

Silver Dressings Augment the Ability of Negative Pressure Wound Therapy to Reduce Bacteria in a Contaminated Open Fracture Model

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Background: Despite a lack of evidence supporting their use, silver dressings are often used with negative pressure wound therapy (NPWT). This study investigates the effectiveness of silver dressings to reduce bacteria in contaminated wounds when used with NPWT.

Methods: Complex orthopedic wounds were created on the proximal left legs of anesthetized goats. The wounds were inoculated with either a strain of bioluminescent *Pseudomonas aeruginosa* or *Staphylococcus aureus*. These bacteria are genetically modified to emit photons, thereby allowing quantification of bacterial concentration with a photon-counting camera system. The wounds were debrided 6 hours after inoculation and were treated with silver impregnated gauze combined with NPWT. Repeat debridements were performed every 48 hours for 6 days. Imaging was performed pre- and postdebridement. These results were compared with standard NPWT controls that used dressings without silver.

Results: There were fewer bacteria in the silver groups than the standard NPWT groups at 6 days. In the groups that were inoculated with *P. aeruginosa*, wounds in the silver group contained $21\% \pm 5\%$ of baseline bacterial load compared with $43\% \pm 14\%$ in the standard NPWT group. The addition of the silver dressings has a more pronounced effect on *Staphylococcus*. Wounds in the silver group contained $25\% \pm 8\%$ of baseline bacterial load compared with $115\% \pm 19\%$ in the standard NPWT group.

Conclusions: The use of silver dressings with NPWT is a fairly common practice with limited literature to support its use in contaminated wounds. This study demonstrates that the addition of a silver dressing to NPWT effectively reduces bacteria in contaminated wounds and is more beneficial on the gram-positive bacteria. These data support the use of silver dressings in contaminated wounds, particularly ones contaminated by *S. aureus*.

Key Words: Contamination, Infection, Open fracture, Negative pressure, Silver dressing.

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This study has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations and in accordance with the principles of the Guidance for the Care and Use of Laboratory Animals.

Smith & Nephew loaned the negative pressure devices to the authors for the studies.

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Initially designed for the treatment of chronic, nonhealing wounds, the indications for negative pressure wound therapy (NPWT) have quickly expanded.^{1–3} It is commonly used in the treatment of large wounds and acute open fractures, and has even been applied to surgical incisions in an effort to minimize wound drainage.^{4,5} Recent reports have demonstrated that NPWT may offer some beneficial effects on bacterial clearance within the wound.^{4,6,7} In an effort to reduce infection, there is a growing interest in combination therapy, which uses NPWT in conjunction with an antimicrobial agent or device.^{8–12}

One adjunctive treatment recently reported in the literature is the addition of silver to the NPWT dressing.^{12–14} Antimicrobial activity of silver is well established and has been used for many years.^{15,16} Silver, although inert in its metallic state, is ionized when in moist environment such as a wound. The ionized form of silver is highly reactive and interacts with negatively charged molecules within a bacterial cell, leading to disruption of DNA replication, as well as cell wall and cell membrane function. Although the use of silver dressings in the treatment of burns and chronic ulcers has demonstrated reduced infection rates, there are no investigations demonstrating the effectiveness of silver combined with NPWT.

The purpose of this study was to determine whether silver dressings in combination with NPWT reduce bacteria using a complex open fracture model in a large animal. We hypothesize that the amount of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the wounds that are treated with NPWT with silver dressings will be less than standard NPWT.

MATERIALS AND METHODS

In Vitro Screening of Silver Dressings

Muller-Hinton plates were inoculated with either *P. aeruginosa* (lux; ATCC 27317) or *S. aureus* (lux) (Xenogen 29; Caliper Life Science, Hopkinton, MA). These are two of the more common infecting organisms in both civilian¹⁷ and combat wounds.¹⁸ These bacteria are genetically engineered to be luminescent by random chromosomal insertion of the luciferase-luciferin construct. *Pseudomonas* was grown to 10^8 cfu/mL concentration and spread evenly over the plates. Small pieces of six silver dressings (~1 cm in diameter) were placed on the plates for 24 hours, and the zone of inhibition

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was measured. The best performing silver dressing on *Pseudomonas* was Acticoat 7 (Smith & Nephew, St. Petersburg, FL) and was chosen to be used in the in vivo experiments. Acticoat 7 and two other dressings (Silverlon; Argentum Medical, LLC, Willowbrook, IL, and V.A.C. GranuFoam; Kinetic Concepts Inc., San Antonio, TX) were evaluated in the same manner using *S. aureus* to confirm effectiveness. The zone of inhibition is the area on an agar plate where growth of the bacteria is prevented. The size of the zone is dependent of the diffusion rate of the antimicrobial and the degree of sensitivity of the microorganism.

