RESEARCH PAPER

Negative Pressure Wound Therapy With Instillation (NPWTi) Better Reduces Post-debridement Bioburden in Chronically Infected Lower Extremity Wounds Than NPWT Alone

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Abstract

OBJECTIVE: An overabundance of bacteria in the chronic wound plays a significant role in the decreased ability for primary closure. One means of decreasing the bioburden in a wound is to operatively debride the wound for wound bed optimization prior to application of other therapy, such as Negative Pressure Wound Therapy (NPWT). We undertook a prospective pilot study to assess the efficacy of wound bed preparation for a standard algorithm (sharp surgical debridement followed by NPWT) versus one employing sharp surgical debridement followed by Negative Pressure Wound Therapy with Instillation (NPWTi).

METHODS: Thirteen patients, corresponding to 16 chronic lower leg and foot wounds were taken to the operating room for debridement. The patients were sequentially enrolled in 2 treatment groups: the first receiving treatment with operative debridement followed by 1 week of NPWT with the instillation of quarter strength bleach solution; the other receiving a standard algorithm consisting of operative debridement and 1 week of NPWT. Quantitative cultures were taken pre-operatively after sterile preparation and draping of the wound site (POD # 0, pre-op), post-operatively once debridement was completed (POD # 0, post-op), and on post-operative day 7 after operative debridement (POD # 7, post-op).

RESULTS: After operative debridement (post-operative day 0) there was a mean of $3 (\pm 1)$ types of bacteria per wound. The mean CFU/gram tissue culture was statistically greater – $3.7 \times 10^6 (\pm 4 \times 10^6)$ in the NPWTi group, while in the standard group (NPWT) the mean was $1.8 \times 10^6 (\pm 2.36 \times 10^6)$ CFU/gram tissue culture ($p = 0.016$); at the end of therapy there was no statistical difference between the two groups ($p = 0.44$). Wounds treated with NPWTi had a mean of $2.6 \times 10^5 (\pm 3 \times 10^5)$ CFU/gram of tissue culture while wounds treated with NPWT had a mean of $2.79 \times 10^6 (\pm 3.18 \times 10^6)$ CFU/gram of tissue culture ($p = 0.43$). The mean absolute reduction in bacteria for the NPWTi group was 10.6×10^6 bacteria per gram of tissue while there was a mean absolute increase in bacteria for the NPWT group of 28.7×10^6 bacteria per gram of tissue, therefore there was a statistically significant reduction in the absolute bioburden in those wounds treated with NPWTi ($p = 0.016$).

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CONCLUSION: It has long been realized that NPWT does not make its greatest impact by bioburden reduction. Other work has demonstrated that debridement alone does not reduce wound bioburden by more than 1 Log. Wounds treated with NPWTi (in this case with quarter strength bleach instillation solution) had a statistically significant reduction in bioburden, while wounds treated with NPWT had an increase in bioburden over the 7 days.

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Introduction

Chronic wounds often harbor a high bacterial burden that negatively impacts tissue healing.¹ Bacteria disrupt the wound healing process by elevating the metabolic requirement, stimulating a pro-inflammatory environment, and secreting detrimental cytokines that cause vasoconstriction and decreased blood flow to the wound bed.^{2,3} Bacteria attract polymorphonuclear neutrophils which in turn release cytotoxic enzymes responsible for degrading existing extracellular matrix, inhibiting cell migration, and preventing wound closure.⁴ Bacteria may even consume the nutrients and oxygen necessary for tissue repair to occur in the wound.² To minimize the detrimental effects of a high bacterial burden on chronic wounds, surgical debridement is often necessary. Aggressive debridement of chronic wounds helps manage the devitalized tissue, decrease the wound bioburden, and may convert the wound back into the “normal” wound healing cycle of an acute wound.³

One method commonly employed to decrease wound bioburden is operative debridement followed by 1 week of negative pressure wound therapy (NPWT) for further wound bed optimization.⁵ The known beneficial effects of NPWT on a wound bed are the increased local blood flow, rate of granulation tissue formation, and nutrient blood flow.^{2,3,6–10} NPWT has also been shown to expedite wound closure rates compared to standard of care dressing.^{3,5,11,12} Bioburden control is not a recognized potential benefit of NPWT.^{12–16} Although Morykwas et al had initial success in decreasing wound bioburden in a porcine model of infected wounds, those results have not been replicated on human wounds.⁶ In fact, there have been reports of wound bioburden increasing with NPWT compared to standard dressings.¹⁴ Fortunately, as technology advances, new products and devices to combat wound bacterial bioburden are being introduced.

