The benefits of negative pressure wound therapy (NPWT) are now well established. Multiple trials have shown improved wound healing and faster wound closure with NPWT devices.\textsuperscript{1–3} There are several mechanisms through which NPWT achieves these outcomes, including increased blood flow to the wound bed and removal of surrounding interstitial fluid, which facilitates granulation tissue growth.\textsuperscript{4} Another mechanism that has been proposed is an increased resistance to infection and improved bacterial clearance in the wound. Early studies in pigs suggested that the vacuum-assisted closure device (V.A.C.\textsuperscript{®} Therapy, KCI, San Antonio, TX)
enhanced bacterial clearance in full-thickness wounds that were inoculated with *Staphylococcus aureus* or *Staphylococcus epidermidis*. Further studies in humans have failed to show this benefit, and it remains unclear whether NPWT exerts an antimicrobial effect.

There are a few theoretical ways in which NPWT could promote bacterial eradication or clearance. Mechanical debridement and removal of exudate could physically clear bacteria and biofilms. Increased blood flow could enhance the tissue’s immune response. Increased oxygen delivery may enhance neutrophil oxidative bursts, and the higher oxygen tension may prevent anaerobic growth. Finally, the use of dressings that incorporate the naturally bactericidal element silver, such as the GranuFoam Silver® (KCI, San Antonio, TX) for the vacuum-assisted closure device may aid in stemming bacterial growth through call wall lysis and poisoning of the intracellular processes of bacteria.

These effects are theoretical, though, and may not apply to the clinical setting. The aim of the present study was to test the antibacterial effects of the vacuum-assisted closure device in a controlled animal study using the standard foam and the silver foam. A porcine model was chosen since pigs have skin structure and physiologic mechanisms of wound healing that are similar to humans.

**Methods**

Infected wounds were created with a porcine model as described elsewhere. Six 3-month-old, 25 kg, Yorkshire pigs were acquired and housed in the animal care facility at St. Luke’s-Roosevelt Hospital Center (New York, NY). The animals were cared for in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the National Research Council and approved by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). After a 1-week acclimation to our animal care facility, the pigs were pre-medicated with an intramuscular injection of atropine (0.05 mg/kg) and sedated with ketamine (20 mg/kg) and xylazine (2 mg/kg), followed by intubation and inhalation of a 1% to 3% isoflurane USP mix with oxygen. In a sterile fashion, four 6-cm diameter full-thickness wounds were created on the pigs’ backs. A suspension containing coagulase negative *Staphylococcus, Pseudomonas aeruginosa*, and *Bacteroides fragilis* in a 2:2:1 ratio at a concentration of 10⁷ CFU/mL was prepared and poured into the wound reservoirs. Gauze soaked in the solution was placed in the wounds, which were then covered with a bio-occlusive dressing. The pigs received pain control consisting of an intraoperative dose of intramuscular buprenorphine and a 72-hour Fentanyl patch (50 mg/hr; Janssen Pharmaceutical Products, LP, Titusville, NJ) applied to the back. Three days later, the dressings were removed and quantitative tissue cultures were obtained to confirm an infection (bacterial counts > 10⁶ CFU/g tissue).

On day 0 (three days after bacterial inoculation), the pigs were sedated as above and the wounds were treated with one of the following regimens: V.A.C. device with the standard NPWT foam, V.A.C. device with silver NPWT foam, or a control dressing (saline-moistened gauze without NPWT). Four animals had 3 wounds treated with the silver foam (12 wounds), while two animals had 3 wounds treated with the standard foam (6 wounds). Each animal had a control wound (6 wounds). All NPWT foam were cut to the size of each wound and a porous, non-adherent, non-absorbent dressing was placed in the wound bed under the foam and the saline-moistened gauze to prevent the foam from adhering to the wound. The vacuum-assisted closure devices were all set to intermittent suction—on 5 minutes and off 2 minutes—at a pressure of -125 mmHg. The pigs were individually housed in our animal care facility and had access to food and water ad libitum.

Wound data were collected on days 0, 2, 4, and 7. All wounds were photographed and traced. Quantitative tissue biopsy cultures with 1-cm, full-thickness punch biopsies were obtained from the granulation bed after cleaning the wound surface. Semiquantitative swab cultures were also taken for comparison with the quantitative cultures. Finally, 1-cm punch biopsies were obtained for histologic analysis; tissue slices were stained with standard hematoxylin and eosin (H&E). All pigs were sacrificed on day 7 of treatment. The dressings were changed three times weekly, which corresponds to the clinical application of these devices in the authors’ facility. Additionally, most of the V.A.C. clinical trials allowed for (and in most cases represented the majority of patients) three dressing changes per week instead of the every other day dressing changes that KCI recommends.

Statistical analysis was not applied to the model. This was a pilot study to assess if there was any appreciable difference in bacterial response and wound closure rate between the standard and silver NPWT foam. If we had sought to appreciate a difference, we would have been able to generate a model for an appropriately powered study with the goal of 3-fold reduction in bacteria and a 20% difference in closure rate over 14 days.
Figure 1. Control wounds: Day 2, 4, and 7.

Figure 2. NPWT without silver: Day 2, 4, and 7.