Animal Procedures

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 the effects of different clinical treatments on *P. aeruginosa* and *S. aureus* in a complex musculoskeletal wound model. The amount of bacteria within the wounds was compared with historical controls that were treated with standard NPWT (without silver) by the same surgeons.⁶

Wound Creation and Bacterial Inoculation

All animals were fasted overnight before all surgical procedures. After adequate anesthesia using both general anesthetic (combination of ketamine hydrochloride 2.75 mg/kg, midazolam 1.25 mg/kg intravenously, maintained with isoflurane 1% to 5%, in 100% oxygen) and epidural injection (Duramorph 0.1 mg/kg), a complex, contaminated musculoskeletal wound was created on the hindlimb of 13 castrated, adult male Boer goats (*Capra hircus*; Byron Stetser, New Braunfels, TX). As previously described,⁶ a 35 cm² trapezoidal portion of skin and fascia covering the anterior tibia was removed, exposing the anterior tibia and musculature. A portion of the periosteum was removed, and a 10-mm cortical defect was created in the metaphyseal region of the proximal tibia using a core reamer to simulate an open fracture without the need for skeletal stabilization. Bovie electrocautery was used to remove ~13 g of muscle from the tibialis anterior, and a freeze injury was performed to a portion of the remaining muscle by applying a 1 cm × 4 cm metal bar, cooled in liquid nitrogen, for two iterations of 30 seconds. Finally, a thermal injury was performed to all exposed muscle, fascia, and periosteum with bovie electrocautery.

Eight animals were inoculated with 1 mL of >10⁸ cfu/mL *P. aeruginosa* (lux) and five were inoculated with 1 mL of >10⁸ cfu/mL of *S. aureus* (lux) which was spread throughout the wound. The wounds were then bandaged with Kerlix (Kendall, Mansfield, MA) and Vetwrap (3 mol/L; Animal Care Products, St. Paul, MN). After surgery, the goats were recovered in their pens and allowed activity ad libitum.

Imaging and Treatment

Six hours after bacterial inoculation, the goats were reanesthetized (combination of ketamine hydrochloride 2.75 mg/kg and midazolam 1.25 mg/kg intravenously, maintained

with isoflurane, 1–5%, in 100% oxygen) and placed supine on an operating table in a custom, light-free imaging chamber. As described previously, a photon-counting camera (Charge Couple Device Imaging System Model C2400; Hamamatsu Photonics, Inc., Hamamatsu-City, Japan) was used to capture the quantitative and spatial distribution of the bacteria within the wound.⁶ After collection of baseline luminescent data, standard debridement and irrigation was performed with 9 L of normal saline using gravity flow low-pressure irrigation. The debridement of nonviable tissue was performed by a surgeon using a scalpel and scissors. The imaging sequence was then repeated to obtain postdebridement and irrigation data. The operative limb was then dressed by placing the Acticoat 7 dressing directly on the wound bed and covering it with a negative pressure dressing. The NPWT units (EZCare system; Smith & Nephew, Key Largo, FL) were placed ~5 feet off the floor on a rotating platform suspended from the top of the cages, and continuous suction at 125 mm Hg was used. Subsequent debridement and irrigations were performed at 48 hours and 96 hours postinoculation with pre- and postirrigation imaging obtained. At 144 hours, the final imaging was obtained, and the goats were killed.

Data Analysis

Gray-scale images were first obtained, and the location and intensity in terms of photon number were created by superimposing the luminescent images onto the gray-scale image. Photon counts at each time point were normalized by baseline photon counts (6-hour predebridement and irrigation). The two treatment groups, those contaminated with *P. aeruginosa* (lux) and those contaminated with *S. aureus* (lux), were compared with historical controls using the same animal model receiving standard NPWT (n = 8 in *P. aeruginosa* and n = 9 for *S. aureus*).⁶ All data were analyzed using two-way analysis of variance with repeated measures and Tukey's post hoc using SAS statistical software (SAS Institute, Cary, NC) with significance set at $p < 0.05$.

