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NEGATIVE PRESSURE WOUND THERAPY (NPWT) AT ALTITUDE FOR COMPLEX WOUNDS

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14. ABSTRACT

Introduction: Negative pressure wound therapy (NPWT) for soft tissue injury (STI). Early post-transfers via AE to definitive care may induce bacterial proliferation (BP). However, NPWT/instillation in limiting BP during post-injury AE has not been studied. We hypothesized instillation NPWT during simulated aeromedical evacuation (SAE) would decrease colonization within STI. **Methods:** For STI, two 4 centimeter (cm) dorsal wounds were created in 34.9 $\pm$ 0.6 kilogram (kg) porcine and inoculated with *Acinetobacter baumanii* (AB) or *Staphylococcus aureus* (SA) 24 hours (h) prior to a 4h SAE or ground control. Randomized models: wet-to-dry (WTD) dressing, NPWT, instillation NPWT-normal saline (NS-NPWT), instillation NPWT-normosol® (NM-NPWT), and RX4-NPWT. Complex wound (CW) were inoculated with AB 24h prior to SAE with WTD or RX4-NPWT dressings. Collected samples at baseline, pre-flight, and 72h post-flight. **Results:** SAE did not affect BP. The STW arm demonstrated a decrease in SA/AB SAE using RX4-NPWT. NS-NPWT during AE effectively prevented BP than WTD dressing. There was no difference in colony forming units (CFU). **Conclusion:** The environment did not independently affect bacterial growth. RX4-NPWT provided effective bacterial reduction post SAE, followed by NS-NPWT. Future research to determine ideal instillation fluids, pressure settings, and dressing change during AE is recommended.

15. SUBJECT TERMS
wound management, aeromedical evacuation, irrigation, Acinetobacter, MRSA, negative pressure wound therapy

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TABLE OF CONTENTS

DSICLAIMER:

The following final technical report provides results regarding the instillation of NPWT during SAE would decrease bacterial colonization within STI and CW. The funded study title " NPWT at altitude for complex wounds". The research efforts involving large pre-clinical models were reviewed and approved by the University of Cincinnati Institutional Animal Care and Use Committee as well as the Air Force Medical Support Agency Office of Research Oversight and Compliance. The final report will include information covering the methods, results for each research activity.

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1.0 INTRODUCTION:

Complex musculoskeletal wounds (CMW) comprised up to 50 percent (%) of all injuries incurred during the Global War on Terror (1). A total of 52,143 veterans were wounded in action during Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) contributing heavily to US health care and personal functional costs (2). Although advances in protective equipment and earlier operative intervention have decreased mortality and limb amputation rates, extremity wounds continue to cause significant morbidity. A large part of wound-related morbidity is due to the high rate of infection observed in CMW upon arrival at tertiary care centers (3). The most commonly isolated bacteria from CMW during military conflicts in Afghanistan and Iraq include AB and SA (3-9). Following complex musculoskeletal injury, military personnel are stabilized by a forward surgical team and treated in the combat theater for the first 24h to 48h. Patients then undergo transport via the AE system which consists of aircraft with cabins pressurized to 8,000 feet (ft) (10, 11). A previous study suggested that exposure to a hypoxic, hypobaric environment during this transport increases bacterial burden within CMW (12). NPWT is ideal for use in austere environments with limited resources and personnel as it allows for less frequent dressing changes and protects the wound from additional contamination while preventing wound desiccation. Prior studies have shown that NPWT is effective in reducing bacterial loads within CMW acquired in combat (13-17). However, there have been limited studies evaluating the effectiveness of NPWT during AE. Retrospective reviews during OIF and OEF have shown that NPWT is safe and effective during flight with minimal complications (18-20). These reports focused primarily on equipment performance and malfunction but did not specifically address the effectiveness of NPWT in reducing bacterial load and promoting wound healing during and after flight to prevent wound deterioration from the AE process.

Furthermore, it has been shown that early irrigation with large amounts of sterile saline or potable water may reduce bacterial burden within combat wounds (21, 22). NPWT with instillation is a relatively new method of wound management that dwells a programmed volume of fluid within the wound for a set amount of time at predetermined intervals. This technique has been shown to decrease bacterial growth rates within soft tissue wounds over time compared to WTD or standard NPWT (23, 24). Similar to instillation NPWT, continuous saline irrigation with NPWT aids wound healing and bioburden reduction (25). The purpose of this study was to evaluate whether NPWT or instillation NPWT is effective in reducing bacterial load within a soft tissue wound (STW) or CMW during the hypoxic hypobaric conditions associated with AE. Our hypothesis was that the instillation of normal saline combined with NPWT during simulated flight would result in a decreased bacterial burden within the wound post-flight.

