Negative Pressure Wound Therapy Reduces *Pseudomonas* Wound Contamination More Than *Staphylococcus aureus*

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Purpose: The purpose of this study is to determine if negative pressure wound therapy (NPWT) treatment results in fewer bacteria than wet-to-dry (WTD) dressings in a contaminated open fracture wound model.

Methods: For Study 1, complex wounds were created on the proximal left leg of goats. The wounds were inoculated with *Pseudomonas aeruginosa*. The wounds were débrided and irrigated 6 hours after inoculation. The first group received WTD dressing changes twice daily; the second and third groups received NPWT using systems from two different companies. All three groups received repeat débridements every 48 hours for 6 days. Bacteria quantification was performed both immediately before and after each débridement. For Study 2, the only changes were that *Staphylococcus aureus* was used and only one NPWT group was included.

Results: In Study 1, there were significantly fewer *Pseudomonas* in both NPWT groups at all imaging sessions after the initial débridement and irrigation. At the 6-day time point, the wounds in the NPWT groups were 43 ± 14% and 68 ± 6% of the baseline amount, respectively. The WTD groups were 464 ± 102% of the baseline amount. In Study 2, NPWT did not reduce the *S. aureus* contamination within the wound. At the 6-day time point, the wounds in the NPWT and WTD groups contained 115 ± 19% and 192 ± 52% of the baseline values, respectively.

Conclusion: NPWT showed a significant and sustained decrease in the *Pseudomonas* levels compared with WTD dressings at all time points. This beneficial effect was seen not seen in *S. aureus*.

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INTRODUCTION

Negative pressure wound therapy (NPWT) has been used for treatment of wounds for over two decades.[1] Since that time, it has become widely accepted in the treatment of large, musculoskeletal wounds such as high-velocity gunshot wounds and high-energy fractures.[2,3] Some of the possible benefits of NPWT include increased blood flow to damaged tissue, decreased interstitial edema, increased granulation of wound beds, and increased bacterial clearance.[4–7] Both clinical and laboratory trials have shown an increase in the rate of granulation of these wounds; however, the effects of NPWT on bacterial colonization and infection of traumatic wounds has not been established.[7–9] A recent clinical trial looking specifically at bacterial clearance in wounds treated with NPWT versus those treated with traditional wet-to-dry (WTD) dressings showed an increase in the counts for Gram-positive bacteria and a decrease in Gram-negative bacterial counts.[10]

In this and other previous studies looking at bacterial clearance from wounds, bacterial contamination was measured using quantitative tissue culture biopsies, swabs from various sections of the wound, or by clinical evidence of infection. These data, unfortunately, are subject to sampling errors and offer little information regarding the total surface area of wound contamination. It is likely because of these limitations that the results of these previous studies have varied so greatly. Recently, transgenic, bioluminescent bacteria were used to evaluate the effectiveness of various irrigation pressures to remove bacteria from a wound. This approach has excellent correlation to quantitative cultures and can quantify the bacteria in an entire wound while avoiding sampling errors.[11] We used this bioluminescent bacteria and imaging technology to compare the effect of NPWT and WTD therapy on bacterial contamination of large, complex musculoskeletal wounds.

MATERIALS AND METHODS

All procedures were performed in a laboratory accredited by the Association for Assessment and Accreditation of Laboratory Animal Care after approval of the protocol was obtained from the Institutional Animal Care and Use Committee. Two separate studies were designed to compare

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the effects of the different clinical treatments on *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Wound Creation

A complex, contaminated wound was created in the proximal tibia of 47 castrated, adult male Boer goats (*Capra hircus*). The animals were then sedated with ketamine/midazolam and intubated. Anesthesia was maintained with isoflurane and supplemental oxygen. An epidural injection of morphine (0.1 mg/kg) diluted in 0.9% sterile saline solution to a volume of 0.13 mL/kg was given both as an adjunct to general anesthesia and for its durable postoperative analgesic effect. The goat was then transported to the operating room and placed supine on the operating table.

A 35-cm^2 trapezoidal-shaped area of skin was excised centered 2 cm distal and 1 cm medial to the tibial tubercle. This excision of skin, subcutaneous tissue, fascia, and periosteum exposes the anteromedial tibia. The incision was carried down through the fascia overlying the anterior compartment muscles using Bovie electrocautery to maintain hemostasis. With the anterior muscle compartment exposed, 13 to 15 g of muscle was removed with electrocautery. A 10-mm defect in the medial cortex was created with a coring reamer in the metaphyseal region without disturbing the lateral cortex. The remaining anterior musculature was then freeze-injured by cooling an aluminum rod in liquid nitrogen and placing the rod onto the muscles for 30 seconds. This was repeated for a total of two freezes with 30 seconds' duration each. Electrocautery was then used to cause thermal damage to the fascia, periosteum, and subcutaneous tissues and thus rendering a complex open fracture model (Fig. 1).