RESULTS

In Vitro

All the silver dressings had a zone of inhibition with *P. aeruginosa* when evaluated in vitro, although Acticoat 7 was largest (Table 1). Acticoat 7, Silverlon, and V.A.C. GranuFoam also demonstrated large zone of inhibitions with *S. aureus* (14, 14, and 7 mm, respectively).

TABLE 1. The Effect of Silver on Growth of *Pseudomonas aeruginosa* (lux)

| Dressing | Standard Susceptibility Test Zone of Inhibition (mm) |
|---------------------|--|
| Acticoat 7 | 15.2 |
| Aquacel Ag | 14.6 |
| Arglaes Film | 12.4 |
| Silverlon | 14.4 |
| Silverseal Hydrogel | 9 |
| V.A.C. GranuFoam | 9.6 |

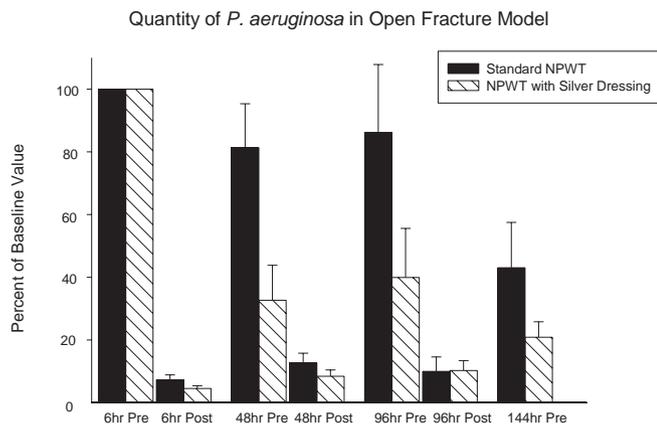


Figure 1. Bacterial quantity in wound compared with baseline levels. Comparison of the percentage of *Pseudomonas* remaining in the wound at various time points. Standard NPWT, negative pressure wound treatment with gauze dressing; NPWT with Silver Dressing, negative pressure wound treatment with a silver dressing.

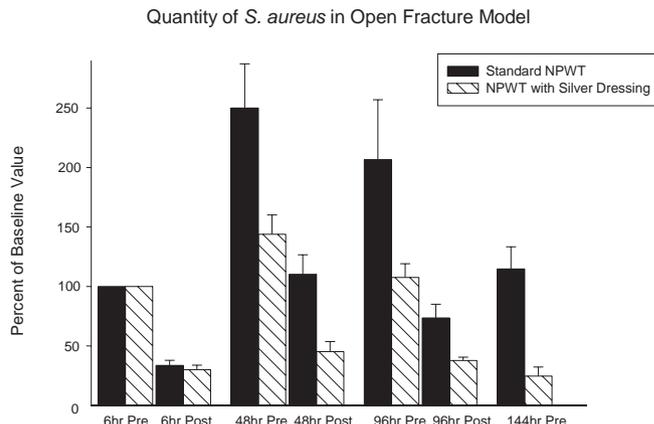


Figure 2. Bacterial quantity in wound compared with baseline levels. Comparison of the percentage of *S. aureus* remaining in the wound at various time points. Standard NPWT, negative pressure wound treatment with gauze dressing; NPWT with Silver Dressing, negative pressure wound treatment with a silver dressing.

In Vivo

Silver dressings effectively reduced the amount of *Pseudomonas* and *Staphylococcus* within the wounds. For wounds inoculated with *P. aeruginosa*, the bacterial photon count after the initial debridement and irrigation was similar between the treatment and historical control group, $5\% \pm 1\%$ and $7\% \pm 2\%$, respectively. There was no group effect ($p = 0.28$) between the standard NPWT and NPWT with silver dressing. Although not statistically significant, there was a trend toward fewer bacteria in wounds treated with silver dressing. At the study endpoint (6 days), the wounds treated with NPWT with silver contained approximately one half of the bacteria when compared with the standard NPWT group ($21\% \pm 5\%$ and $43\% \pm 14\%$, respectively; Fig. 1).