2.0 METHODS:

Animal Model

This study was reviewed and approved by the University of Cincinnati Institutional Animal Care and Use Committee and by the United States Air Force Medical Support Agency Office of Research Oversight and Compliance. Animals were cared for by a program approved by the Association for Assessment and Accreditation of Laboratory Animal Care International and in compliance with the National Research Council's 2011 Guide for the Care and Use of Laboratory Animals as well as Department of Defense Instruction 3216.01. Female yorkshire pigs weighing 34.9 $\pm$ 0.6 kg were obtained from Isler Genetics (Prospect, OH) and acclimated in the animal facility for 48-72h before experimentation. Animals were housed alone or in pairs and provided with food and water without restriction, except for the night prior to study initiation to prevent aspiration during induction of anesthesia. Pigs were sedated with tiletamine hydrochloride (Telazol) and xylazine hydrochloride (each 5 milligram per kilogram (mg/kg) administered intramuscularly; Henry Schein Animal Health, Dublin, OH). Sedated pigs were placed in a supine position and orotracheally intubated, then maintained on a ventilator (Ohmeda, Madison, WI) in pressure control mode during non-altitude portions of the experiment and transferred to a Zoll 731 Series Ventilator for simulated AE due to approved performance at altitude for ventilation.

Bacterial Inoculation

Clinical strains of AB and SA were acquired from a collaborating lab at Shriners Children's Hospital (Cincinnati, OH). These samples were aliquoted into 200 microliter (μL) portions and stored in a -80 degrees Celsius (°C) freezer. Four days prior to inoculation, the AB samples were thawed and mixed with 5 milliliter (mL) portions of tryptic soy agar broth (Thermo Fisher Scientific, Waltham, MA). SA samples were thawed and mixed with 5 mL of Luria-Bertani broth (Thermo Fisher Scientific, Waltham, MA). Stock cultures were grown for approximately 92 h in a 37°C incubator at which time a dilution of 10^8 CFU)/mL was obtained and confirmed via spectrometry.

Porcine Injury Model and Wound Treatment Groups

Swine were assigned into one of two wound model arms: STW or CMW. Each arm had a ground and flight component. Pigs within the STW arm of the study underwent creation of a dorsal STW on the cephalad portion of the torso as depicted in **Figure 1A**. Sharp dissection was utilized to create two 4x4 cm soft tissue defects to the level of the fascia with a connecting bridge between to facilitate NPWT placement with a single wound vacuum post-injury. After hemostasis was achieved, 200 μL of 10^8 CFU/mL AB was inoculated into one side of the wound and 200 μL of 10^8 CFU/mL SA was inoculated into the other side of the wound (**Figure 1A**). The wound was then dressed in a WTD dressing for the first 24h post-surgery to simulate a point-of-injury battlefield dressing.

Swine in the complex wound arm underwent an extremity injury model adapted from the United States Army Institute of Surgical Research (21, 26). Briefly, the left hind leg was shaved, prepped, and draped. Models were anesthetized prior to any procedures being performed. A 5x2 cm area of skin and fascia was removed using sharp dissection exposing the peroneus tertius muscle. Crush injury was induced by clamping the exposed peroneus tertius muscle with two Kelly clamps for 1 minute duration. A 4x2 cm portion of the peroneus tertius muscle lying immediately anterolateral to the tibia was then removed using electrocautery (**Figure 1C**). Kerrison rongeurs were utilized

to create a superficial 1x1 cm defect in the periosteum and anterior cortex of the mid-tibia to allow for muscle and bone injury without inhibiting independent post-injury ambulation. After hemostasis was achieved, 200 μL of 10^8 CFU/mL AB was distributed within the wound and a WTD dressing was applied. AB was selected based on its higher prevalence in recent combat wounds and based on the initial data from the simple tissue wound arm of the study. Only WTD and RX-4 NPWT were tested in complex wounds based on the bacterial analysis of the simple tissue wound model. No ground controls were completed for CMW based on the lack of differences in the simple tissue wound arm and to minimize animal utilization.

Three different vacuum-assisted closure (V.A.C.) systems were utilized within this study including: V.A.C. ULTA (Kinetic Concepts Inc., St. Paul, MN), V.A.C. VERAFLO (Kinetic Concepts Inc., St. Paul, MN), and V.A.C. RX-4 (Kinetic Concepts Inc., St. Paul, MN). Swine within the simple tissue wound arm were separated into five groups: WTD dressing, V.A.C. ULTA (NPWT), V.A.C. RX-4 NPWT (RX4-NPWT), V.A.C. VERAFLO instillation NPWT with either NS-NPWT or NM-NPWT (**Figure 2A**). Swine within the CTW arm were separated into two groups: RX4-NPWT and WTD based on the initial data from the simple tissue wound arm of the study (**Figure 2B**). Pigs within the NPWT, NS-NPWT, NM-NPWT, and RX4-NPWT groups each had pre-cut black sponge pieces placed within the wound and covered with sterile plastic drapes (**Figure 1B**). Negative pressure was set to -125 millimeter of mercury (mmHg) on each device as this is the most commonly used setting in military applications. NPWT was initiated just prior to simulated flight to ensure adequate seal to the device across each wound. Pigs within the NS-NPWT and NM-NPWT groups underwent instillation of 36 mL of the designated crystalloid fluid with 10 minutes of dwell time for each hour of vacuum therapy. Following simulated AE, swine were recovered and monitored for 72h prior to euthanasia. During this time, pigs within the WTD groups were maintained in WTD dressings, which were changed at 24h. Pigs in the NPWT, NS-NPWT, NM-SPWT, and RX4-NPWT groups were maintained in a NPWT system using a black sponge with a V.A.C. PREVENA PLUS (Kinetic Concepts Inc., St. Paul, MN) suction device that was secured to the dorsum with a mesh vest, tape, and staples.

