NAVAL MEDICAL RESEARCH UNIT SAN ANTONIO

THE EFFECT OF A THIRD GENERATION HEMOSTATIC DRESSING IN A SUBCLAVIAN ARTERY AND VEIN TRANSECTION PORCINE MODEL

BJORN K SONG, PHD; FOREST R. SHEPPARD, CDR, MC, USN;
ANTONI MACKO, PHD; AND RENE ALVAREZ, PHD

EXPEDITIONARY AND TRAUMA MEDICINE DEPARTMENT
COMBAT CASUALTY CARE AND OPERATIONAL MEDICINE DIRECTORATE

NAMRU-SA REPORT # 2014-22

DISTRIBUTION A – Approved for public release; unlimited distribution.

Declaration of Interest

The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

This work was funded by work unit number G1102.

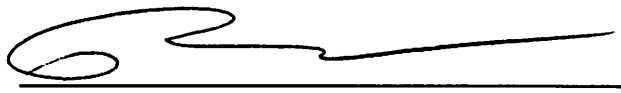
The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996.

CDR Forest R. Sheppard is a military service member; Bjorn K. Song, Antoni Macko and Rene Alvarez are employees 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 or employee of the US Government as part of that person's official duties.

Acknowledgements

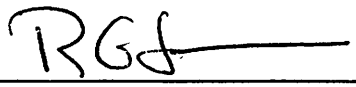
We are grateful for the technical assistance provided by Ramon Cestero, Jennifer Cox, and Jason Rall.

Reviewed and Approved by:



4/16/2014
Date

Rene Alvarez, Ph.D.
Director, Combat Casualty Care & Operational Medicine
Chair, Scientific Review Board
Naval Medical Research Unit San Antonio
3650 Chambers Pass, BLDG 3610
Fort Sam Houston, TX 78234-6315



16 May 14
Date

CAPT Rita Simmons, MSC, USN
Commanding Officer
Naval Medical Research Unit San Antonio
3650 Chambers Pass, BLDG 3610
Fort Sam Houston, TX 78234-6315

Table of Contents

Abbreviations 4
Executive Summary 5
Introduction 7
Materials and Methods 8
Results 13
Discussion 14
Study Limitation 16
Summary 16
References 17

Abbreviations

ABL	Application Blood Loss
ANOVA	Analysis of Variance
BL	Baseline
CBC	Complete Blood Counts
CoTCCC	Committee on Tactical Combat Casualty Care
IM	Intra-Muscular injection
$ETCO_2$	End Tidal Carbon Dioxide
LRS	Lactated Ringer's Solution
MAP	Mean Arterial Pressure
MSD	Minisponge-Based Dressing
NAMRU-SA	Naval Medical Research Unit San Antonio
Pre-TBL	Pre-treatment Blood Loss
Post-TBL	Post-treatment Blood Loss
SCA	Subclavian Artery
SCV	Subclavian Vein
SEM	Standard Error of the Mean
SOCOM	Special Operations Command
SCHD	Standard-of-Care Hemostatic Dressing
TCCC	Tactical Combat Casualty Care

Executive Summary

Background: Hemorrhage is the leading cause of preventable death on the battlefield. Non-compressible injuries to junctional regions such as the axilla, neck, and groin account for 20% of these deaths and present a challenge to first responders and medical care providers. Timely and effective, point-of-care treatments for junctional hemorrhage could help save lives on the battlefield and in civilian trauma. A novel product, the minisponge-based dressing (MSD), has been developed to treat junctional hemorrhage. The MSD is comprised of a syringe applicator filled with compressed chitosan coated sponges which are delivered into the wound at the site of the junctional injury and hemorrhage. Although initial published results seem promising, an independent evaluation and comparison of this product has yet to be performed.

Objective: The objective of the current study was to evaluate the hemostatic effectiveness of a novel MSD product to control bleeding in a porcine model of junctional hemorrhage.

Methods: A swine subclavian artery (SCA) and vein (SCV) transection model of uncontrolled junctional hemorrhage was utilized. All animals were anesthetized, instrumented, and underwent splenectomy before the SCA and SCV were isolated via a small skin incision. The exposed subclavian vessels were treated with lidocaine, transected and allowed to free bleed for thirty seconds prior to application of the hemostatic dressing. Animals were randomly assigned into one of three treatment groups: 1). Minisponge-based dressing (MSD; n=10), 2). standard-of-care hemostatic dressing with three minutes of manual compression (SCHD-WC; n = 10) and 3). standard-of-care hemostatic dressing no manual compression (SCHD-NC; n = 10). Following dressing application, a "wait period" of 4 minutes for MSD and 3 minutes for both SCHD-WC and SCHD-NC groups transpired. Subsequently a 60-minute observation period commenced (T = 0-60 minutes). All animals were euthanized while under anesthesia at T = 60 minutes. Primary endpoints were animal survival, blood loss, hemostasis, and application time. Statistical significance was compared at each 5 minute interval from T = 0 through T = 60 minutes using one-way ANOVA. A p-value of less than 0.05 was considered significant.

Results: Baseline values for weight, blood pressure, cavity volume, and hematocrit were not different between groups. Additionally, there were no differences in the diameters of both the SCA and SCV, or blood loss prior to hemostatic dressing application between treatment groups. Pack time was shorter ($p < 0.05$) in the MSD group (31.1 ± 4.1 s) compared to both SCHD-WC (65.0 ± 5.7 s) and SCHD-NC (60.3 ± 2.0 s) which were not different from one another. The

amount of blood loss during the time of application was also significantly lower in the MSD group (1.3 ± 0.3 g/kg, $p < 0.05$) compared to both the SCHD-WC (5.1 ± 0.9 g/kg) and SCHD-NC (5.1 ± 0.7 g/kg) groups. There was no difference in survival, hemostasis or total blood loss during the observation period (T0-T60) among groups.