Bacterial Preparation and Inoculation

Once the open fracture model was created, the goats were inoculated with either 1 mL of greater than 10^8 colony-forming units of *P. aeruginosa* (lux) (ATCC 27317)[11] or 1 mL of greater than 10^8 colony-forming units/mL *S. aureus* (lux) (Xenogen 29; Caliper Life Science, Hopkinton, MA) each genetically engineered to be luminescent by random chromosomal insertion of the luciferase–luciferin construct lux-CDABE. Twenty-seven animals were inoculated with *P. aeruginosa* (lux) and 20 animals were inoculated with *S. aureus* (lux). The wound was bandaged with Kerlix (Kendall, Mansfield, MA) and Vetwrap (3M Animal Care Products, St. Paul, MN) dressings.

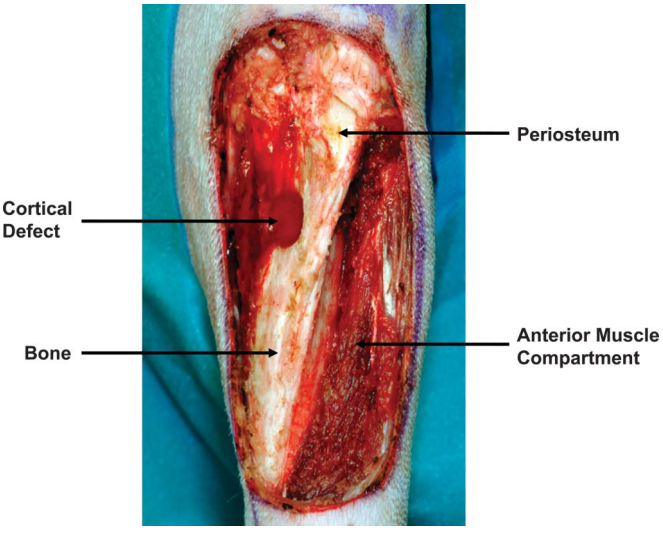

FIGURE 1. Standard wound over the anterior tibia. A complex and reproducible wound involving substantial injury to muscle, fascia, periosteum, and bone was created.

Imaging and Treatment

Six hours after bacterial inoculation, the animals were anesthetized and the wound was imaged to determine the baseline quantity of bacteria. A photon-counting camera was used to capture the quantitative and spatial distribution of the bacteria in the wound.[12]

Once the baseline luminescent data were collected, the wound was surgically débrided and irrigated with 9 L normal saline delivered with cystogram tubing. After treatment, repeat images were obtained of the wound. The limb was then dressed according to its assigned group: WTD, NPWT with sponge, and NPWT with gauze for the wounds inoculated with *P. aeruginosa;* and WTD and NPWT with gauze in the wounds inoculated with *S. aureus*. The WTD dressing consisted of 4-inch Kerlix gauze that had been moistened with normal saline. Dry 4-inch Kerlix gauze was wrapped over the moistened gauze and leg and then completely covered with Vetwrap bandaging tape. Dressings were changed twice daily. The Vacuum Assisted Closure system (Kinetic Concepts, Inc, San Antonio, TX) was used in the NPWT with sponge group and the EZCare system (Smith & Nephew, Key Largo, FL) was used in the NPWT with gauze group. The plastic adhesive drape was placed over the gauze or sponge, and the dressings were then covered with dry 4-inch Kerlix gauze and Vetwrap bandaging tape. The NPWT units were placed approximately 5 feet off the floor on a rotating platform suspended from the top of the cages. Débridement and irrigation of the wounds in all three groups were repeated at 48 and 96 hours as were the pre- and postirrigation imaging. At 144 hours, the wounds were imaged and the animals were euthanized.

Samples of muscle within the wound and from the contralateral tibialis anterior were then obtained and lyophilized for wet-to-dry weight ratios and to calculate the edema index (ratio of muscle wet-to-dry weight of experimental leg/ratio of the wet-to-dry muscle weight of the contralateral leg).