The NPWT device was originally developed with the goal of providing a moist wound-healing environment while protecting the wound from outside bacteria.^{17–19} However, the first model did not sufficiently address wound bioburden. Wound irrigation with the goal of decreasing bioburden is a fundamental next step in the management of open wounds.² Combining NPWT with a therapeutic solution that acts to cleanse the wound may lessen the bacterial bioburden and be a more effective therapy than NPWT alone.²⁰ The NPWT with instillation (NPWTi) device

provides continuous negative pressure therapy followed by delivery of a regulated amount of topical solution to the wound for a short, pre-determined interval, and finally, removal of the solution from the wound site as negative pressure therapy is reinstated. We believe this novel mode of therapy will provide an added benefit to standard NPWT by introducing a bactericidal topical solution that acts to decrease wound bioburden.²¹

While we have used the original instillation model in the past, the NPWTi device has undergone further modifications since its introduction to the market and now offers a more user-friendly console with improved dressing fluid retention.²⁰ This therapy has been used in small, single center pilot studies for treating diverse wounds with differing instillation protocols and various outcomes evaluated.^{2,3,20,22}

The primary objective of this study was to assess the difference in chronic wound planktonic bioburden after operative debridement and 1 week of treatment with either standard NPWT or NPWT with instillation using a mild concentration of Dakin's solution (Century Pharmaceuticals, Inc., Indianapolis, IN), a bactericidal agent. There is literature supporting that Dakin's solution, when used at concentrations below 0.025%, is efficacious in its bactericidal effect while simultaneously having a non-toxic effect on tissues.²³

Methods

Study Design

A single center, prospective pilot study of contemporary case series was conducted between October 2012 and October 2013. The goal was to assess change in bacterial bioburden in chronic lower extremity wounds before and after operative debridement used in conjunction with NPWT versus NPWTi therapy. Patients were sequentially enrolled at random into 2 treatment groups: the first group received treatment with operative debridement followed by 1 week of NPWTi with quarter strength Dakin's solution; the second group received standard therapy consisting of operative debridement and 1 week of NPWT.

A total of 13 patients, corresponding to 16 chronic lower extremity wounds demonstrating significant bioburden (quantitative tissue cultures at baseline were $>10^5$ CFU

per gram tissue) were included for analysis. One patient was involved in both study groups after negligent management of a split thickness skin graft (STSG) at a nursing facility, resulting in failure and a recurrent open wound. Two patients had bilateral lower extremity wounds; each wound was considered individually in the study.

Wound Treatment Protocol

Both groups underwent operative debridement under regional or general anesthesia. Quantitative planktonic tissue cultures were taken both before and after operative debridement on Day 0 and on postoperative day 7 after operative debridement, prior to wound closure with split thickness skin graft. Immediately after debridement on POD 0, either NPWT or NPWTi was initiated for a period of one week. The procedure of instillation for the NPWTi group involved regular delivery of quarter strength Dakin's solution into the wound bed. The instillation therapy setting for all patients in this group consisted of the following: instillation of a predetermined amount of Dakin's solution and a 10 min dwell time during which the wound was bathed in solution, followed by 60 min of negative pressure therapy. The volume of instillation was individualized based on wound surface area as recommended by the manufacturer (0.2 cc per cm² wound area).²⁴ The vacuum-assisted devices were set to a negative pressure of 125 mm Hg in both NPWT and NPWTi groups. There has been data to suggest that using variable or intermittent modes of therapy may be more beneficial than continuous negative pressure, however in order to not confound our experimental variables (namely, the effect of instillation versus no instillation), we opted to utilize a standard level of continuous negative pressure.²⁵

Wound Instillation Agent

Dakin's solution, whose active ingredient is sodium hypochlorite, is a topical therapeutic agent that has been used for wound cleansing since World War I.²⁶ The highest concentration tolerable on skin is 0.5% sodium hypochlorite, which is known as "full strength." The preparation used in this study was 0.125% or "quarter strength," which was chosen for its in vitro efficacy against MRSA, *Pseudomonas* and *Enterococcus*, among others, as well as its cost, availability and ease of use.^{23,27}

Data Collection

This study was reviewed and approved by the Institutional Review Board. All patients provided informed consent in accordance to the Declaration of Helsinki. Patient demographics, including age, sex, comorbidities, were collected from the medical records. Wound data such as etiology, location, surface area, and chronicity were recorded at the first visit to the operating room for

debridement, tissue biopsy, and NPWT or NPWTi placement.