Conclusion: The current study demonstrates the potential benefits of a novel MSD hemostatic device and supports clinical translation for field application. However, further work is needed to more comprehensively evaluate this novel approach and determine optimal guidelines for its use in tactical medicine.

Introduction

Hemorrhage is responsible for 90% of potentially preventable deaths on the battlefield, with the majority of these deaths occurring prior to arrival at medical treatment facilities (Eastridge et al., 2012). Of the casualties who die from potentially survivable hemorrhage before reaching a medical treatment facility, 50% die from non-compressible truncal hemorrhage and 20% die due to junctional (i.e. axillary, neck, or groin) hemorrhage that is not amenable to tourniquet application (Kelly et al., 2008; Eastridge et al., 2012; Eastridge et al., 2011). Although the increased use of field tourniquets and hemostatic dressings by the military has resulted in a reduction in deaths from extremity hemorrhage (Kragh et al., 2009; King, 2011), the management of non-compressible truncal and junctional hemorrhage remains a significant challenge to medical providers both in military and civilian settings (Holcomb et al., 2007; Kelly et al., 2008; Eastridge et al., 2011).

Anatomically, junctional injuries occur at the intersections of anatomically distinct body regions and are traversed by major vascular structures. Junctional vascular structures are often not amenable to external compression either due to their depth or overlying boney structures. The subclavian artery and vein are in a defined junctional region and injuries to these vessels present a unique challenge as a result of their relatively protected and non-compressible location underneath the clavicle. The standard military recommendation of direct pressure to achieve hemorrhage control (Butler & Blackbourne, 2012) is typically ineffective in this region because pressure over the clavicle will not be transmitted effectively to the deep subclavian vessels to stop hemorrhage. As a result of their non-compressible nature and paucity of effective field techniques or equipment for controlling hemorrhage from this region, penetrating injuries to the subclavian vessels have been associated with a high mortality, up to 50% in some studies (Demetriades et al., 1999).

Researchers have recently developed a novel hemostatic dressing intended to treat junctional, non-compressible, hemorrhage. The dressing is comprised of rapidly expanding minisponges which are administered into a wound through a lightweight syringe applicator. The minisponges expand upon contact with blood, do not require manual compression and result in direct compressive hemorrhage control of the bleeding vessel (Mueller et al., 2012). In a recent publication using a swine subclavian injury model of lethal non-compressible junctional hemorrhage, the minisponge-based dressing (MSD) was compared to the current Committee on

Tactical Combat Casualty Care's (CoTCCC) standard-of-care hemostatic dressing (SCHD). The SCHD is used to "control life-threatening hemorrhage from external bleeding at sites that are not amenable to tourniquet use" (Butler & Blackbourne, 2012). In comparison to SCHD, MSD treated animals were reported to have significant improvements in both hemostasis and survival, along with reduced blood loss, resuscitation fluid requirements, and treatment time for application of the product.

The Naval Medical Research Unit San Antonio (NAMRU-SA) was selected by Special Operations Command (SOCOM) to evaluate the efficacy of MSD compared to SCHD using the swine subclavian injury model of junctional, non-compressible, hemorrhage. The purpose of the present investigation was to specifically evaluated these dressings for overall survival, time of application, blood loss, and hemostasis.

Materials and Methods

Animals.

Thirty male Yorkshire Swine (Oak Hill Genetics, Ewing, IL) weighing 50 to 80 kg were used in this study. Animals were randomly assigned to 1 of 3 treatment groups, each with an n = 10. Temperature, humidity, and husbandry care were in accordance with the current Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences. Animals were housed individually, maintained in a 12 hour light and 12 hour dark cycle, fed a commercial diet and given water ad libitum. All animal procedures were approved by the Fort Sam Houston Tri-Service Research Lab Institutional Animal Care and Use Committee (Protocol: NAVY-11-06).

Anesthesia, Surgical Manipulation and Euthanasia.

Twelve hours prior to the procedure, swine were ordered *nil per os* with the exception of water *ad libitum*. Animals were sedated with 8 mg/kg Telazol (Fort Dodge Animal Health, Fort Dodge, IA) via intra-muscular (IM) injection. During surgical preparation and prior to anesthesia, 0.01 mg/kg buprenorphine IM and 0.004 mg/kg glycopyrrolate IM were provided for pain management and reduction of mucosal secretions, respectively. After intubation, general anesthesia was conducted utilizing isoflurane delivered at 2-4% in pure oxygen for initial anesthesia induction and then reduced to 1-2% thereafter to maintain a surgical plane of anesthesia. The ventilator was adjusted to maintain an end tidal CO_2 ($ETCO_2$) partial pressure between 38 and 42 mmHg.

A marginal ear vein was accessed for collection of a baseline venous blood gas sample and to be used as an alternate access for fluid delivery. The right carotid artery was catheterized with a 7fr vascular catheter (Harvard Apparatus, Holliston, MA) and used for arterial blood sampling and for monitoring of blood pressure and heart rate. The right jugular vein was catheterized with an Intracath 16ga catheter (Deseret Co, Sandy, UT) for fluid infusions. Subsequently, a midline laparotomy was performed followed by a splenectomy to reduce the potential hemodynamic effects of autotransfusion during the experiment. The spleen was weighed and 3 ml/gram of spleen weight of warmed Lactated Ringer's Solution (LRS) was administered at a rate of 100 mL/min to compensate for the splenic blood loss, which was performed to maintain consistency with a previous MSD study (Mueller et al., 2012). A cystostomy was performed for urinary drainage prior to laparotomy closure.

Standard vital telemetry was used for continual monitoring of heart rate, blood pressure, respiration, temperature, electrocardiogram, $ETCO_2$ and oxygen saturation (SpO_2) by Cardiocap/5 (GE Healthcare, Salt Lake City, UT) every 15 minutes prior to the experimental injury and 5 minute intervals thereafter.