Data Analysis

The location and intensity in terms of photon number were created by superimposing this image onto a gray-scale background. Photon counts at each time point compared with the baseline photon counts were calculated to include the mean and standard error of the mean. All data were analyzed using one-way analysis of variance with repeated measures. SAS statistical software (SAS Institute, Cary, NC) was used for the bioluminescence. The edema index data from the two studies were pooled and analyzed with a *t* test. Significance was set at $P < 0.05$.

TABLE 1. Baseline Photon Counts of *Pseudomonas**

WTD	$3.88 \times 10^5 \pm 7.30 \times 10^4$
NPWT with sponge	$3.75 \times 10^5 \pm 7.02 \times 10^4$
NPWT with gauze	$4.60 \times 10^5 \pm 5.64 \times 10^4$

*The photon counts before initial wound débridement and irrigation.
WTD, wet-to-dry; NPWT, negative pressure wound therapy.

RESULTS

Bacterial Luminescence

For the wounds inoculated with *P. aeruginosa,* the bacterial photon count after the initial débridement and irrigation was similar between all the three groups (Table 1). There were significantly fewer bacteria ($P < 0.001$) in both NPWT groups at all imaging sessions after the initial débridement. At the 144-hour time point, the wounds in the NPWT with gauze, NPWT with sponge, and WTD groups contained 43 ± 14%, 68 ± 6%, and 464 ± 102% of the baseline values, respectively (Fig. 2). This quantitative difference between WTD and NPWT is illustrated in images of the different groups at the 144-hour time point (Fig. 3). Two animals required euthanasia before completion of the study and were excluded from the analysis. One animal, in the WTD group, had a pre-existing lung infection that was exacerbated by the procedure and one animal, in the NPWT with sponge group, had an inadvertent spinal injury with placement of the epidural. An additional animal was excluded from the NPWT with sponge group because of operator error and a malfunction in the Vacuum Assisted Closure system group. The evacuation tubing was inadvertently clamped from the 6-hour débridement until the 48-hour débridement, and the alarm did not function.

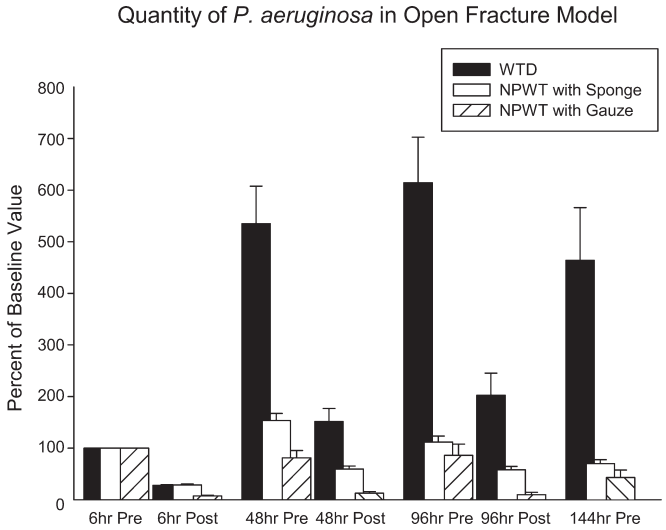

FIGURE 2. Bacterial quantity in wound compared with baseline levels. Comparison of the percentage of *Pseudomonas* remaining in the wound at various time points. WTD, wet-to-dry; NPWT, negative pressure wound therapy.

For the wounds contaminated with *S. aureus*, the bacterial photon count after the initial débridement and irrigation were similar between the two groups (Table 2). Unlike *Pseudomonas,* NPWT did not reduce the amount of *S. aureus* within the contaminated wounds when compared with WTD dressings ($P = 0.37$). At the 144-hour time point, the wounds in the NPWT with gauze and WTD groups contained 115 ± 19% and 192 ± 52% of the baseline values, respectively (Fig. 4). Three animals were excluded from data analysis in this study as well. One animal from each group died before study completion. Postmortem examination confirmed that the deaths were the result of pneumonia. In addition, one animal in the WTD group had no bacteria in the wound starting on Days 4 and 6. A secondary fungal infection was identified with deep wound cultures taken on Day 6 after final imaging. The absence of bacteria within the wound has not been seen in any of the previous studies and was believed to be caused by competitive inhibition resulting from the fungal infection.

Edema

The edema indices for the muscle within the WTD and NPWT wounds were 1.62 ± 0.07 and 1.50 ± 0.05, respectively ($P = 0.16$).