Statistical Analysis

Wilcoxon signed rank test with Hodges-Lehmann estimates were used to determine significance of reduction of planktonic bioburden at post-operative day 7 from baseline (after post-operative debridement) and the maintenance of the reduction of bioburden after debridement to closure. All statistical analyses were carried out using MedCalc, Version 12.7.1.

Results

Patient Demographics

There were a total of 13 patients, with 7 patients in the NPWTi group, corresponding to 8 wounds, and 7 patients in the standard NPWT group, corresponding to 8 wounds (Table 1). The NPWTi group was composed of 2 female and 5 male patients. The mean age was 57. The standard NPWT group was composed of 5 female and 2 male patients. The mean age was 61. The majority of patients in both arms had significant comorbidities: diabetes mellitus (DM), chronic kidney disease (CKD), and hypertension (HTN). Two patients corresponding to 3 wounds in the NPWTi group had a history of deep vein thrombosis (DVT), while 1 patient with 1 wound in the NPWT group had a history of DVT. There were no statistical differences between groups (NS).

Wound Characteristics

Patients in the standard NPWT group had a mean wound chronicity of 30 months while in the NPWTi group had a mean wound chronicity of 23 months ($p = 0.31$) (Table 2). The mean wound surface area was 63 cm² prior to debridement in the NPWTi group and 123 cm² prior to debridement in the NPWT group ($p = 0.94$).

Wound Bacteriology

After operative debridement (post-operative day 0) there was a mean of 3 (± 1) types of bacteria per wound. The most common types of bacteria for both groups were *Staphylococcus aureus*, *Corynebacterium*, and *Pseudomonas aeruginosa*. The mean CFU/gram tissue culture was 3.7×10^6 ($\pm 4 \times 10^6$) in the NPWTi group, while in the NPWT group the mean was 1.8×10^6 ($\pm 2.36 \times 10^6$) CFU/gram tissue culture (Fig. 1). There was a statistically greater number of bacteria in the NPWTi cohort than the NPWT group at baseline ($p = 0.016$), however, at the end of therapy there was a non-statistically significant difference between the two groups ($p = 0.44$).

Table 1 Patient Demographics

Patient	Initials	Age	Gender	Comorbidities	Wound Etiology	Treatment Group
1	DW	57	F	None	Chronic venous stasis	Standard NPWT
2	CA	65	M	DM, HTN, CKD, COPD	Diabetic foot ulcer	NPWTi/standard NPWT
3	FA	48	F	HTN, h/o DVT, hypothyroidism	Unclear etiology, possibly cocaine-induced vasospasm	Standard NPWT
4	CBM	74	F	DM, HTN, CHF, HLD, CAD, A.fib	Diabetic foot ulcer	Standard NPWT
5	VC	57	F	HTN, RA	Chronic venous stasis	Standard NPWT
6	RP	66	M	DM, HTN, HLD, Obesity, A.fib	Chronic venous stasis	Standard NPWT
7	NS	59	F	DM, HTN, HLD	Chronic venous stasis	Standard NPWT
8	WC	56	M	DM, HTN, h/o DVT, OA, CHF	Necrotizing soft tissue infection	NPWTi
9	NA	34	F	DM, HTN, CKD	Diabetic foot ulcer	NPWTi
10	TD	64	M	HTN, BPH, seizure disorder	Trauma	NPWTi
11	TC	54	M	DM, HTN, h/o DVT, hepatitis	Chronic venous stasis	NPWTi
12	BL	64	F	HTN, h/o CVA, liver cirrhosis	Chronic venous stasis	NPWTi
13	TM	62	M	DM, HTN, CKD	Arterial insufficiency	NPWTi

DM, diabetes mellitus; HTN, hypertension; CHF, congestive heart failure; DVT, deep vein thrombosis; CKD, chronic kidney disease; BPH, benign prostatic hypertrophy; CVA, cerebrovascular accident; COPD, chronic obstructive pulmonary disease; OA, osteoarthritis.

At 7 days, the mean number of bacterial species per wound was 2 (± 1) in the NPWTi group, with a decreased number demonstrating *S. aureus* as compared to the NPWT group. Wounds treated with NPWTi had a mean of 2.6×10^5 ($\pm 3 \times 10^5$) CFU/gram of tissue culture while wounds treated with NPWT had a mean of 2.79×10^6 ($\pm 3.18 \times 10^6$) CFU/gram of tissue culture ($p = 0.43$) (Fig. 1). The mean absolute reduction in bacteria for the NPWTi group was 10.6×10^6 bacteria per gram of tissue

while there was a mean absolute increase in bacteria for the NPWT group of 28.7×10^6 bacteria per gram of tissue, therefore there was a statistically significant reduction in the absolute bioburden in those wounds treated with NPWTi ($p = 0.016$).

