

**Title:** Does negative pressure wound therapy influence subjacent bacterial growth? A systematic review

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## **Abstract**

### *Background*

Negative pressure wound therapy is a ubiquitous wound management resource. The influence of NPWT on the bacterial bioburden of the subjacent wound remains unclear. We sought to examine the evidence.

### *Datasources*

MEDLINE, Embase, PubMed, the Cochrane Database of Systematic Reviews and the Cochrane Controlled Trials Register were searched for articles quantitatively evaluating bacterial load under NPWT.

### *Results*

Twenty-four studies met the inclusion criteria including 4 randomised controlled trials, 8 clinical series and 12 experimental studies. Twenty studies evaluated conventional NPWT while 4 evaluated Infiltration-based NPWT. While 8 studies using conventional NPWT failed to demonstrate an observable effect on bacterial load, 7 studies reported that NPWT was inherently bacteriostatic and 5 others reported species selectivity with suppression of non-fermentive Gram-negative bacilli (NFGNB), including *pseudomonas* spp. Simultaneously, there was some evidence of enhanced proliferation of Gram-positive cocci where the niche was cleared of NFGNB. Two of the 4 studies using infiltration-based NPWT also reported selectively impaired proliferation of *pseudomonas* spp.

## *Conclusion*

The assumption that NPWT suppresses bacterial proliferation is oversimplified. There is evidence that NPWT exhibits species selectivity, suppressing the proliferation of NFGNB. However, this may depopulate the niche for exploitation by Gram-positive cocci. This, in turn, has implications for the use of NPWT where highly virulent strains of Gram-positive cocci have been isolated, as well as the duration of NPWT therapy and frequency of dressing changes.

## Introduction

Since the publication of the seminal papers in 1997 by Argenta and Morykwas <sup>1,2</sup>, negative pressure wound therapy (NPWT) has found widespread application for almost every type of open wound and, more recently, also over closed incisions. Based largely on these papers the purported mechanisms of action, namely promotion of granulation tissue formation, increased blood flow to adjacent tissue and bacterial clearance at the wound bed have become accepted and are often repeated, despite the emergence of experimental studies that have challenged some of these assertions. More recent work has revealed a complex rationale for the apparent clinical benefits of NPWT based on micromechanical deformation with fibroblast proliferation, VEGF-mediated neo-angiogenesis, and modulation of local and systemic expression of cytokines, growth factors and matrix metalloproteinases <sup>3,4</sup>.

The influence of NPWT on the microbiological environment of the subjacent wound is shrouded in confusion owing to the plethora of variables in studies designed to answer the question. Acute, chronic and pre-contaminated wounds have all been investigated. Various sampling methods and qualitative outcome measures have been employed. Intermittent surgical debridement and the simultaneous use of systemic antibiotics have also complicated data analysis. The purpose of this study was to systematically analyse the existing experimental and clinical data to establish if NPWT acts in part by improving bacterial clearance of the wound.

## Patients and methods

### *Search strategy*

Pubmed (no date limit), OVID Medline (1997 to March 2016), Embase (1997 to March 2016), the Cochrane database of systematic reviews and the Cochrane controlled trials register (searched 24<sup>th</sup> March 2016) were searched using medical subject heading (MeSH) terms and free text terms. Articles were searched from 1997 as this year corresponds with the first description of the therapy as it used in contemporary practice. The search strategy and terms used are shown in **supplemental table 1**. Articles were cross-referenced to identify others of interest. Additionally, the online trials registers ClinicalTrials.gov and the national research register (NRR) were scrutinised for completed, discontinued and on-going trials relating to NPWT and bacterial growth within the subjacent wound. The search strategy was performed in accordance with the Cochrane Highly Sensitive Search Strategy guideline in the Cochrane Handbook for Systematic reviews of Interventions. The review is reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement <sup>5</sup>. This study was registered prospectively on the PROSPERO database (CRD42016036914).

### *Inclusion criteria*

Studies that quantitatively or semi-quantitatively evaluated the influence of NPWT bacterial growth within the subjacent wound were included. We included *in-vivo* and *in-vitro* studies, animal models, human clinical studies and randomised controlled trials. Study quality was assessed using the Grading of Recommendations Assessment, Development and Evaluation

(GRADE) approach where study design, risks of bias, imprecision, inconsistency and indirectness to draw reasoned conclusions about the quality of evidence <sup>6,7</sup>.