Hemorrhage was induced by transection of the subclavian artery (SCA) and vein (SCV) as described previously by Mueller et al. (2012). The left SCA and SCV were accessed via a 4.5 cm skin incision approximately 4 cm lateral to the sternum, and directly over the left pectoralis major muscle. The pectoralis major muscle was transected using electro-cautery to expose both the SCA and SCV. Using a combination of blunt and sharp dissection, a 5 cm segment of both the SCA and SCV were isolated and dissected circumferentially from surrounding tissues. Once all pre-calculated splenic replacement fluid was delivered, measurements were taken of the vessel diameters, cavity depth, and cavity volume. The subclavian vessels were then immersed in 30 mL of 2% lidocaine (Phoenix Pharmaceuticals, St. Joseph, MO) for 10 minutes to promote vasodilation. This ten-minute period was also designated as a stabilization period in which mean arterial pressure (MAP) was maintained above 60 mmHg. When the stabilization period was completed, excess lidocaine in the cavity was removed and vessels were measured again. The SCA and SCV injuries were then created by complete transection of the SCA and SCV using a scalpel, followed by a 30-second period of free bleeding prior to hemostatic dressing application.

All treatment groups received 500 mL of Hextend (Hospira Inc., Lake Forrest, IL) immediately following the 30-second free bleed, subsequently LRS was infused as needed to

maintain a MAP above 65 mmHg. All resuscitation fluids were delivered by a Belmont Rapid Infuser (Belmont Instruments, Billerica, MA) at a rate of 100 mL/min.

During the 60 minute observation period, signs of hemostasis, bleeding and vital signs were continuously recorded. Blood escaping the edges of the wound was collected by vacuum suction to measure total blood loss throughout the experiment. Pre-treatment blood loss (pre-TBL) was defined as all blood collected from the vacuum during the 30 second bleed prior to hemostatic dressing application plus the measured volume of the cavity. Application blood loss (ABL) was defined as any blood which exited the wound during application of the test materials after the 30 second free bleed period and prior to the start of the 60-minute observation period (T0). Post-treatment blood loss (post-TBL) was defined as any blood collected on pre-weighed absorbent pads, blood absorbed by the hemostatic agents, and blood collected during the observation period (T0 – T60). As defined in this study, hemostasis was achieved when blood ceased to drain from the wound site. Re-bleed was defined as failure of hemostasis that occurred during the observation period (between T0 and T60). At the end of each experiment, the test materials were removed and weighed, the final cavity volume was recorded and, if not already deceased, the animals were euthanized with a lethal dose of Beuthasol (100 mg/kg), Sodium Pentobarbital, (JA Webster, Sterling, MA). Death criteria for the study was defined as MAP less than 20 mmHg and $ETCO_2$ less than 15 mmHg for more than two minutes.

Hemostatic Dressings.

The current standard-of-care hemostatic dressing (SCHD) to control hemorrhage in the US military (Butler & Blackbourne, 2012) is a gauze (50% Rayon and 50% Polyester) impregnated with kaolin, an aluminosilicate clay, which activates the intrinsic coagulation cascade when in contact with blood (Lawton et al., 2009; Granville-Chapman et al., 2011). The SCHD was purchased from the manufacturer (Z-Medica Corporation, Wallingford, CT) as sterile, individually packaged, z-folded, 3-inch by 12-foot strips from the manufacturer (Z-Medica Corporation, Wallingford, CT). Manufacturer directions for SCHD include packing of the product into the wound, followed by three minutes of compression to the packed dressing. The mini-sponge dressing (MSD) is a novel product that utilizes compressed, chitosan-coated, cellulose sponges that expand upon contact with liquid. The compressed sponges are packaged into a 30mm diameter applicator syringe that allows for injection of the mini-sponges into the

external injury site. The MSD used in this study was manufactured by REVMEDX (REVMEDX, Wilsonville, OR) and provided by SOCOM.

Dressing Treatment Groups and Randomization.

Animals were randomly assigned to one of three treatment groups: MSD with no compression (n=10), SCHD with no compression (SCHD-NC, n=10), and SCHD with manual compression (SCHD-WC, n=10). The investigators involved in surgical procedures and hemostatic application were blinded to the randomization of the hemostatic dressing treatment until immediately prior to the creation of the subclavian injury. The time-course for hemostatic dressing application of MSD and SCHD is depicted in a diagram (Figure 1), and also described in detail below for all three treatment groups.

In the MSD group (n = 10), MSD was injected into the wound cavity until the cavity was filled with MSD and additional MSD could not be contained within the wound cavity. The time of MSD administration into the wound was noted as "pack time." Multiple MSD syringe applicators could be used to accomplish cavity filling. Once the cavity was filled with MSD, a 4 minute wait period occurred prior to T0 and the start of the 60-minute observation period (T0 – T60).

The entire pack of the SCHD was administered into the wound cavity followed by a backing of additional gauze (Kerlix™; Kendall, Miami, FL) until the cavity was filled. The time of SCHD and Kerlix application was noted as "pack time". No manual compression was administered to the dressing following its application (SCHD–NC). Once the cavity was filled with SCHD, a 3 minute wait period occurred prior to T0 and the start of the 60 minute observation period (T0 – T60). In the event that hemostasis was not achieved following initial application of the dressing and prior to the end of the 3-minute wait period, the SCHD and Kerlix™ were removed and a second, final, dressing with SCHD and Kerlix™ was applied. The time required to re-apply SCHD and Kerlix™ was noted as "2nd pack time." Following the second application, a 3 minute wait period occurred prior to T0 and the start of the 60 minute observation period (T0 – T60).

In arm 3, an entire pack of SCHD was administered into the wound cavity followed by a backing (Kerlix™; Kendall, Miami, FL) until the cavity was filled. The time of SCHD and Kerlix^{TM} application was noted as "pack time." Manual compression was then administered to the dressing for 3 minutes (SCHD–WC) once the cavity was filled with SCHD. Following 3

minutes of compression the 60 minute observation period (T0 – T60) was commenced. In the event that hemostasis was not achieved following initial application of the dressing and compression and prior to the end of the 3 minutes of compression, the SCHD and Kerlix™ were removed, and a second, final, application of SCHD and Kerlix™ with subsequent compression for 3 minutes was performed. The time required to re-apply SCHD and Kerlix™ was noted as "2nd pack time." Following the second application and the 3 minute compression time the 60 minute observation period (T0 – T60) was commenced.

