210-916-1174

TABLE OF CONTENTS

1.0 SUMMARY

This study was a unique in-vivo study that examined the long-term ability of novel antibiotic grafts to resist infection and maintain patency. Our previous work had assessed a short < 1 month model for survival; however, a variety of questions remained. These questions included what the long-term antibiotic sustained presence would be in a long-term survival model and whether the findings in our previous Dacron model could be translated to ePTFE, a more commonly used graft. A 6-month survival model with 5 proposed groups that included sham, a positive control group ePTFE, a positive control group for Dacron, experimental group bonded with ePTFE, and an experimental group bonded with Dacron was developed with technical and veterinary staff; the longest survival models our group has used to date. To prevent substantial animal growth, feeding was limited in these test animals, a variance from previous survival models used. A prolonged period of non-surveillance was established to replicate normal daily activity following surgery. At the 6-month endpoint, a necropsy was conducted. However, due to unforeseen technical issues with animal growth, a number of amendments were necessary yielding an overall decreased number of total animals enrolled. While not ideal, we identified issues that would not have yielded meaningful results and worked with the IACUC to modify this study. A decision was made to remove the positive control Dacron group and experimental group bonded with Dacron. With such low number of test animals, a definitive comparison of groups could not be reached. However, none of the test animals with antibiotic-bonded vascular grafts (ePTFE) revealed infectious changes or showed evidence of rejection.

2.0 INTRODUCTION

Combat-related extremity vascular injuries represent a common cause of hemorrhage and loss of limb[1]. Diligent efforts to ameliorate the deadly impact of ballistic and explosive devices have yielded body armor that more effectively shields the head and torso but leaves the extremities vulnerable[2]. While this innovation contributes to a reduced rate of mortality in current conflicts relative to Vietnam, soldiers that would have otherwise likely perished now present with wounds in unprotected extremities[3,4] There is a perception notion that an extremity can tolerate 6 - 8 hours of ischemia, with little persistent functional deficit, allowing surgeons the opportunity to address orthopedic and other wounds preferentially or delay restoration of flow to the extremity until transportation to a facility with greater resources is possible[5]. The current clinical paradigm is for definitive vascular repair to take place at these better-equipped, higher echelon, military treatment facilities. There are times when combat-injured soldiers cannot tolerate the longer procedure needed to harvest veins or there is no vein available to restore blood flow to the limb. Typical reconstruction of choice is usually desired using a patient's native vein. However, often, native veins are destroyed during explosive impact requiring instead the use of prosthetics for temporary repair with synthetic graft reconstruction. Additionally, infection remains a major subsequent complication when repairing these types of vascular injuries with either method. Our goal was to identify a usable infection-resistant graft for vascular repair that maintains patency and performs well long-term.

Previously, our team developed a short-term, porcine vascular graft infection model to evaluate antibiotic-bonded Dacron, a woven nylon, ability to acutely withstand and maintain patency in an infected field. While that study did show improved patency, we did not evaluate the long-term performance of it nor did we compare its' performance against other types of antibiotic-bonded synthetic grafts, otherwise known as ePTFE, particularly since PTFE remains the readily available graft in theater. This study was a natural continuation of ongoing efforts to examine

long-term patency and integrity of novel antibiotic-bonded vascular grafts as well as close military gaps on preventing wound infection and maintaining vascular access for the combat-injured service member. Despite the challenges experienced with this study and the low number of test animals, the team noted that none of the test animals with bonded grafts (ePTFE) revealed infectious changes or showed evidence of rejection.

3.0 METHODS, ASSUMPTIONS, AND PROCEDURES

Methods:

At the beginning of this study, our goal was to evaluate the long-term sustainability of antibiotic-bonding to the graft and the long-term patency of the antibiotic-bonded Dacron and antibiotic-bonded ePTFE at 6 months after insertion. A 6-month survival porcine model with 5 proposed groups that included sham, a positive control group ePTFE, a positive control group for Dacron, experimental group bonded with ePTFE, and an experimental group bonded with Dacron was developed with technical and veterinary staff; the longest survival model our group has used to date. We anticipated the use of 3 animals during model development and 6 animals in each of the groups for a total of 33 total animals (see Table 1).