#### *Exclusion criteria*

Publications prior to the contemporary description of NPWT (pre-1997), descriptive studies reporting qualitative data and studies lacking a control intervention were excluded. Studies using non-proprietary forms of NPWT such as Redon drains, wall-mounted suction or vacuum chambers were also excluded.

#### *Outcome measures*

The primary outcome measures were quantitative bacterial counts from tissue specimens or surface swabs of the subjacent wound following the application of NPWT or control dressing. Secondary outcome measures were comparative analyses of the floral spectrum from the same specimens following NPWT or control dressings and quantitative bacterial counts using the modified form of NPWT which utilises periodic instillation of saline or antibacterial solutions.

#### *Study selection*

Two authors (GG and GM) independently performed an abstract review. Full text review was undertaken for studies that met the inclusion criteria and additional studies that

possibly met the inclusion criteria. Discrepancies were resolved by consensus with each other and the senior author (JN).

#### *Data extraction*

Data were retrieved from all full text articles accepted for final analysis by 2 authors (GG and GM). A standardised proforma was used to record data variables including the model (experimental model, randomised controlled trial or clinical series), species, type of wound (acute, chronic or surgical), surgical debridement, magnitude of negative pressure, type of wound filler, control intervention, method of quantitative bacteriological analysis, time points, duration of intervention and simultaneous use of systemic antibiotics or local antibacterial preparations. Due to diversity in clinical and methodological aspects of the included studies and variations in outcome measures and the inclusion of semi-quantitative outcome data, it was not possible to perform a meta-analysis.



## Results

### *General*

The selection of articles for inclusion is summarised in **figure 1**. A total of 24 papers were accepted for full analysis and formed the basis of the main review <sup>1,4,8–25</sup>. These included 10 experimental studies <sup>1,4,8–13,24,25</sup>, 4 randomised controlled trials <sup>14–17</sup> and 6 clinical studies <sup>18–23</sup>. Four studies evaluating NPWT with periodic installation were also included <sup>26–29</sup>. Study quality, assessed using the GRADE system, is summarized in **supplemental table 2**.

### *Experimental studies*

The review identified 10 experimental studies, the key characteristics of which are summarised in **supplemental table 3**. Four studies <sup>4,10,24,25</sup> described significant reductions in bacterial counts at the wound bed under NPWT relative to controls and one study (Zhou et al) also found this effect irrespective of the magnitude of NPWT applied <sup>10</sup>. Interestingly, in each of these investigations a decrease in the bacterial count over time in the control intervention was also observed, which was at odds with the trend towards an increase in bacterial count over time in the control intervention reported in the other studies. Morykwas et al demonstrated a significant reduction in bacterial count at the wound bed under NPWT relative to control dressings at one time point only (between days 4 and 5) <sup>1</sup>. Statistical significance was achieved because the mean bacterial counts at this single time point were atypical of the trends observed overall. By contrast, both Boone et al and Assadian et al demonstrated no difference in bioburden between NPWT and control dressings <sup>8,9</sup>. Lallis et al and Stinner et al demonstrated that the growth kinetics of

*staphylococcus aureus* were not influenced by NPWT over 6 days but, when compared with controls dressings, NPWT reduced the growth of *pseudomonas* species over the same period <sup>11,13</sup>. Using two different quantification methods, Liu et al also concluded that NPWT significantly reduced growth of *pseudomonas* species relative to control dressings <sup>12</sup>. The results for experimental studies are summarised in **table 1**.