Regardless of randomized treatment, "pack time" was defined as the time required to apply the hemostatic dressing into the wound. In SCHD groups there were potentially two pack times, depending on whether a second dressing application was required due to failure of the first application to achieve hemostasis during the 3 minute wait period. Hemostasis was achieved if no blood came through the hemostatic dressing and out of the cavity. "Application time" was defined as the time during which the technician was in physical contact with the animal (ex. pack-time + manual compression time). "Application time" did not include wait periods; however, it did include the 3 minutes of compression in the SCHD-WC treatment group. If SCHD was applied twice, then "application time" was the cumulative time for both applications; for example, the MSD and SCHD-NC "pack time" was the same as "application time" if SCHD was only applied once. The "wait period" was implemented for the MSD and SCHD-NC animals to standardize the start of the observation period (T0) to SCHD-WC animals that required 3 minutes of manual compression. Therefore, SCHD-NC animals had a 'wait period' of 3 minutes to match the time of manual compression required for SCHD-WC animals. However, MSD animals had a 4 minute 'wait period' as opposed to 3 minutes, because the MSD "pack time" was generally 30 seconds to 1 minute shorter than the "pack time" of SCHD.

Laboratory Analysis.

A baseline (BL) blood sample was collected immediately post-splenectomy (T = 0) and additional samples were collected at T = 5, 15, 30, 45 and 60 minutes following the spelenctomy. Analyses included complete blood counts (CBC) (LH500, Beckman Coulter, Inc., Brea, CA) and blood chemistry analysis (Abl 830 Flex; Radiometer America, Westlake, OH).

Statistical Analysis.

Data are presented as mean ± standard error of the mean (SEM). Chi-square tests were used to analyze categorical variables. Analysis of Variance (ANOVA) with Tukey *post hoc*

analysis was used to determine differences between treatment groups. Data analysis was performed using Origin 8.0 (OriginLab, Northampton, MA). Statistical significance was determined when $p < 0.05$.

Results

Baseline (BL) Parameters.

Table 1 demonstrates the relationship between BL conditions and treatment groups: MSD, SCHD-NC, and SCHD-WC. All parameters evaluated at baseline including; weight, hemoglobin levels, hematocrit, mean arterial pressure (MAP), platelet counts, pH, splenic weight, artery diameter, vein diameter, cavity volume, and pre-treatment blood loss were not different between groups.

Pack and Application Time.

Pack time during the initial application was shorter in the MSD (31.1 ± 4.1 s, $p < 0.05$) than the SCHD-NC (65.0 ± 5.7 s) and SCHD-WC (60.7 ± 3.7 s) treatment groups. There was no difference in pack-time between SCHD groups during the first or second packing (Figure 2). The MSD treatment group had a shorter ($p < 0.05$) overall application time (31 ± 7 s) than the SCHD-NC (100 ± 18 s) and SCHD-WC (307 ± 27 s) treatment groups. The SCHD-NC treatment group had a shorter application time compared to SCHD-WC ($p < 0.05$) (Figure 2).

Hemostasis and Mortality.

Hemostasis for each experimental group was determined in three categories: hemostasis at T0, hemostasis at T60, and whether a re-bleed occurred (Table 2). In evaluating hemostasis at T0, which was defined as absence of active bleeding at the onset of the observation period, there were no differences between the MSD (60%), SCHD-NC (50%), and SCHD-WC (50%) treatment groups. There were no differences between treatment groups with regard to hemostasis at the end of the 1-hour observation period (T60) MSD (90%), SCHD-NC (100%), and SCHD-WC (70%) (Table 2). Furthermore, there were no differences among the three treatment groups with regard to re-bleed, where MSD, SCHD-NC and SCHD-WC had re-bleed rates of 0, 11 and 14 %, respectively. All of the animals in the MSD and SCHD-WC groups survived the observation period; however, one animal in the SCHD-NC group was intravenously administered a lethal does of Beuthasol (100 mg/kg) (sodium pentobarbital) because the animal reached the death criteria for the study.

Blood Loss.

Pre-TBL and post-TBL were not different ($p < 0.05$) between the MSD, SCHD-NC and SCHD-WC treatment groups (Figure 3), in which pre-TBL for MSD, SCHD-NC and SCHD-WC were 12.3 ± 0.9, 13.8 ± 1.5, and 13.1 ± 0.8 g/kg, respectively, while the post-TBL values were 7.0 ± 5.0, 3.5 ± 2.0, and 8.7 ± 3.2 g/kg, respectively. However, ABL was lower in the MSD group (1.3 ± 0.3 g/kg, $p < 0.05$) compared to both SCHD-WC (5.1 ± 0.9 g/kg) and SCHD-WC (5.1 ± 0.7 g/kg) (Figure 3).

Resuscitation with Lactated Ringer's Solution (LRS).

LRS was administered intravenously, as required, to maintain a minimum MAP of 65 mmHg in all animals during the observation period of the study. When normalized to the weight of the animal, MSD, SCHD-NC and SCHD-WC groups received LRS volumes that were not different from each other: 32.5 ± 11 ml/kg, 55.6 ± 10 ml/kg and 62.7 ± 7 ml/kg respectively.

Discussion

Non-compressible truncal and junctional hemorrhage is the underlying cause of the majority of potentially survivable deaths on the battlefield (Eastridge et al., 2012; Eastridge et al., 2011). The current CoTCCC field treatment recommendations for hemorrhage not amenable to tourniquet use include the application of SCHD followed by three minutes of compression. However, junctional injuries are typically under bony structures, deep and/or adjacent to vital structures, making effective application of manual compression difficult and complicating attempts to control hemorrhage. MSD, which was specifically developed to address non-compressible, junctional, sites of hemorrhage, is composed of compressed cellulose minisponges which expand upon contact with blood to plug the wound cavity by causing internal tamponade. Using a lethal model of subclavian artery (SCA) and vein (SCV) transection, this study compared MSD with the current TCCC standard-of-care hemostatic dressing (SCHD) both with (SCHD-WC) and without manual compression (SCHD-NC).