For wounds inoculated with *S. aureus*, the bacterial photon count after initial debridement and irrigation was also similar between the treatment and historical control group, $30\% \pm 4\%$ and $33\% \pm 4\%$, respectively ($p = 0.72$). There was significantly less bacteria within the wounds treated with silver dressing (group effect, $p = 0.0002$). At the end of the study, the wounds treated with NPWT with silver dressings contained much less bacteria than the standard NPWT ($25\% \pm 8\%$ and $115\% \pm 19\%$, respectively; $p = 0.001$; Fig. 2).

DISCUSSION

Silver dressings used in conjunction with NPWT reduced both gram-negative (*P. aeruginosa*) and gram-positive (*S. aureus*) bacteria in this contaminated open fracture model. In wounds contaminated with *P. aeruginosa*, the addition of silver resulted in a 50% reduction in the overall bacterial load when compared with NPWT alone ($43\% \pm 14\%$ vs. $21\% \pm 5\%$). The reduction in bacterial load was even more pronounced in wounds contaminated with *S. aureus* where the bacterial load in wounds treated with NPWT was almost five times higher than that seen in wounds treated with silver used in conjunction with NPWT ($25\% \pm 8\%$ vs. $115\% \pm 19\%$).

Initial reports demonstrating the effectiveness of NPWT on bacterial clearance in contaminated wounds showed a decrease in bacterial load after five days of treatment.² Although one recent prospective, randomized clinical study demonstrated a significant reduction in infections in severe lower extremity open fractures with the soft tissues managed with NPWT,⁴ these results have not been duplicated in the literature. Our previous work, using the same musculoskeletal wound model, demonstrated superiority in bacterial clearance in wounds contaminated with *P. aeruginosa* that were treated with NPWT compared with wet-to-dry dressing changes.⁶ Moreover, these beneficial results were seen in <2 days of treatment. However, this difference was not seen in wounds contaminated with *S. aureus*.

Antibacterial effect of silver is well established in the literature. It is routinely used in topical formulations for the treatment of burns and chronic wounds. However, these older formulations require frequent dressing changes because of rapid inactivation by the wound environment. This led to the development of newer silver formulations,¹⁵ such as the nanocrystalline silver-coated high-density polyethylene mesh, Acticoat, the dressing used in this study. A recent meta-analysis compared infection rates in burn patients treated with nanocrystalline silver dressings to those treated with older silver formulations, such as silver sulfadiazine, and identified a significant reduction in infection rates in those treated with nanocrystalline silver dressings.¹⁶ One advantage to the newer silver dressings is that they have a controlled release of silver, which leads to less frequent dressing changes. In addition, the newer formulations are available as dressings which can then be used in conjunction with other treatment modalities, such as NPWT, as demonstrated in this study.¹²⁻¹⁴

Similar to all preclinical studies, this study has several limitations. Although only one silver dressing was tested in this study, this method of combination therapy could potentially be used with any silver dressing. It must also be mentioned that treatment with a silver dressing can result in

darker tissue discoloration, known as argyria, which may mislead the treating physician to suspect tissue necrosis. This discoloration may not be reversible, so care and judgment must be used to avoid disfigurement to the patients. In these situations, muscle or tissue perfusion, contractility, and consistency must be evaluated before proceeding with debridement. In addition, the effect the silver dressing had on tissue viability was not assessed formally as it was beyond the scope of this study. In addition, although systemic antibiotics are typically administered in the treatment of complex musculoskeletal wounds, they were not used in this study in an effort to minimize confounding variables and to allow independent assessment of the tested interventions.

Open wounds caused by trauma often become infected despite meticulous surgical care and use of systemic antibiotics. Other treatment options are often used to help with NPWT being one of the most common because it helps manage the soft tissue wounds and is believed to reduce infection rates by improving circulation. Silver dressings in conjunction with NPWT are used because of its antimicrobial ability; this is done despite of the dearth of data demonstrating its effectiveness. This study demonstrates the effectiveness of this form of combination therapy, and the results suggest that it may help in the management of more contaminated wounds.

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