Figure 1

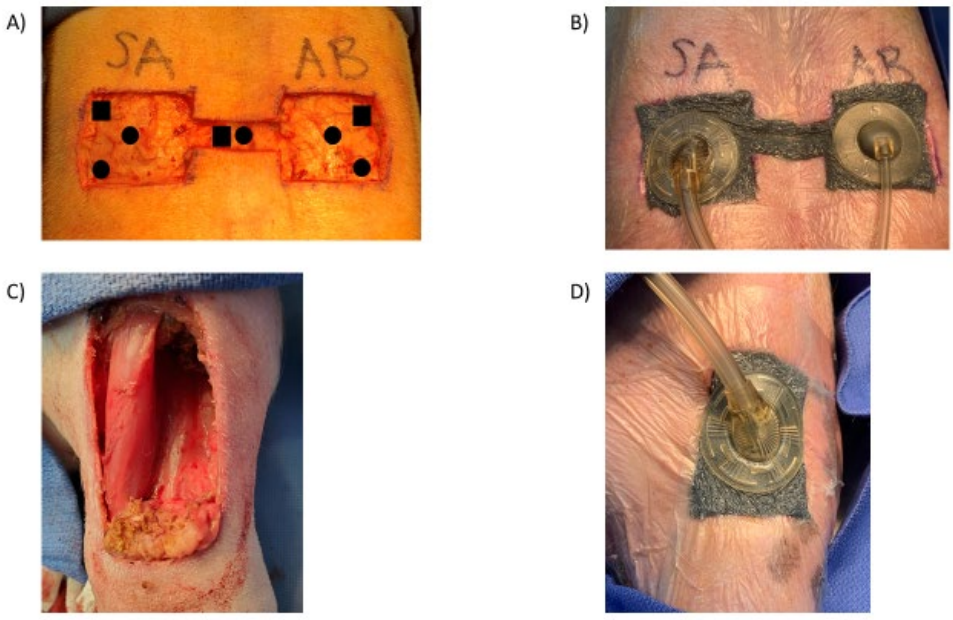


Figure 1: A) Soft tissue wound with overlying diagram showing biopsy locations at 24 and 72 hours post simulated AE. B) Soft tissue wound with negative pressure wound therapy in place. C) Complex musculoskeletal wound on left anterior hindleg demonstrating removal of a portion of the peroneus tertius muscle. D) NPWT is applied to the complex musculoskeletal wound with black sponge.

Figure 2

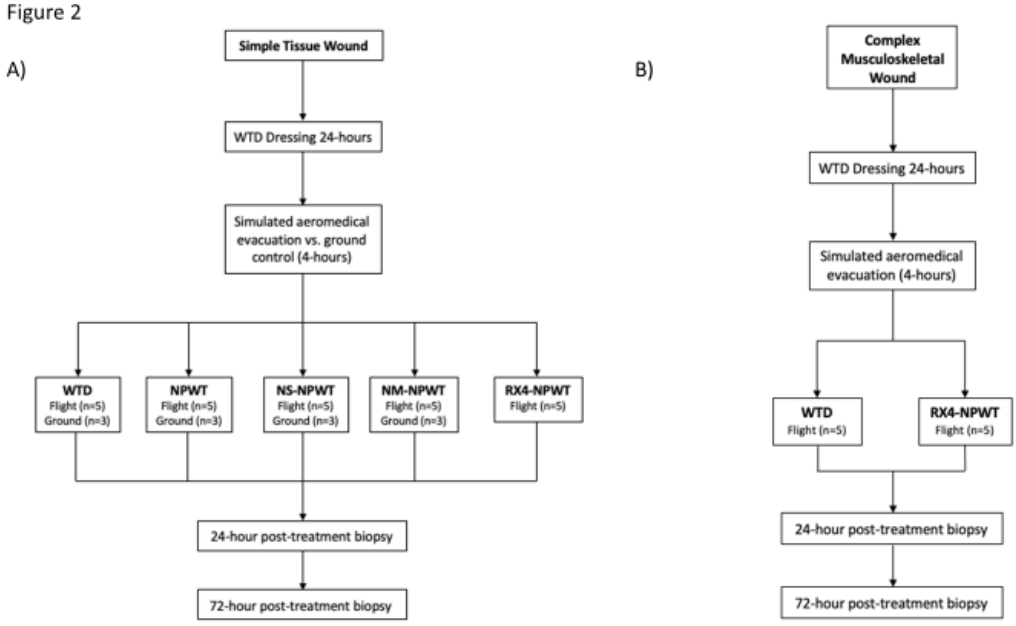
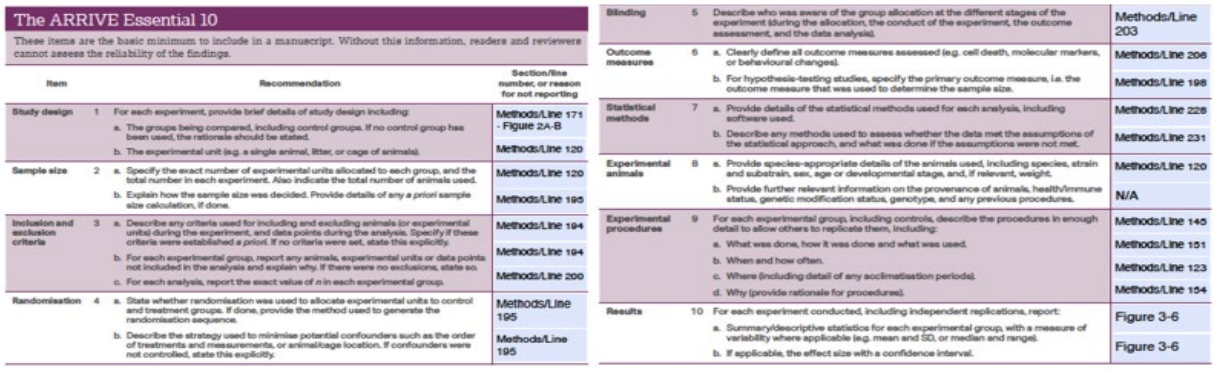