Due to the rapid lethal nature of non-compressible injuries and anatomic differences between humans and swine, no standardized research models of non-compressible junctional hemorrhage exist. The porcine subclavian injury model used in this study entailed complete transection of the SCA and SCV, both of which are junctional vessels which bleed significantly at lethal rates of 1.0 to 1.5 liters/minute, similar to rates in humans (Demetriades et al., 1999). In

humans, SCA and SCV injuries represent non-compressible, junctional hemorrhage that is not amenable to tourniquet placement. However, swine as opposed to humans, lack a clavicle over these vessels, thereby allowing some degree of compression of the SCA and SCV. Though the swine model is not an exact representation of a non-compressible human subclavian vascular injury, it does represent a junctional vascular injury not amenable to tourniquet placement. Therefore, successful control of hemorrhage in this swine model, by a hemostatic dressing without the requirement of compression, has promising implications for translation to hemorrhage control of a SCA and/or SCV and potentially other non-compressible injury sites in humans.

In this study, using a swine SCA and SCV transection injury model, MSD, SCHD-NC, and SCHD-WC all had similar efficacy with regard to hemostasis, re-bleed, and mortality. Overall, MSD, SCHD-NC, and SCHD-WC worked equally well in preventing mortality as well as achieving both immediate and overall hemostasis. There were no differences between groups during the 30-second free bleed or the observation period (T0 – T60). However, there was a difference noted in the amount of blood lost during product application (application blood loss): MSD-treated animals lost significantly less blood (1.3 g/kg) compared to both SCHD-WC (5.1 g/kg) and SCHD-NC-treated animals (5.1 g/kg). The difference in blood loss during the application period may be a result of differences in pack-time and application time as both pack-time and application time were significantly shorter for MSD than for either SCHD-NC or SCHD-WC (Figure 2) and the application blood loss measurement was not normalized to account for the observed differences in product application time. The observed measurable differences in blood loss during pack time and application time determined in this study between the single application of MSD as compared to SCHD were potentially a result of the method of application of MSD; no requirement for physical compression or additional gauze (Kerlix™) application. The clinical implications of the observed reductions to pack-time, application time, and blood loss in the MSD group appear promising but additional evaluation is required before definitive conclusions can be drawn.

Further investigation is warranted to more completely evaluate the potential of MSD and to permit continued reassessment of the SCHD. Continued research and development efforts remain a priority in order to develop, refine and assess effective hemostatic dressings for non-

compressible truncal and junctional hemorrhage control for our wounded warfighters in austere and combat environments.

Study Limitation

The current study compared MSD and SCHD in a porcine model of non-compressible junctional hemorrhage. As noted in the discussion, although the model used in this study recapitulates several aspects of non-compressible hemorrhage in humans, it differs in that the subclavian vessels in swine are not covered by the clavicle bone as they are in humans. However, the results of the current study support the potential efficacy of MSD to control hemorrhage in human injuries where manual compression cannot be effectively applied.

Study subjects were observed for a one-hour time period after injury, therefore, it is possible that longer observation may reveal higher re-bleed rates than those identified in this study. Lastly, safety issues including potential retained sponge products, possible embolization of particles, or direct effects on tissues in contact with the hemostatic dressings were not addressed in this study. A more comprehensive study should include; a larger sample size, longer observation periods after injury, standardization of bleeding measurements to application time, safety evaluations, and histological analyses to determine the contributions of tamponade and enhanced local clotting on the hemostatic effects of MSD.

Summary

In the current study utilizing a porcine model of SCA and SCV transection, MSD provided similar initial and overall hemostatic efficacy as the current Committee on Tactical Combat Casualty Care standard-of-care hemostatic dressing (SCHD), however, application time and blood loss during application were significantly lower with MSD and furthermore, these observed results were achieved without manual compression. The potential benefits of these findings as they translate to field application are clear. However, further work is needed to more comprehensively evaluate this novel hemostatic approach and determine optimal guidelines for its use in tactical medicine.

References

1. Butler FK, Jr., Blackbourne LH. (2012). Battlefield trauma care then and now: a decade of Tactical Combat Casualty Care. *J Trauma Acute Care Surg*. 73(6 Suppl 5):395-402.
2. Demetriades D, Chahwan S, Gomez H, Peng R, Velmahos G, Murray J, … Bongard F. (1999). Penetrating injuries to the subclavian and axillary vessels. *J Am Coll Surg*. 188(3):290-295.
3. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, … Blackbourne LH. (2012). Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg*, 73(6 Suppl 5): p. S431-437.
4. Eastridge BJ, Hardin M, Cantrell J, Oetjen-Gerdes L, Zubko T, Mallak C, … Blackbourne LH. (2011). Died of wounds on the battlefield: causation and implications for improving combat casualty care. *J Trauma*. 71(1 Suppl): p. S4-8.
5. Granville-Chapman J, Jacobs N, Midwinter MJ. (2011). Pre-hospital haemostatic dressings: a systematic review. Injury. 42(5):447-459.
6. Holcomb JB, McMullin NR, Pearse L, Caruso J, Wade CE, Oetjen-Gerdes L, … Butler FK. (2007). Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001-2004. *Ann Surg*. 245(6): p. 986-991.
7. Kelly JF, Ritenour AE, McLaughlin DF, Bagg KA, Apodaca AN, Mallak CT, … Holcomb JB. (2008). Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003-2004 versus 2006. *J Trauma*. 64(2 Suppl): p. S21-26; discussion S26-27.
8. King DR. (2011). Thirty consecutive uses of a hemostatic bandage at a US Army combat support hospital and forward surgical team in Operation Iraqi Freedom. *J Trauma*. 71(6): p. 1775-1778.
9. Kragh JF, Jr., Walters TJ, Baer DG, Fox CJ, Wade CE, Salinas J, Holcomb JB. (2009). Survival with emergency tourniquet use to stop bleeding in major limb trauma. *Ann Surg*. 249(1): p. 1-7.
10. Lawton G, Granville-Chapman J, Parker PJ. (2009). Novel haemostatic dressings. *J R Army Med Corps*. 155(4):309-314.
11. Mueller GR, Pineda TJ, Xie HX, Teach JS, Barofsky AD, Schmid JR, Gregory KW. (2012). A novel sponge-based wound stasis dressing to treat lethal noncompressible hemorrhage. *J Trauma Acute Care Surg*. 73(2 Suppl 1):S134-139.