Figure 3. NPWT with silver: Day 2, 4, and 7.
Results

Eleven of the 12 wounds treated with silver foam were fully evaluable, as one became completely contaminated. Four of the 6 wounds treated with regular foam were fully evaluable, secondary to problems with animal behavior and dressing failure. There were 6 control wounds. Contraction and some degree of re-epithelialization occurred in all wounds, but none of the wounds closed during the study period. The decrease in wound area during the study was between 28% and 55%, which did not correlate with the provided treatment. On gross examination, wounds receiving NPWT with either dressing showed considerable clinical improvement. Compared to controls, these wounds had fewer areas of necrosis, less exudate, and improved granulation (Figures 1-3). Histologic examination confirmed this improvement, with the NPWT-treated wounds showing faster granulation, improved cell organization, and faster clearance of neutrophils (Figures 4, 5).

Microbiologic data were obtained for all four wounds receiving standard NPWT therapy, eight wounds receiving NPWT therapy with the silver foam, and all four control wounds. All wounds started the study with significant bacterial counts (> 10^5 CFU/g tissue) and all wounds maintained significant counts throughout the study.
regardless of the treatment received (Figure 6). The average bacterial count rose for all treatment groups. By day 7, all wounds that received NPWT treatment had > 10^8 CFU/g tissue, regardless of whether standard NPWT foam or silver NPWT foam was used. Although the control group started and ended with a lower average bacterial count, it had a faster growth rate; even so, bacterial counts were not significantly different statistically for either treatment compared to the controls. Bacterial counts peaked at day 4 and then dropped by day 7.

While the wounds continued to grow significant amounts of all three of the introduced bacterial strains, all of the wounds also became contaminated with other bacteria, most commonly Proteus vulgaris, Escherichia coli, and beta hemolytic Streptococcus. This infiltration and the bacterial strains involved were consistent despite treatment.

Discussion

As past studies have shown, wounds receiving V.A.C. therapy improve clinically compared to controls. The primary goal of the present study was to assess NPWT’s capability of decreasing the bacterial burden of infected wounds. In this regard, NPWT failed to achieve a significant reduction in bacterial load. Instead, the bacterial load was higher after 1 week of treatment than it was at the start of the therapy in both the NPWT-treated wounds and control wounds. Although the bacterial counts began to trend down by day 7, they were still noticeably high, and the clinical improvement seen in wounds treated with NPWT cannot be attributed to an improvement in bacterial burden.

While this supports the results of human trials that question NPWT’s ability to clear bacteria, they do not provide a definitive answer. The present trial included a limited number of pigs and wounds. While in all cases the bacterial counts were higher at the end of the study than at the beginning, NPWT may have slowed this growth. However, a much larger number of wounds would be necessary to show a significant difference. Also, the downward trend in bacterial counts that began at the end of the treatment week may have continued with further therapy. These data question the absolute rule that bacterial counts alone have a negative role in tissue closure. The idea that 10^5 bacterial is an absolute is obviously becoming a thing of the past. The wound response to bacteria is beginning to be recognized as a composite response to biofilms, local virulence factors, and host response. The porcine model used in the present study explored an acute wound infection model, not a chronic wound model, in a competent host with a defined point of inoculation. Despite bacterial load, the clinical wound improvement clearly demonstrates that we need to look to other markers of clinical infection other than absolute bacterial count.

Another consideration is that perhaps this study may not adequately represent a true clinical situation. We chose to test NPWT in wounds that were obviously clinically infected and had high bacterial counts. While we cleaned the wound surface with saline and gauze prior to biopsy, the wounds were not sharply debrided nor pulse irrigated; a step that is normally performed in our patients before further NPWT therapy. Treatment was stopped at 7 days, a point at which no wound has time to close.

Some clinicians may wonder why we used a non-stick, porous material as the interface between the NPWT foam and the wound. First, such a layer under the V.A.C. must always be used. Generally, it increases comfort and decreases bleeding at the time of dressing change. While some data contradict this practice, the authors believe while many of the properties of the foam itself are important, direct contact with the foam is not mandatory. Also, with a porous, non-stick layer that is only one layer thick, the wound bed maintains (microscopically) all of the macrodeformational characteristics as seen when using NPWT foam alone. The wound also benefits from the foam’s ability to maintain open-air channels even while under significant negative pressure. Despite these limitations, there are several conclusions that can be made: 1) NPWT therapy did not decrease the bacterial burden in the infected wounds; 2) the use of silver NPWT foam did not alter wound outcomes; 3) in a grossly infected wound, the silver NPWT foam did not reduce the bacterial burden when compared to NPWT foam without silver.

Conclusion

The increase in bioburden most likely represents the virulence of the combination of bacteria that were used, and that the dressings were placed directly on infected wounds that had not been debrided. However, wounds treated with NPWT appeared much healthier on gross examination and histology, but this did not correlate with bacterial load. Therefore, the benefit of the V.A.C. should not be attributed to its control of bacterial burden. Clinical appearance is important, despite bacterial burden, because clinical appearance of a wound remains the
primary indicator of wound progress used in real world practice. Clinical appearance is collected and tabulated in all clinical wound trials, and while two wounds may have the same bacterial burden, one might be full of slough and the other granulating. Further research will be needed to test whether NPWT can prevent or slow bacterial growth in wounds that have been adequately treated with means other than NPWT to decrease bioburden. More importantly, the importance of the initial debridement and its effect on wound closure and systemic infection rates in patients being treated with NPWT devices must be determined.

Acknowledgements

The facility performing this work was in part supported by an unrestricted grant from Nitric Bio, Inc (Bristol, Pa). The V.A.C. devices used for the study were provided by KCI (San Antonio, Tex).

References