Figure 2: A) Flowchart demonstrating treatment groups for the simple tissue wound arm. B) Flowchart demonstrating treatment groups for the complex musculoskeletal wound arm.

An altitude chamber (Abbess Instruments and Systems, Ashland, MA) was utilized to simulate AE to an altitude of 8,000 ft for 4h (27). Ground control animals were placed within the altitude chamber without a change in altitude. Heart rate (HR), respiratory rate (RR), systolic arterial pressure (SBP), and oxygen saturation (SpO_2) were monitored continuously and noninvasively throughout simulated AE. Swine in simulated AE were maintained at an SpO_2 of 82-85% to simulate a hypoxic environment consistent with an altitude of 8,000 ft (10).

The study was designed to conform with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and a complete checklist has been uploaded as Supplemental Digital Content (**SDC-1**). No pigs were excluded from the study or the analyses. The determination of sample size was derived from the primary outcome of measure being bacterial counts in the wound based on our previous investigation of wounds after altitude exposure. Using previously published data, we estimated that wound bacterial counts would be 50% lower in NPWT compared to WTD-treated wounds, with a 33.3% variance, so that a minimum sample size of four animals per NPWT group was established (12). We included five pigs in each of the flight treatment groups and three pigs in each ground-treated group as the intent of the study was primarily to compare wound dressing groups with simulated altitude exposure.

SDC-1 – ARRIVE Checklist



The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Methods/Line 171 - Figure 2A-B
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods/Line 120
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Methods/Line 120
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods/Line 195
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly.	Methods/Line 194
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	Methods/Line 194
		c. For each analysis, report the exact value of *n* in each experimental group.	Methods/Line 200
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	Methods/Line 195
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods/Line 195
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Methods/Line 203
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Methods/Line 206
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	Methods/Line 198
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Methods/Line 228
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods/Line 231
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Methods/Line 120
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	N/A
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	
		a. What was done, how it was done and what was used.	Methods/Line 146
		b. When and how often.	Methods/Line 151
		c. Where (including detail of any acclimatisation periods).	Methods/Line 123
		d. Why (provide rationale for procedures).	Methods/Line 154
Results	10	For each experiment conducted, including independent replications, report:	
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	Figure 3-6
		b. If applicable, the effect size with a confidence interval.	Figure 3-6

SDC-1: ARRIVE Checklist

Bacterial Quantification

Bacteria were quantified via previously described methods (13, 28). Briefly, tissue samples were collected from each wound at 24h and 72h post-flight. Biopsies were obtained using a 6-millimeter(s)(mm) punch biopsy (Integra LifeSciences, Princeton, NJ) at a depth of approximately 5-mm. Samples were obtained from the lateral inferior aspect of the simple tissue wound at 24h and subsequently from the lateral superior aspect and center of the wound at 72h (**Figure 1a**). Samples were placed in 2mL of Dulbecco's phosphate-buffered saline (Fisher Scientific, Hampton, NH) prior to homogenization. Homogenized samples then underwent a series of dilutions prior to being plated on trypticase soy agar (TSA) plates (Fisher Scientific, Hampton, NH). Plates from 24h samples were incubated at 37°C for 24h prior to quantification of CFUs. Plates from 72h samples were incubated at 37°C for 72h prior to quantification of CFUs. Swine were euthanized on post-injury day 4.

Serum Analysis

Blood samples were collected prior to surgery, prior to simulated AE, and at 72h post AE. Whole blood was placed in serum separator tubes (BD Bioscience, San Diego, CA) and centrifuged at 1000 grams for 10 min. Serum was collected and subsequently analyzed for pro-inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α using a Qplex Porcine Chemokine High Sensitivity enzyme linked immunosorbent assay according to manufacturer protocol (Quansys Bioscience, Logan, UT).