Groups	**Number of Animals**					**Total**
	SHAM (negative control)	ePTFE (positive control)	Dacron (positive control	Antibiotic-bonded ePTFE (experimental)	Antibiotic-bonded Dacron (experimental)	
Experimental development	0	1	0	1	1	3
Number of Animals	6	6	6	6	6	30
Total	**6**	**7**	**6**	**7**	**7**	**33**

Table 1: Initial 6-month survival porcine model with 5 groups

Following anesthesia, the iliac artery was surgically exposed through a small cut in the belly of the animal, a standard retroperitoneal exposure of the iliac vessels. Following dissection and heparin therapy, a 2cm section of iliac artery was removed and reconstructed with a 6mm graft. Sham arms were also done in this fashion. Genetically labeled bacteria (Polymicrobial wash containing *Pseudomonas aeruginosa* and *Staphylococcus aureus*) was placed into the region and on the grafts. The animal was closed and monitored post-surgery. Following postoperative monitoring (Days 1, 7, 14), the animals were sent to pasture for a total of approximately six months at which point they returned for evaluation and necropsy (Day 180). A prolonged period of non-surveillance was established to replicate normal daily activity following surgery. Pre-operative US, blood draws, and other testing were done and repeated at final necropsy as noted in Table 2.

	Preop	Immed Postop	POD1	POD7	POD 14	POD 180	Necropsy
CBC	X						
ABG	X	X					
Blood CX							X
Doppler US	X	X	X	X	X	X	
Tarlov Gait Scale Measurement	X	X	X	X	X	X	
Path/Cultures							X
Histology							X

Table 2. Preoperative and postoperative testing protocol outcome measures

Major Changes to Study Design:
Despite food restriction, growth of the swine test animals was unprecedented in addition to other technical challenges. The long-term model was modified with the assistance of the IACUC to the following completion of the model development phase. A major amendment to the study included removal of the positive control group– Dacron and the experimental antibiotic-bonded Dacron group. Additionally, a reduction of overall animals approved (Table 3).

Groups	**Number of Animals**			**Total**
	SHAM (negative control)	ePTFE (positive control)	Antibiotic-bonded ePTFE (experimental)	
Experimental development	1	2	0	3
Number of Animals	3	1	2	6
Total	**4**	**3**	**2**	**9**

Table 3. Major amendment and change in enrollment number of study animals

Assumptions:
Porcine growth was anticipated with this study based on previous models; however, it was underappreciated and created a variety of technical challenges that required a reassessment of study groups and the feasibility of total animal enrollment. To address the concern for continued animal growth during the survival time period of six months, food restriction was implemented. By limiting overall growth, it was assumed that ultrasound evaluations of the vascular graft would be feasible and accurate. A second assumption was that reconstruction of the iliac artery could serve as the primary arterial flow to the leg which would aid in clinical identification of thrombosis.

Major Study Endpoints:
Our clinical endpoints were to obtain graft patency with ultrasound prior to necropsy, observe patency at necropsy and determine any evidence of remaining antibiotic on bonded graft and/or infection present using histopathology. The endpoints of this study relied heavily on our assumptions noted above and technical success of the implant.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

- IACUC Approved, July 2016
- Kick-off meeting conducted, September 2017
- Model development and 2 test animals completed (Phase I –implantation), May 2018
- Vast unexpected animal growth observed, April – August 2018
- Multiple revisions and discussions with IACUC, July – September 2018
- Major protocol amendment, August – September 2018
- Phase II experiments (harvesting) completed November 2018
- Pathology results received, February 2019
- Data Analysis, February – June 2019
- Study Closeout, June 2019

5.0 RISK ASSESSMENT

5.1 Risk Analysis

The major risk for this study was rapid growth of animal body size experienced in previous models. To slow animal growth, we proposed limited feeding. Unfortunately, limited feeding was not enough to slow the growth of the animal in this study. The excessive body mass of each test animal created a need to reevaluate study design and our ability to conduct the necessary testing (i.e. ultrasound to evaluate graft patency) and graft harvesting.

5.2 Technical Challenges

This study attempted to examine the long-term ability of novel antibiotic grafts to resist infection and maintain patency. The length of study time and animal selected for this study proved challenging for this project and will need to be considered for any future or similar projects. We fully appreciated that substantial growth would cause difficulty, but underappreciated the difficulty of handling such large animals. The growth of the animals made Necropsy in an OR suite near impossible which compromised sterility. Fortunately, in this case, we do not believe surgical removal of the graft on the surgical floor due to animal size impacted primary endpoints. During the conduct of the study, obtaining reliable US imaging on animals this size proved to be equally challenging. The iliac interposition is placed from a retroperitoneal position. It appears that as the animal grows this section of vessel become relatively more proximal and enters an area near intersection of the torso and limb. This makes US very difficult as the artery is turning and deep. Previous models had not shown this issue, but this size and depth of what we visualized in this study was more distal than the actual interposition graft. This observation was evident through differences noted between necropsy and US of the test animals on the final day.