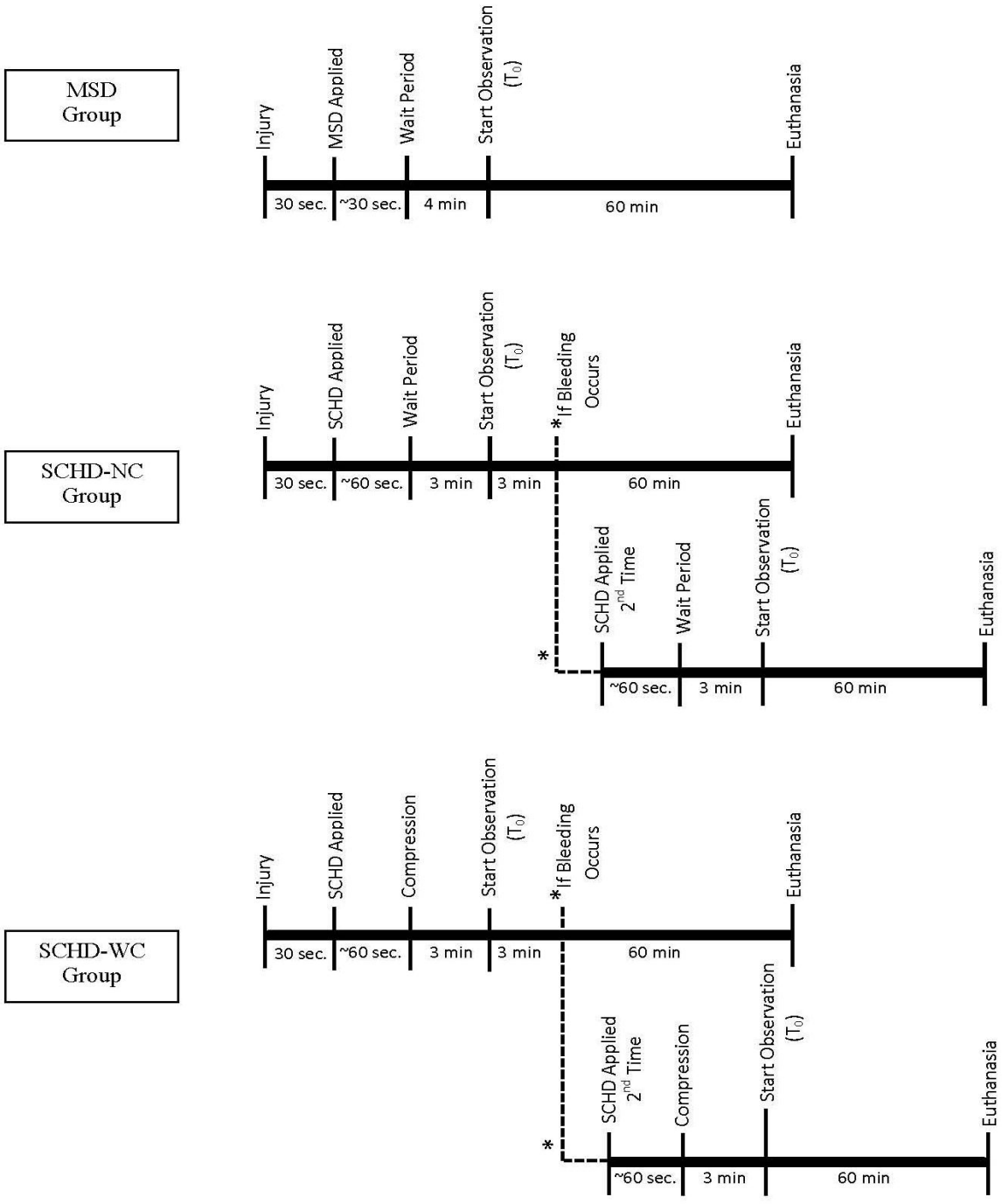


Figure 1. Application Timeline. The injury was accomplished by transecting the subclavian artery and vein. The observation period was 60 minutes (T = 0–60 min). If bleeding was observed in the first three minutes of the wait period, standard-of-care hemostatic dressing (SCHD) was removed and a new SCHD and backing was applied.