However this significance was not as great when examined as a percent in change from the wound bioburden at baseline, with each wound acting as its own control. The percentage change in the planktonic bacteria after

Table 2 Wound Characteristics

Number	Initials	Wound Etiology	Wound Site (s)	Wound Duration (months)	Wound Surface Area (cm ²)	Treatment Group
1	DW	Chronic venous stasis	Right lateral lower extremity	36	3.96	Standard NPWT
2a	CA	Diabetic foot ulcer	Right plantar heel	18	1.5	Standard NPWT
2b	CA	Diabetic foot ulcer	Right plantar heel	18	1.5	NPWTi
3	FA	Unclear (cocaine-induced vaso-occlusive disease?)	Right transmetatarsal amputation site	10	7.1	Standard NPWT
4	CBM	Diabetic foot ulcer	Right plantar foot	12	88	Standard NPWT
5	VC	Chronic venous stasis	Right lateral ankle	10	5	Standard NPWT
6a	RP	Chronic venous stasis	Right lower extremity, circumferential	60	323	Standard NPWT
6b	RP	Chronic venous stasis	Left lower extremity, circumferential	60	500	Standard NPWT
7	NS	Chronic venous stasis	Right ankle	36	54	Standard NPWT
8	WC	Necrotizing soft tissue infection	Medial left lower leg	3	294.5	NPWTi
9	NA	Diabetic foot ulcer	Left plantar foot	2	12.25	NPWTi
10	TD	Trauma	Medial right lower leg	30	52.5	NPWTi
11a	TC	Chronic venous stasis	Anterior left lower leg	36	43.55	NPWTi
11b	TC	Chronic venous stasis	Anterior right lower leg	36	5.29	NPWTi
12	BL	Chronic venous stasis	Right ankle	36	5.2	NPWTi
13	TM	Arterial insufficiency	Left transmetatarsal amputation site	24	70	NPWTi

Figure 1 Bacterial bioburden change over time with NPWTi versus NPWT.

debridement was 90% (84%–100%) (absolute bacterial count mean reduction 2.62×10^7 to 2.51×10^6) for the NPWTi group versus 88% (68%–100%) (absolute bacterial count mean reduction 2.14×10^6 to 4.76×10^5) for the NPWT group ($p = 0.87$). The ability to maintain the post-debridement reduced bioburden was a 16% increase in quantitative bacterial count in one week (81% increase to 2% reduction) (absolute mean increase from 4.76×10^5 to 5.74×10^6) for the NPWT group; when the wound was treated with NPWTi there was only a 4.6% increase in bacterial count from post debridement levels (53% increase to 15% further reduction) (absolute mean increase from 2.51×10^6 to 3.15×10^6) ($p = 0.078$). From baseline the NPWT group had a 16% increase in bacteria (8200% increase to 100% reduction) over the course of therapy while the NPWTi group had an 87% reduction (45%–100% reductions) ($p = 0.078$).

Discussion

The use of NPWTi in wound healing has been demonstrated to be useful by many authors. In 1998 Fleischmann proposed instillation of wounds with various solutions to enhance readiness for wound closure or spontaneous healing.²² More recently, NPWTi has been used to treat chronic wounds, infected wounds, osteomyelitis, and surgical implant infections.^{3,19,28,29} The majority of these studies did not investigate the role of NPWTi in reducing bacterial bioburden on quantitative tissue culture. Gabriel et al conducted a study to evaluate the efficacy of NPWTi in bioburden reduction using silver nitrate, and found promising results in clinical infection reduction compared to standard wet to moist dressings.³

The goal of this study was to determine the difference in wound bed preparation, with regard to bacterial bioburden, using surgical debridement in combination with novel NPWTi versus standard NPWT. We were able to

demonstrate that surgical wound debridement followed by one week of NPWTi resulted in a significantly decreased wound bioburden as compared to wound debridement followed by one week of standard NPWT.