Statistical Analysis

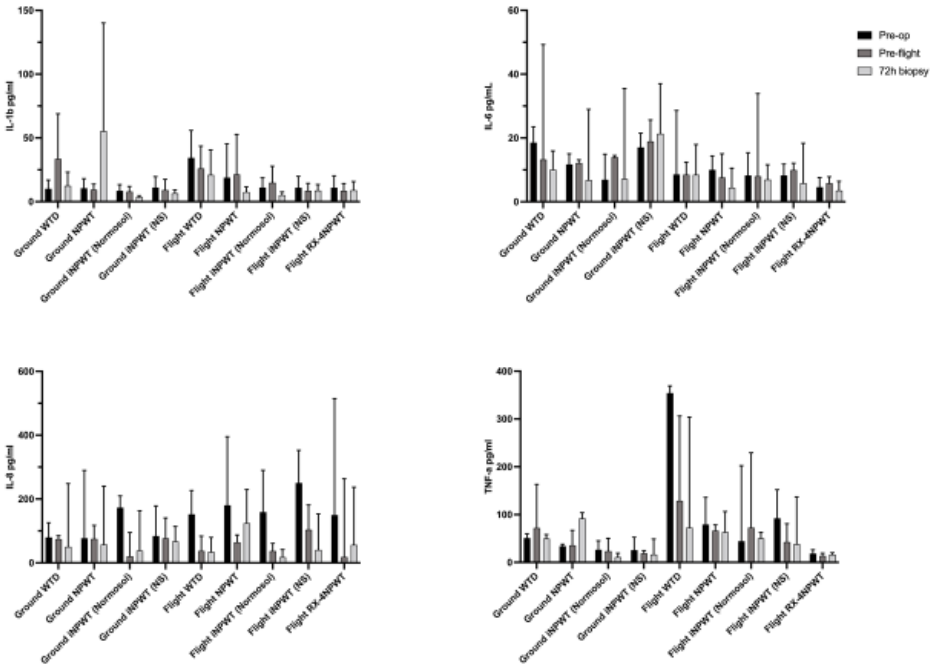
JMP Pro 16 (JMP, Cary, NC) was used for all statistical analysis. Prism 6 (GraphPad Software, La Jolla, California) was utilized to produce graphical figures. Data are presented as the median and interquartile range. Two-way Analysis of Variance (ANOVA) or non-parametric one-way ANOVA was utilized to determine significance between groups based on D'Agostino and Pearson Normality Test. A power (p) value less than 0.05 was considered significant.

3.0 RESULTS

Systemic Inflammatory Response

There were no significant differences in heart rate or temperature among any of the groups for STW or CWM. No differences in serum IL-1β, IL-6, IL-8 or TNF-α were noted between any of the simple or complex wound treatment groups whether at ground or during simulated AE (**SDC-2**). There were also no differences within each treatment group over time during the study, suggesting a lack of systemic response to the bacterial colonization of the wounds.

SDC-2



SDC-2: Bar graph demonstrating cytokines IL-1β, IL-6, IL-8 and TNF-α for all ground and flight animals within the simple tissue wound arm pre-operatively, prior to flight, and at 72 hours post simulated aeromedical evacuation. (median + interquartile range)

Ground Controls

There were no significant differences in SA or AB quantification at 24 hours amongst the various wound management techniques at ground **(Figure 3A-B)**. There were also no differences in SA or AB bacterial load at 24 hours between flight animals and ground controls for each specific treatment modality **(Figure 3A-B)**

There were no significant differences in SA or AB colonization in the simple tissue wound model at 72 hours amongst ground treated groups **(Figure 4, 5).** When comparing each wound management strategy at ground level versus simulated-AE, there were no significant differences in SA or AB bacterial counts at 72 hours post flight **(Figure 4, 5).**

Simulated Aeromedical Evacuation

There were no significant differences in SA or AB quantification at 24-hours between the various wound management techniques during simulated flight **(Figure 3A-B).** The simple tissue wound arm demonstrated a significant decrease in both AB and SA CFU at 72 hours after simulated AE for flight RX4-NPWT treated pigs compared to each of the following groups: WTD, NPWT, NS-NPWT, and NM-NPWT **(Figure 4, 5).** There was also a significant decrease in AB bacterial counts at 72 hours post-flight in NS-NPWT treated animals compared to WTD treated animals **(Figure 5)**. By contrast, there were no significant differences in AB bacterial load at 24- or 72-hours after simulated flight between WTD and RX4-NPWT treated complex wounds **(Figure 6).**