DISCUSSION

NPWT has become the standard of care for many different types of wounds and was used before clinical evidence of benefit.[1–3,5,10] The believed benefits include increasing blood flow around the injured tissue, which may prevent necrosis of the marginal tissues left behind after initial débridement. Furthermore, deformation of the cells under the negative pressure causes an increase in the rate of granulation of these wounds, which may decrease the need for extensive soft tissue coverage procedures.[8,9] The NPWT dressing involves placement of a plastic sheet over the wound to maintain negative pressure in the wound. There is concern, however, that sealing a wound that is contaminated may create an environment that allows further growth of bacteria. The initial studies using this device in a swine model showed a significant decrease in the amount of bacteria in the wound after 5 days of treatment.[4] These results have not been duplicated in the literature despite attempts to collect quantitative cultures in wounds randomized to receive NPWT or standard WTD treatment. Furthermore, these studies evaluated the effect of NPWT on bacteria used superficial wounds and were not complex musculoskeletal wounds.

The possible reasons for the inability of further studies to show a change in the bacterial counts of wounds in controlled experiments are several. First, the variety of wounds and level of initial contamination treated in these studies likely play a role in the clearance of bacteria. The animal model used in this study creates a well-controlled wound, which limits the variations of wounds seen clinically that likely affect infection rates. The technique of quantifying the bacteria can also lead to spurious results. Quantitative cultures used by all of these previous studies sample only portions of the wound. If certain areas of the wound are more contaminated than other areas, wide ranges of bacterial counts can be seen leading

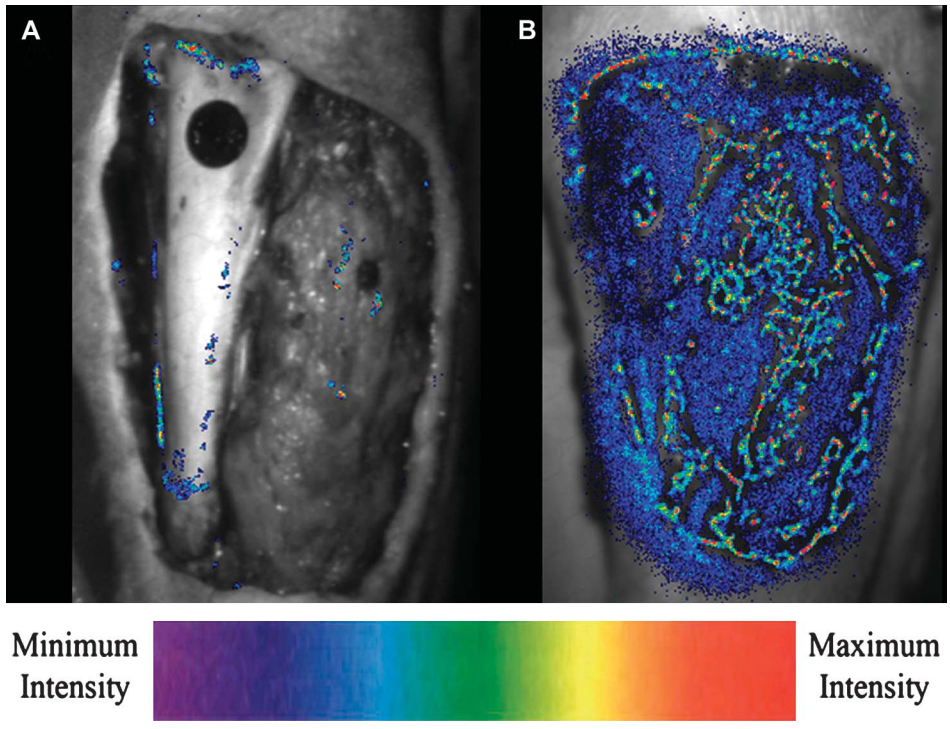

FIGURE 3. (A) Image of the luminescent bacteria within a wound in Study 1 at the 144-hour time point from the negative pressure wound therapy (NPWT) group. (B) Image of the luminescent bacteria within a wound at the 144-hour time point from the wet-to-dry (WTD) group.

to potentially high levels of variance. The type of bacteria contaminating the wounds can also lead to disparities in the results of infection rates between wounds treated with NPWT and WTD. Our results show that distinct bacteria behave differently with each form of treatment.