6.0 TRANSITION PLAN

6.1 Military Relevance

The military needs effective tools that can help prevent wound infections and improve limb salvage and vascular outcomes associated with ballistic and explosive events. Our goal was to evaluate an off-the-shelf, antibiotic-bonded vascular graft that could resist infection and maintain patency long term, ultimately improving overall patient outcomes and potentially fill these research gaps. This was the first and only study to date that has used an antibiotic-bonded vascular graft (bonded ePTFE) in a long-term model that complimented our previous short-term study with a related graft (bonded Dacron). Although this study had tremendous limitations, we

can look at our previous short-term study Dacron results and postulate that the addition of antibiotic bonding to Dacron is safe in a live model and that even with time (6 months), the bonding process of antibiotics on graft material does not appear to cause harm. While it is hard to promote additional long-term survival testing for this type of study, this project has positively promoted the use antibiotic-bonding on other materials such as Extracorporeal Membrane 0xygenation (ECMO) circuits and other types of indwelling equipment. Furthermore, as we move into other studies looking at antibiotic-coating or antibiotic-bonded conduits, the particular lessons learned from this study should be strongly considered.

6.2 Transition Strategy

There are a variety of FDA-approved antibiotic medications that can be considered for bonding onto various conduits. For this study we maintained a relationship with MD Anderson; the bonding process is their intellectual property. Although further animal studies will not be continued, our refinement of technical application in sewing these grafts should not be overlooked. During our last discussion regarding this product, MD Anderson was in active discussion with industry for potential clinical trials for compassionate use application in humans. We anticipate that in the near future, antibiotic-bonded grafts will be made available for trauma and vein-limited patients. From a practical standpoint, this option is appropriate for patients initially bypassed in theater. Even if this type of graft is used in temporarily, the potential for improved patient outcomes in a far forward care setting is promising.

7.0 RESULTS

The initial protocol submission received local Institutional Animal Care & Use Committee (IACUC) approval in June 2016. Following experimental development, we completed graft insertion for 2 subjects using negative control (sham), 1 subject for positive control PTFE, and 2 subjects experimental antibiotic-bonded ePTFE. Of the 5 subjects, one of the negative control (sham) subjects was euthanized 14 days postoperatively for respiratory distress. Of the remaining 4 subjects, the positive control PTFE subject was euthanized postoperatively within 24 hours of surgery due to bilateral sciatic hematoma complications. The remaining 3 subjects reached day 180. Ultrasound conducted was difficult and necropsy had to be completed on the surgical floor due unanticipated subject weight. After IACUC amendments were complete, 4 additional subjects were completed: 2 negative controls (sham) and 2 positive controls (ePTFE). Of the 2 positive controls, 1 subject was euthanized on postoperative day 4 for respiratory distress. The remaining 3 animals reached day 180. At the end of the study, 6 total subjects' data were evaluated. Ultrasound conducted remained a difficult task and necropsy had to be completed on the surgical floor due to unanticipated subject weight.

8.0 CONCLUSION/DISCUSSION

The aims of this study were to evaluate the ability of antibiotic-bonded vascular grafts to withstand a polymicrobial infection and remain patent in a contaminated field compared to the current grafts available. Of the results available, necropsy of the sham subjects were able to maintain patent vessels but had an immense inflammatory response. Interestingly, no abscess collections were seen in the specimens, which likely shows the immune response of swine. In the positive control subjects, the graft specimens had more inflammation, but this is not unusual or unexpected since foreign bodies often enhances inflammatory response. However, notably, the positive control subjects showed no evidence of abscess, fluid, or overt infection. Pathology results indicate mineralization but no neutrophil evidence or other infectious changes. It would

be of interest to know whether these findings were due to the unique swine immune response. We do know from our previous study and other historical studies that non-bonded Dacron graft sites do become infected, and that typically, the presence of a foreign body heightens the chance of infection. With such a low completion number for our subjects, definitive conclusions cannot be made. However, of the subjects that were complete, none of the antibiotic-bonded grafts revealed infectious changes. Additionally, none of the subjects showed evidence of rejection of the antibiotic-bonded material.

Important lessons were learned in this study that impact a long-term study using this animal model. Our experience noted that Yorkshire swine are not a good choice for long-term projects, particularly if necropsy is needed. The starting size makes them ideal for 6mm grafts; however, their final size at 6 months is prohibitive and frankly, too heavy for the OR tables to sustain. Limited feeding to slow swine growth is good in theory but extremely difficult in practice. In fact, we noted that one particular animal grew more readily and was essentially domesticated by six months.

9.0 DELIVERABLES

To date, we have not published any articles; however, we have shared our technical experience with others, namely the team at MD Anderson. The information we provided was deemed helpful as they move toward a clinical trial geared toward trauma and compassionate use in humans. Based on the results received and low number of enrolled subjects, we do not anticipate a manuscript for publication; however, a technical paper may be considered describing surgical technique of vascular graft replacement and removal in challenging environments.
Since completion of our project, additional studies using large swine have been developed and planned for other materials. This study has served to provide lessons learned and issues of consideration during the planning of those studies to avoid similar outcomes.