Figure 3

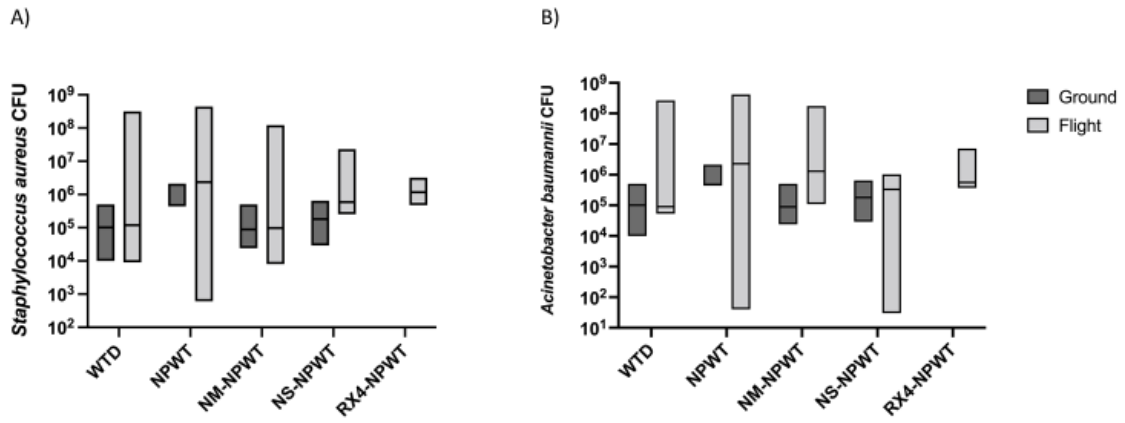


Figure 3: A) Box and whisker plot demonstrating no significant differences in *Staphylococcus aureus* CFU between treatment groups in ground and flight treated animals at 24-hours post-simulated aeromedical evacuation/ground control in simple tissue wounds B) Box and whisker plot demonstrating no significant differences in *Acinetobacter baumanii* CFU between treatment groups in ground and flight treated animals at 24-hours post-simulated aeromedical evacuation/ground control in simple tissue wounds. (median $\pm$ interquartile range)

Figure 4

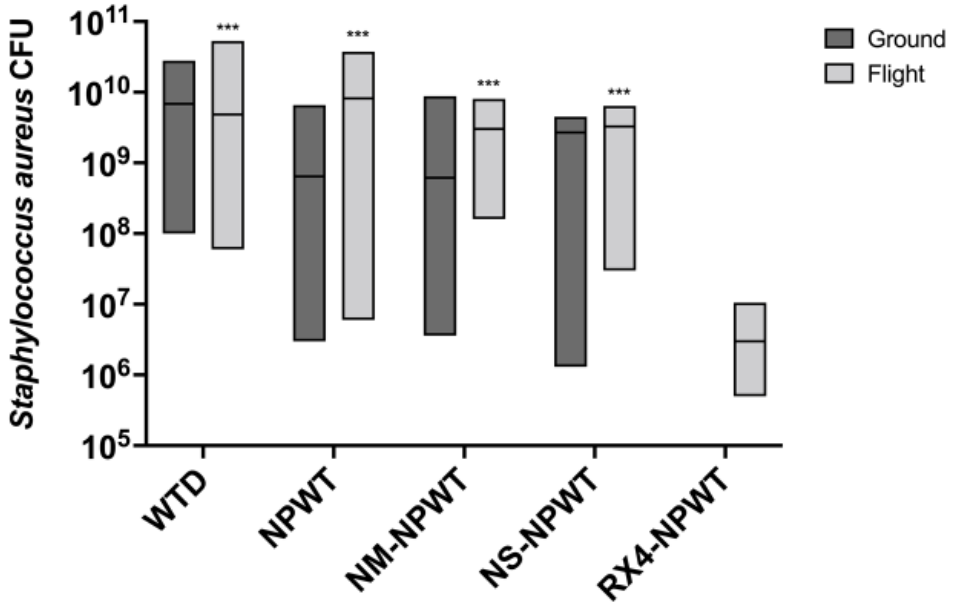


Figure 4: Box and whisker plot demonstrating significant difference in *Staphylococcus aureus* CFU between RX4-NPWT treated simple tissue wounds and all other wound management groups (* above each group denote significant difference between that group and RX4-NPWT). (median $\pm$ interquartile range). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure 5

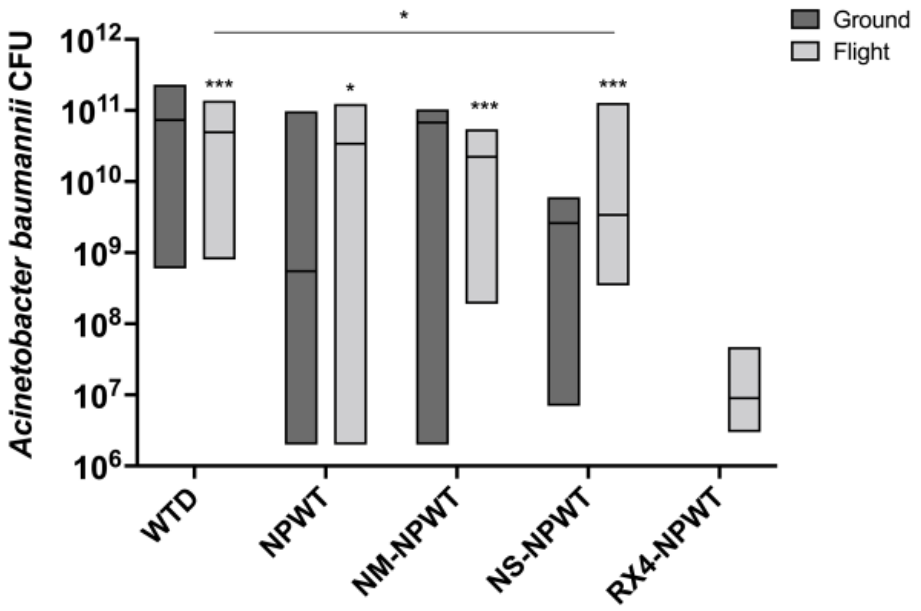


Figure 5: Box and whisker plot demonstrating significant difference in *Acinetobacter baumanii* CFU between RX4-NPWT treated simple tissue wounds and all other wound management groups (* above each group denote significant difference between that group and RX4-NPWT). There is also a significant difference in *Acinetobacter baumanii* CFU between WTD dressing and NS-NPWT at 72-hours post-flight in simple tissue wounds. (median $\pm$ interquartile range) * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure 6