Using this model, we have shown that NPWT decreases *Pseudomonas* bacterial counts in an open fracture with soft tissue loss as early as 42 hours after initiating therapy. Although the wounds contaminated with *S. aureus* also had fewer bacteria in the NPWT treatment group than the WTD treatment group, this difference was not significant; a post hoc power analysis indicates that 30 animals would be required in each group. The results from this study are in agreement with Mouës et al who also found a difference between Gram-positive and Gram-negative bacteria.[10] These results are not surprising when you consider that *Pseudomonas* is an opportunistic bacteria. It poses a significant clinical problem in those who are immunocompromised such as diabetics, burn patients, and the elderly,[13] whereas *S. aureus* is virulent in all patient populations. Treatment with NPWT creates a better wound healing environment by increasing blood flow and granulation tissue within the wound bed,[6,7] which can augment the host's response to injury and bacterial contamination.

TABLE 2. Baseline Photon Counts of *Staphylococcus aureus**

WTD	$1.91 \times 10^5 \pm 3.33 \times 10^4$
NPWT with gauze	$1.89 \times 10^5 \pm 3.36 \times 10^4$

*The photon counts before initial wound débridement and irrigation.
WTD, wet-to-dry; NPWT, negative pressure wound therapy.

The wound that was treated with the NPWT, which was not receiving suction for the first 42 hours, showed some interesting results worth discussion. The wound bacterial counts at the 48-hour débridement were approximately three times higher than the NPWT group. This indicates that maintaining suction with the device is vital, and the use of suction equipment that cannot detect leaks in the system should be avoided in a clinical setting. Despite thorough débridement and reapplication of NPWT, the bacteria level

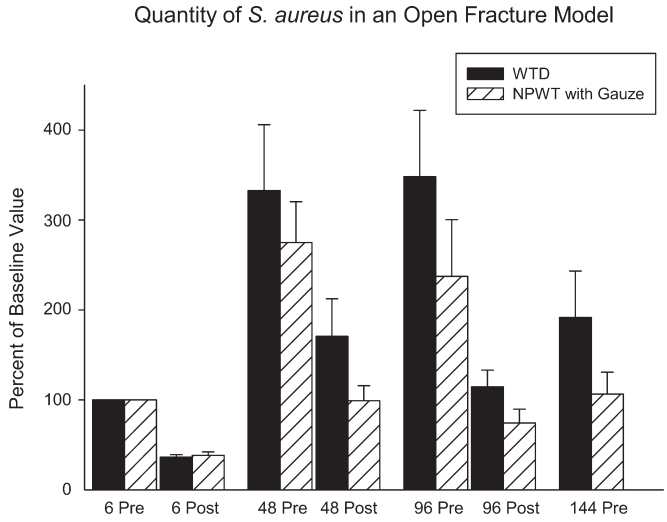

FIGURE 4. Bacterial quantity in wound compared with baseline levels. Comparison of the percentage of *Staphylococcus aureus* remaining in the wound at various time points.

remained higher than both of the NPWT groups and was 10-fold higher at the 144-hour time period. This problem underscores the importance of maintaining suction to the device as much as possible and a warning system should suction be lost for extended periods of time.

We found no difference in the effects of the different devices used to deliver the NPWT. Both the Vacuum Assisted Closure system and the EZCare system showed similar results indicating that the delivery of negative pressure to the wound is the key to its benefit and not the particular device or dressing. The wounds treated with NPWT appeared less edematous and had more granulation tissue. The edema index trended toward being lower in tissue from NPWT groups. Although not statistically significant, this may be biologically relevant reduction in edema because very small changes in tissue edema can have a significant effect on tissue pressure and thus perfusion.[14]

There are limitations to this study. An animal model study with a single type of wound and only two species of bacteria was used. The NPWT units were placed in an elevated position to prevent the animals from interfering with their normal function; therefore, more demand was placed on the pumps to remove fluid. Antibiotics were not given to the animals to eliminate this as a confounder, and the period of treatment was relatively short. This being said, the data clearly demonstrate that wounds treated with NPWT, regardless of the device manufacturer or dressing, had a reduction in *Pseudomonas* bacteria and not in *S. aureus*. This clinically relevant data could only be demonstrated using an animal model such as this.

CONCLUSION

The effectiveness of this type of treatment on the bacterial clearance of infected and contaminated wounds has not been completely elucidated. This study demonstrates that in a contaminated animal open fracture model, NPWT can reduce *Pseudomonas* as early as 42 hours after initiation of therapy and that the benefits are technology-dependent not manufacturer-dependent. This beneficial effect was not seen in the wounds contaminated with *S. aureus.*

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