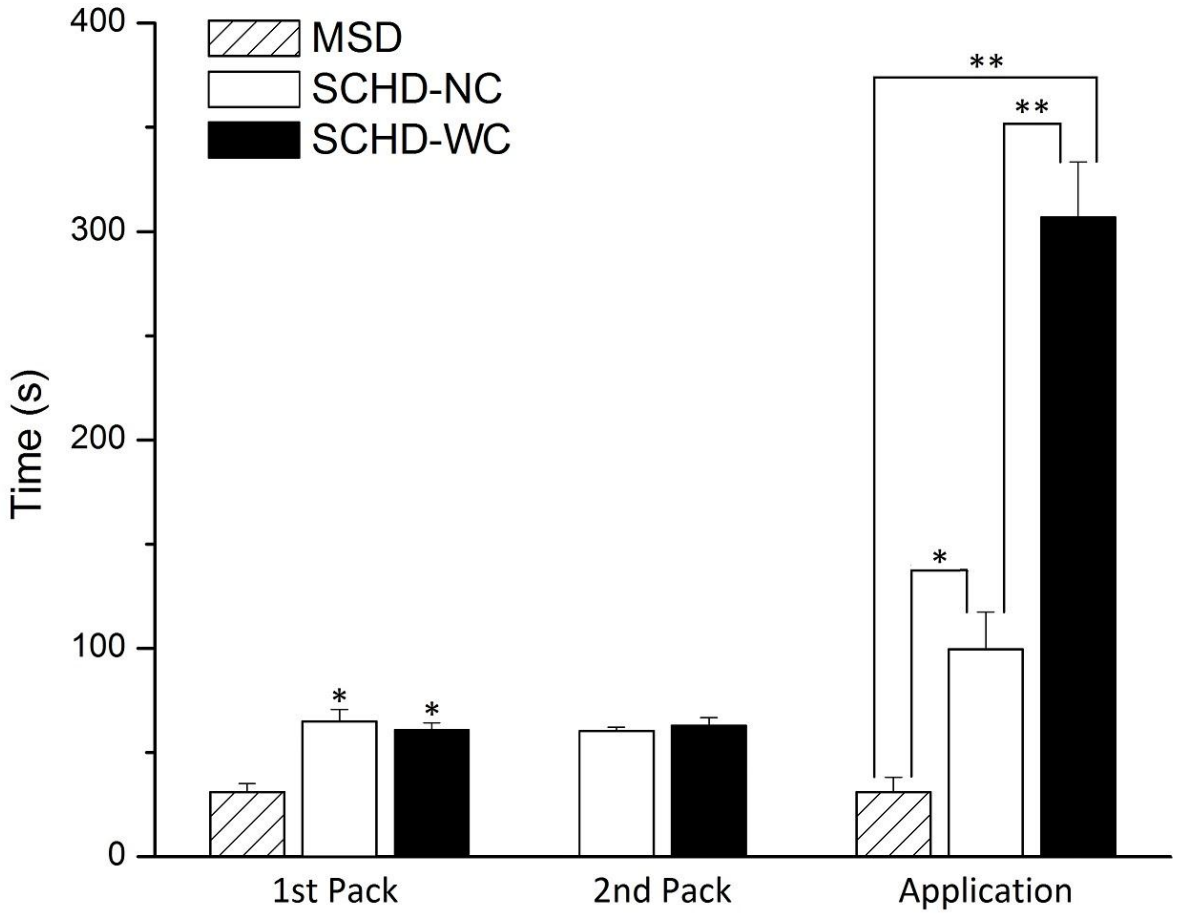


Figure 2. Packing and Application times. Pack time is defined as the time required to administer the agent into the wound, while application time is the total time of physical contact (administration time + compression time).
* Indicates a statistically significant difference ($p < 0.05$) compared to MSD.
** Indicates a statistically significant difference ($p < 0.01$).

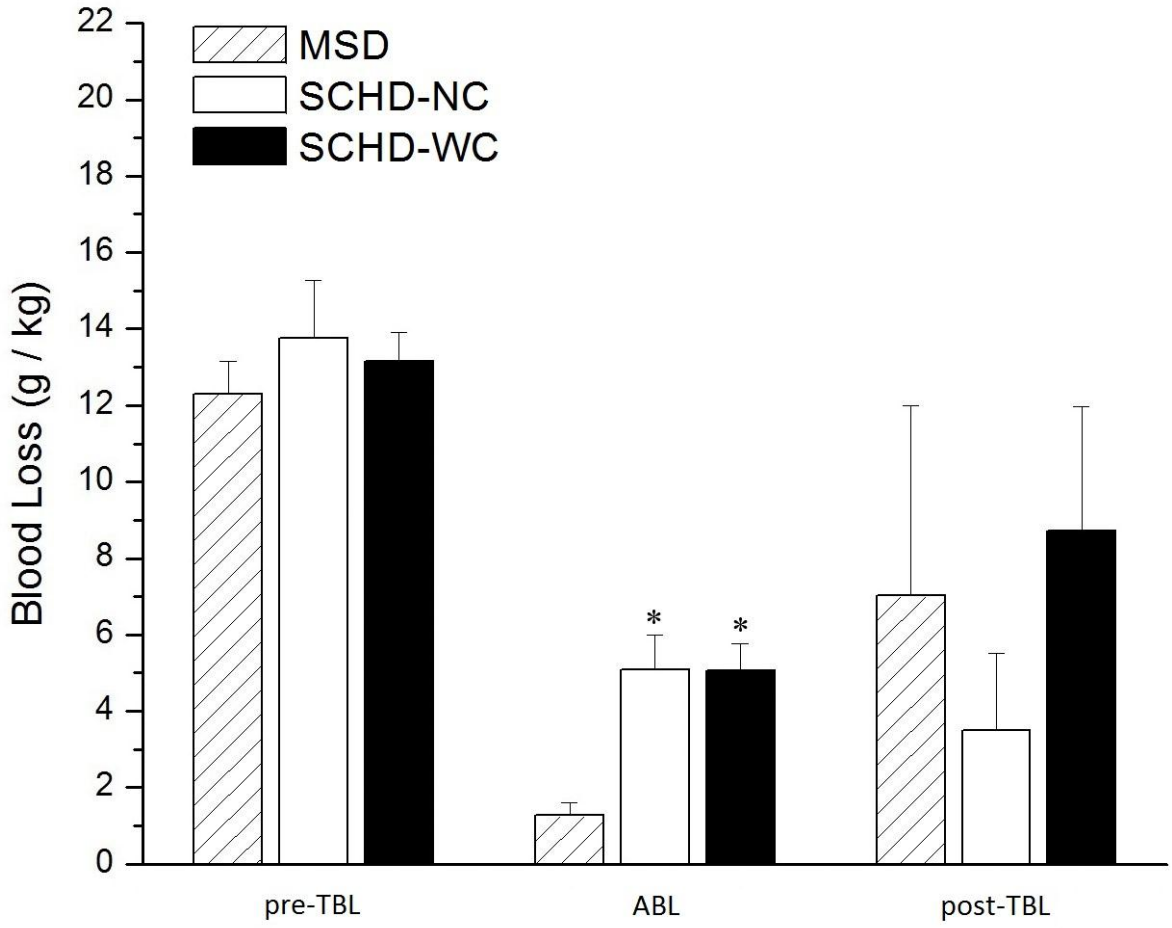


Figure 3. Blood Loss. Blood loss was measured separately for three distinct study periods: pre-treatment total blood loss (pre-TBL), application blood loss (ABL), and post-treatment blood loss (post-TBL).
* Indicates a statistically significant difference between groups within each study period ($p < 0.05$) compared to MSD.