Our study confirmed the findings of other authors in that NPWTi reduced the quantity of bacteria, while standard NPWT resulted in an increased bacterial load.^{2,10,12,14,16,17} With regard to bioburden reduction, NPWTi may indeed provide superior therapy when compared to NPWT. In the past, we have found debridement alone to be insufficient in decreasing bacterial bioburden. The wound bacterial load reduction after sharp surgical debridement alone was not more than 1 Log.³⁰ It follows that further wound treatment may be needed in order to achieve optimal bioburden reduction. On post-operative day 7 of this study, following operative debridement and one week of NPWTi, there was a statistically significant reduction of CFU per gram of tissue culture from baseline. This suggests that instillation therapy may provide further eradication of bacterial bioburden, above the ability of sharp debridement. Debridement must not be disregarded, however, as it is a fundamental step in wound care to first remove devitalized, damaged, or infected tissue.⁵ Following appropriate debridement we have shown that 7 days of NPWTi with Dakin's can act to better prepare wounds for healing and closure. It should be noted, that while the bioburden level increased in the wounds treated with NPWT, there were no apparent signs of active clinical infection and NPWT did not preclude split thickness skin grafting (STSG) on this group, on post-operative day 7.

New medical technology is often associated with a period of acclimation until practitioners have identified the optimal usage to best benefit their patients. The NPWTi is a novel method of treatment currently undergoing clinical study. The manufacturer does not recommend any particular topical agent for wound instillation, but rather there are multiple approved solutions including hypochlorite-based

solutions, silver nitrate (0.5%), sulfur-based solutions (sulfonamides), biguanides (polyhexanide), cationic solutions (octenidine, benzalkonium chloride), and isotonic solutions.²⁴

The ideal solution for topical instillation would be efficacious at reducing wound bioburden with limited cytotoxicity.^{2,31} This is a difficult balance to achieve as bactericidal agents commonly inhibit the cellular growth of normal, healthy tissues.^{1,32,33} Povidone-iodine, silver sulfadiazine, and mafenide acetate cream have shown to be cytotoxic to components of wound healing in rodent models.^{1,32} Dakin's solution (0.025%) was shown in vitro not to promote fibroblast toxicity, although keratinocyte monolayers were sensitive to the solution with varying response.³¹ Neodermal thickness of porcine wounds was significantly increased after 7 days of Dakin's treatment, with an early trend toward re-epithelialization.³⁴

In our clinical experience, Dakin's solution has demonstrated success at bacterial clearance of large lower extremity wounds with little obvious toxicity.³⁵ We therefore elected to utilize quarter strength Dakin's solution at the upper level of concentration acceptability as provided in the NPWTi manual.²⁴ Other clinical pilot studies have utilized various agents in infected or colonized wounds (culture directed topical antibiotics, dilute lidocaine mixed with topical antibiotics, polyhexanide antiseptic solution, PHMG, silver nitrate) with promising results.^{2,3,22,28,29,36} Interestingly, sterile water instillation has been successfully used to enhance complex wound closure in non-clinically infected wounds.³⁷ This suggests that it is the action of repeated cycles of instillation and negative pressure that may enhance wound closure, rather than the specific instillation solution. To our knowledge, there have been no clinical studies evaluating the efficacy of multiple types of instillation solution on bacterial bioburden of chronic wounds.

The cost of new medical devices or technology is an increasingly growing concern for both physicians and patients. At this time, the standard protocol at our institution for wound bed preparation is surgical debridement followed by one week of NPWT. The NPWT system that we currently use is a combined NPWT and NPWTi machine (NPWT/NPWTi; V.A.C. Ulta™ Therapy; KCI USA, Inc., San Antonio, TX) and therefore the only additional cost of using the instillation mode lies in the choice of instillation solution. Dakin's solution is a cheap and cost-effective wound treatment solution in the United States. Absolute costs were not assessed in this study, though it would appear that the benefits of bioburden reduction in large chronic wounds in this patient population would provide a socioeconomic benefit as compared to conventional therapeutic modalities.

There are three main limitations that can be traced in our approach. This study was a small, non-randomized study. The type of instillation solution utilized for treatment in this study was limited to Dakin's solution; we did not

compare different types of instillation solutions. Finally, as was alluded to previously, a large prospective trial would better clarify other authors' findings with other instillation solutions. Studies in the future need to examine to efficacy of utilizing various dwell times and cycles of instillation for optimal results.

Conclusion

Bioburden reduction was significantly decreased in chronic wounds using 7 days of NPWTi following operative debridement. Chronic wounds treated with 7 days of NPWT actually demonstrated an increase in bacterial load. In the future, studies should evaluate this therapy not only in larger, randomized controlled trials, but also to assess the impact of NPWTi on wound biofilm.

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