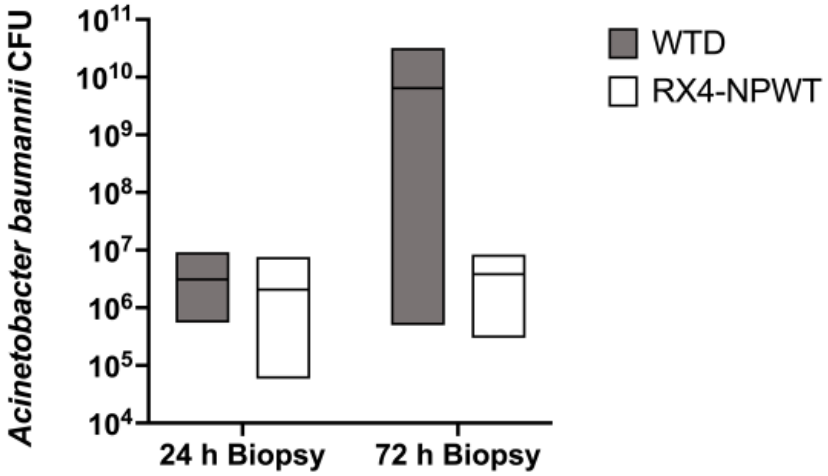


Figure 6: Box and whisker plot demonstrating no significant differences in *Acinetobacter baumanii* CFU between WTD dressing and RX4-NPWT treatment groups at 72-hours post-flight in complex musculoskeletal wounds. (median $\pm$ interquartile range)

4.0 DISCUSSION

In the present study, examined the effect of simulated AE and NPWT on bacterial colonization within STW and CTW. We found that there were no significant differences in SA clearance between ground and flight wounds at 24- or 72h following simulated AE regardless of wound management treatment strategy. These data demonstrated that use of the RX-4 NPWT system is more effective than other treatment modalities at reducing gram-negative bacterial load during simulated. Furthermore, NPWT with NS instillation was more effective than WTD dressings at reducing AB bacterial load by 72h after simulated AE. No differences were observed between simulated flight animals and ground controls treated with the same wound management dressing. Previous work from our research group has demonstrated an increase in bacterial growth during the hypoxic, hypobaric environment innate to AE (12). By contrast, the current study failed to demonstrate similar bacterial growth during AE compared to ground controls. This may be due to the difference in bacteria utilized in the study, as the current study utilized AB and SA whereas the previous study which inoculated *Pseudomonas aeruginosa* in a complex wound. SA is a facultative anaerobe while AB and *Pseudomonas* spp. are obligate aerobes. Therefore, the hypoxic environment during simulated AE may be better tolerated by SA than the other two types of bacteria. The present study supports this theory as there was no significant decrease in SA CFU at 24- or 72h after simulated AE regardless of wound management strategy. However, there was a decrease in AB at 72h within certain groups, which could be caused by the disruption in bacterial growth of AB during simulated AE due to relative hypoxia. Lalliss et. al. have similarly demonstrated no change in SA bacterial load with NPWT while *Pseudomonas aeruginosa* bacterial counts decreased (29). Mouës et. al. performed a randomized clinical trial evaluating bacterial clearance with WTD dressings versus NPWT which actually revealed an increase in gram positive bacteria when using NPWT (30); this may suggest that anaerobic bacteria retain the ability to proliferate even in a NPWT dressing better than aerobic bacteria. Our previous study also utilized a caprine model instead of a porcine model which may have contributed to differences in bacterial growth within the wound.

Since the initial publications in 1997, NPWT has been considered to reduce bioburden within wounds (31). However, multiple studies since that time have shown no change or an increase in bacterial counts within wounds treated with NPWT (23, 30, 32-35). Our results showed that while NPWT limits gram negative proliferation better than WTD dressings on the ground and in flight, bacterial CFU still increase from the time of inoculation to 72h post AE. This may be related to the fact that some previous studies have included wounds that were copiously irrigated prior to NPWT. We chose not to irrigate wounds after inoculation in our study to allow for bacterial growth prior to simulated AE as would occur from the battlefield prior to definitive care.

Instillation NPWT is a relatively new approach to the NPWT technology which allows fluid to dwell within a wound bed based on a preset duration and frequency. This technique has been shown to decrease bacterial growth rates within soft tissue wounds over time compared to WTD or standard NPWT (23, 24). Instillation NPWT demonstrated less bacterial growth than NPWT alone or WTD dressings during flight, but this was only significant for instillation with normal saline. This study utilized Normosol to compare to normal saline to determine if the inherent composition and power of hydrogen (pH) of the instillation fluid would influence the ability to inhibit bacterial proliferation. The results suggest that instillation NPWT with normal saline may reduce bacterial proliferation, which could be due to the pH of 5.0 of normal saline. Davis et. al.

previously demonstrated less *Pseudomonas* growth within a soft tissue wound at 21 days using NPWT with NS compared to WTD or standard NPWT (23). Our results support this finding as NS-NPWT limited bacterial proliferation during flight compared to WTD dressing. Giri et. al. also demonstrated increased bacterial clearance within human wounds at 10 days using NPWT with NS instillation (24). While our study only utilized NPWT with instillation during the 4h simulated AE period, a more significant difference in bioburden may have been observed if the wounds had been treated with instillation NPWT for a longer period of time. However, continuous instillation was not possible in the current ambulatory porcine model, so instillation was only utilized during the simulated flight period with anesthesia used.