Table 1. Baseline Animal Characteristics.

	MSD	SCHD - NC	SCHD - WC	p-value
Weight (kg)	66.1 ± 1.2	63.7 ± 3.4	67.9 ± 2.2	0.48
Hemoglobin (g/dL)	11.7 ± 0.4	11.2 ± 0.2	11.1 ± 0.2	0.31
Hematocrit (%)	36.0 ± 1.1	34.6 ± 0.7	34.3 ± 0.5	0.32
MAP (mmHg)	90.5 ± 5.2	80.9 ± 3.7	84.9 ± 4.3	0.33
pH	7.46 ± 0.01	7.47 ± 0.01	7.46 ± .01	0.59
Splenic Weight (g)	2029 ± 77	1687 ± 128	1749 ± 119	0.08
Artery Diameter (mm)	6.7 ± 0.2	7.2 ± 0.3	7.2 ± 0.2	0.28
Vein Diameter (mm)	9.8 ± 0.1	9.6 ± 0.1	9.3 ± 0.1	0.67
Cavity Volume (ml)	131 ± 8	121 ± 7	113 ± 7	0.27
Pre-treatment Blood Loss (mL/kg)	12.3 ± 0.9	13.8 ± 1.5	13.1 ± 0.8	0.64

Note. Data expressed as mean ± SEM.

Table 2. Hemostasis Rate for Each Treatment Group.

	MSD	SCHD - NC	SCHD - WC	p-value
Hemostasis at T0	60 %	50 %	50 %	0.88
Re-bleed	0 %	11 %	14 %	0.53
Hemostasis at T60	90 %	100 %	70 %	0.15
Mortality	0 %	10 %	0 %	0.36

Note. Values are shown as mean ± SEM. Re-bleed is defined as hemostatic failure after T0. Statistical comparison between treatment groups was performed via ANOVA.

REPORT DOCUMENTATION PAGE

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB Control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. Report Date (DD MM YY)	2. Report Type	3. DATES COVERED (from - to)
04 01 14	Technical Report	01 2013 - 10 2013

4. TITLE AND SUBTITLE
The Effect of a Third Generation Hemostatic Dressing in a Subclavian Artery and Vein Transection Porcine Model

5a. Contract Number:
5b. Grant Number:
5c. Program Element:
5d. Project Number:
5e. Task Number:
5f. Work Unit Number: G1102

6. AUTHORS
Bjorn K Song, PhD; Forest Sheppard, CDR, MC, USN; Antoni Macko, PhD; Rene Alvarez, PhD

7. PERFORMING ORGANIZATION NAME AND ADDRESS
Naval Medical Research Unit San Antonio
3650 Chambers Pass, Bldg. 3610
JBSA, Fort Sam Houston, TX 78234-6315

8. SPONSORING/MONITORING AGENCY NAME AND ADDRESS
DoD Hemorrhage and Resuscitation Research and Development Program
Combat Casualty Care Research Program US Army Medical Research and Materiel Command
Fort Detrick, Maryland

9. PERFORMING ORGANIZATION REPORT NUMBER
Report No. 2014-22

10. SPONSORS/MONITOR'S ACRONYM(S)
JPC-6

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

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution A

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Background: Hemorrhage is the leading cause of preventable battlefield death. Non-compressible injuries to junctional regions account for 20% of these deaths and present a challenge to medical care providers. A novel minisponge-based dressing (MSD), has been developed to control junctional hemorrhage. The MSD is a syringe applicator filled with compressed chitosan coated mini-sponges which are delivered directly into the wound cavity. Initial published results for MSD are promising but further evaluation of safety and efficacy is warranted. **Methods**: In a swine model of uncontrolled junctional hemorrhage by subclavian artery (SCA) and vein (SCV) transection, animals were randomly assigned to three hemostatic dressing treatment groups (n = 10 per group); MSD and standard-of-care hemostatic dressing with and without three minutes of manual compression (SCHD) and (SCHD-NC), respectively. Blood loss was measured during; a 30-second free bleed immediately following injury, dressing application, a 3-4 minute wait period and a 60 minute observation period. Primary endpoints were application time, blood loss, hemostasis, and survival. Statistical significance was determined by one-way ANOVA. **Results**: Baseline values for weight, blood pressure, hematocrit, injury cavity volume and SCA and SCV diameters, were not different between groups. Blood loss during free-bleed, overall blood loss and hemostasis were also not different between groups. However, pack time and blood loss during application were lower ($p<0.05$) in the MSD group compared to the SCHD-WC and SCHD-NC groups. **Conclusion**: No differences in survival, hemostasis or overall blood loss were observed between the three groups, however, application time and blood loss during application were lower with MSD compared to the two SCHD groups.

15. SUBJECT TRMS
Hemostatic dressing/agent, mini-sponge based dressing, MSD

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	b. THIS PAGE			Commanding Officer, NAMRU-SA
UNCL	UNCL	UNCL	UNCL	24	**19b. TELEPHONE NUMBER (INCLUDING AREA CODE)** COMM/DSN: (210) 539-5334 (DSN: 389-5334)