10.0 COST

AC6 funding received ($150K) at the 59 MDW 23-Jun-16. AC7 Funding received ($153K) at the 59 MDW 8-Aug-17.

11.0 REFERENCES

1. Gawande A. Casualties of war--military care for the wounded from Iraq and Afghanistan. *N Engl J Med*. 2004 Dec; 351(24):2471–5.
2. Kosashvili Y, Hiss J, Davidovic N, Lin G, Kalmovic B, Melamed E, et al. Influence of personal armor on distribution of entry wounds: lessons learned from urban-setting warfare fatalities. *J Traum*. 2005 Jun; 58(6):1236–40.
3. Rich NM, Baugh JH, Hughes CW. Acute arterial injuries in Vietnam: 1,000 cases. *J Traum*. 1970 May; 10(5):359–69.
4. Fox CJ, Starnes BW. Vascular surgery on the modern battlefield. *Surg Clin North Am*. 2007 Oct; 87(5):1193–211, xi.
5. Gifford SM, Eliason JL, Clouse WD, Spencer JR, Burkhardt GE, Propper BW, et al. Early versus delayed restoration of flow with temporary vascular shunt reduces circulating markers of injury in a porcine model. *J Traum*. 2009 Aug; 67(2):259–65.
6. Peck M a, Clouse WD, Cox MW, Bowser AN, Eliason JL, Jenkins DH, et al. The complete management of extremity vascular injury in a local population: a wartime report from the 332nd Expeditionary Medical Group/Air Force Theater Hospital, Balad Air Base, Iraq. *J Vasc Surg*: 2007 Jun; 45(6):1197–204; discussion 1204–5.
7. CJ, Gillespie DL, O'Donnell SD, Rasmussen TE, Goff JM, Johnson C a, et al. Contemporary management of wartime vascular trauma. *J Vasc Surg*: 2005 Apr; 41(4):638–44.
8. Dahl SLM, Kypson AP, Lawson JH, Blum JL, Strader JT, Li Y, et al. Readily available tissue-engineered vascular grafts. Sci Transl Med. 2011 Feb 2; 3(68):68ra9.
9. Prichard HL, Manson RJ, DiBernardo L, Niklason LE, Lawson JH, Dahl SLM. An early study on the mechanisms that allow tissue-engineered vascular grafts to resist intimal hyperplasia. *J Cardiovasc Transl Res*. 2011 Oct; 4(5):674–82.
10. Burkhardt GE, Spencer JR, Gifford SM, Propper B, Jones L, Sumner N, et al. A large animal survival model (Sus scrofa) of extremity ischemia/reperfusion and neuromuscular outcomes assessment: a pilot study. *J Traum*. 2010 Jul; 69 Suppl 1(1):S146–53.
11. Hospenthal DR, Crough HK, English JF, Leach F, Pool J, et al. Multidrug-resistant bacterial colonization of combat-injured personnel at admission to medical centers after evacuation from Afghanistan and Iraq. *J Traum*. 2011 Jul; 71(1 Suppl):S52-7.
12. White JM, Stannard A, Burkhardt GE, Eastridge BJ, Blackbourne LH, Rasmussen TE. The epidemiology of vascular injury in the wars in Iraq and Afghanistan. *Ann Surg*. 2011 Jun; 253(6): 1184-9.
13. Watson J, Houston R, Reinsvold R, Morrison J, Sokol K, Rasmussen T. Short-term patency of human tissue-engineered vascular grafts in porcine model of hind-limb ischemia reperfusion injury.
14. Clemens MS, Stull MC, Heafner TA, Hata KW, Arthurs ZM, Propper BW. PC230. Comparison of Antibiotic-Bonded Graft Patency to Standard Techniques in the Setting of a Polymicrobial Infection in Swine (Sus Scrofa). *J Vasc Surg*. 2016 Jun; 63(6): 223S-223-S. doi:10.1016/j.jvs.2016.03.373

12.0 LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

59th MDW/ST	59th Medical Wing, Office of the Chief Scientist
ABG	Arterial Blood Gases
AFMSA	Air Force Medical Support Agency
Blood CX	Blood Cultures
CBC	Complete Blood Count
cm	centimeters
Dacron	Polyethylene terephthalate
Doppler US	Doppler Ultrasound
DTIC	Defense Technical Information Center
ECMO	Extracorporeal Membrane Oxygenation
EM	Expeditionary Medicine
ePTFE	Expanded Polytetrafluoroethylene
FDA	Food & Drug Administration
FY	Fiscal Year
IACUC	Institutional Animal Care and Use Committee
mm	millimeters
OR	Operating Room
Path	Pathology
PI	Principal Investigator
POD	Postoperative Day
PTFE	Polytetrafluoroethylene
SG5	Office of the Surgeon General Medical Operations and Research
US	Ultrasound