The V.A.C. RX-4 system was designed for the military with the purpose of being able to simultaneously manage up to four wounds with one device. It is the first NPWT system approved by the U.S. Air Force for in-flight use since the V.A.C. Freedom therapy unit during the early 2000s (18). A significant limitation in AB bacterial proliferation in the simple tissue wound was noted in RX4-NPWT treated animals undergoing simulated flight compared to all other flight treatment groups. Interestingly, we noted a difference in AB CFU between wounds treated with V.A.C. ULTA NPWT and V.A.C. RX-4 NPWT. Per technical specifications, the V.A.C. RX-4 and the V.A.C. ULTA devices are rated for pressures between 700 Hectopascal Pressure Unit (hPa) and 1060 hPa, which is equivalent to atmospheric pressure between -389.1 meter (m) (1253 f) and 3,010 m (9878 ft). Initially, we attributed the decrease in bacterial count in the RX4-NPWT groups to the RX-4's improved ability to maintain full negative pressure at altitude, however this may not be the case as both systems are rated for the same altitude. There may be other proprietary engineering differences that separate the V.A.C. ULTA and the V.A.C. RX-4 systems, however these are not published. The RX-4 system was designed specifically for Critical Care Transport teams in the U.S. Airforce. Based on our results, the RX-4 NPWT system outperformed the V.A.C. ULTA NPWT system during simulated AE for increased clearance of gram negative bacteria.

There are limitations to our study that must be considered. We chose to study AB and SA based on these being two of the most common isolated bacterial species within contaminated combat wounds (3-9). Other bacteria such as *Escherichia coli, Enterococcus faecium* and *Pseudomonas aeruginosa* are common within military wounds and may require dedicated investigation. This study implemented various types of wound management techniques only during the 4h simulated AE period. During the other time points of the survival period, wounds were either dressed in WTD dressing or standard NPWT using a PREVENA PLUS V.A.C. system (Kinetic Concepts Inc, St. Paul, MN). Although this mirrors clinical practice, it does not completely isolate the portion of AE for bacterial analysis. Some of the differences between WTD and variations of NPWT may be due to NPWT being applied during the entirety of the post-flight survival period. There were also occasional equipment malfunctions with the PREVENA PLUS V.A.C. systems which led to periods of time during which there was a lack of effective negative pressure on the wound. These periods, although infrequent and relatively short are not uncommon clinically as well but could contribute to bacterial growth in an anaerobic environment under the dressing. Lastly, there is little data on ideal instillation volume, duration, and frequency during NPWT. Further investigation will be needed to determine how instillation settings affect bacterial growth within the wound.

5.0 CONCLUSION

Based on our study, utilization of NPWT and instillation NPWT during AE is safe and effective. The hypoxic, hypobaric environment of AE did not independently affect bacterial growth after simple or complex wounds. RX-4 NPWT during flight demonstrates increased bacterial clearance compared to WTD-treated animals for both SA and AB. The RX-4 NPWT system provided the most effective bacterial reduction following simulated AE. While we demonstrate improvement in bioburden, further research will be necessary to establish the effectiveness of RX-4 NPWT at altitude with various injury patterns and types of bacterial contamination. Future studies will focus on determination of ideal instillation fluids, negative pressure settings, and dressing change frequency prior to and during AE.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	percent
±	plus or minus
AB	*Acinetobacter baumanii*
AE	Aeromedical Evacuation
AFRL	Air Force Research Laboratory
ANOVA	Analysis of Variance
ARRIVE	Animal Research: Reporting of In Vivo Experiments
BP	Bacterial Proliferation
CCATT	Critical Care Air Transport Teams
cm	Centimeter
CMW	Complex musculoskeletal wounds
ft	Feet
h	hours
HPW	711th Human Performance Wing
IL-1β	Interleukin 1-beta cytokine
IL-10	Interleukin 10 cytokine
IL-6	Interleukin 6 cytokine
IL-8	Interleukin 8 cytokine
kg	Kilogram
m	meter
mL	milliliter
mm	millimeter(s)
NPWT	Negative pressure wound therapy
NS	normal saline
OEF	Operation Enduring Freedom
OIF	Operation Iraqi Freedom
RR	respiratory rate
SA	*Staphylococcus aureus*
SAE	simulated aeromedical evacuation
SDC-1	Supplemental Digital Content
SpO_2	oxygen saturation
STI	soft tissue injury
STW	soft tissue wound
TNF-α	*tumor necrosis factor alpha* (V.A.C.) vacuum-assisted closure
WTD	wet-to-dry