### *Randomized controlled trials*

Five papers describing 4 randomised-controlled trials were identified. Moues and colleagues presented the same trial data in 2007 as in 2004 and, therefore, the latter paper was excluded <sup>30</sup>. Patients were randomised to receive NPWT or an alternative dressing for the management of a heterogeneous collection of acute, subacute or chronic wounds <sup>14–16</sup>, or peri-prosthetic groin infections following vascular surgery <sup>17</sup>. The control dressing differed between trials and, in one case <sup>15</sup>, within the same trial. The characteristics of each study are summarised in **supplemental table 3**. Three of the 4 trials found that neither NPWT nor controls dressings significantly reduced the bacterial growth or number of positive cultures. The only study to quantify bacterial proliferation and perform species analysis found that NPWT selectively reduced non-fermentive Gram-negative bacilli (NFGNB) but increased the proliferation of *staphylococcus aureus* <sup>14</sup>. The total number of patients recruited to these trials was 171 (90 and 81 randomised to NPWT and conventional dressings, respectively). A meta-analysis of the randomised controlled trial data was not possible owing to trial and reporting heterogeneity. The results are summarised in **table 2**.

### *Clinical studies*

Six clinical studies were identified <sup>18–23</sup>. These included 2 case series of chronic wounds totalling 17 patients <sup>20,21</sup>, 1 non-randomised trial comparing NPWT with conventional dressings following debridement and systemic antibiotics for the management of osteomyelitis <sup>18</sup>, 1 retrospective series of acute and chronic wounds <sup>19</sup>, 1 retrospective series of adults with intra-abdominal sepsis treated with laparotomy, systemic antibiotics and NPWT <sup>23</sup>, and 1 retrospective review of all incisional biopsy specimens harvested for microbial analysis during the management of combat extremity trauma <sup>22</sup>. Only 1 study demonstrated a clear trend towards reduction of microbial load under NPWT <sup>18</sup>. By contrast, 3 studies demonstrated an increase in general bacterial proliferation under NPWT over the duration of the study <sup>21–23</sup>, while 2 <sup>19,20</sup> found no clear trend. Sheppard demonstrated a reduction in *pseudomonas*-positive biopsy specimens from wounds treated with NPWT <sup>22</sup>. The results for clinical studies are summarised in **table 3**.

### *Bacterial species analysis*

Of the 10 non-experimental (non-inoculated) studies, only 5 performed bacterial species analysis <sup>14,20–23</sup>. Therefore, only the authors of these studies were able to comment on the dynamic influence of NPWT on species selectivity. Two of these studies represented small patient cohorts <sup>20,21</sup> while another <sup>23</sup> was a cohort of intra-abdominal sepsis with dissimilar baseline microbiology to the more frequently studied acute and chronic cutaneous wounds. Of the remaining studies, data by Moues et al supported a selective suppression of NFGNB (of which *pseudomonas* and *acinetobacter* are probably the most clinically significant) <sup>14</sup>

while Sheppard et al demonstrated possible suppression of *pseudomonas spp*, but not *acinetobacter* in combat extremity trauma treated with debridement and NPWT <sup>22</sup>. Of the 10 experimental studies, 4 were inoculated with *pseudomonas spp*. prior to commencement of the experimental protocol <sup>8,11–13</sup>. Three of these studies reported selective suppression of *pseudomonas spp.*, two of which also reported the simultaneous proliferation of *staphylococcus aureus* by contrast. In the fourth <sup>8</sup>, the specific growth dynamics of *pseudomonas spp.* were not delineated.

#### *Evidence from modified NPWT regimens*

Two studies <sup>26,27</sup> compared the effect of NPWT with intermittent instillation versus conventional NPWT and untreated control on *pseudomonas* bioburden. Phillips et al concluded, based on their *ex-vivo* porcine model, that whereas conventional NPWT did not significantly reduce *pseudomonas* bioburden compared to polyurethane foam dressing control, additional periodic instillation antimicrobial solutions (1% and 10% povidone-iodine, 0.05% chlorhexidine gluconate, 0.1% Polyhexanide biguanide (PHMB) or 0.2% polydiallyldimethylammonium chloride) led to a significant reduction in bioburden at 24 hours <sup>26</sup>. By contrast, Davis et al found that at 21 days in their *in-vivo* porcine model, conventional NPWT did significantly reduce *pseudomonas* bioburden when compared to polyurethane foam dressing control and they observed a further significant reduction in bioburden with periodic instillation of 0.01% PHMB or 0.9% saline, but this did not correlate with any significant change in wound healing <sup>27</sup>.

Of the two case series comparing NPWT against NPWT with instillation of antimicrobials, Daeschlein et al <sup>28</sup> found no difference in microbial bioburden between the two groups, whereas Goss et al <sup>29</sup> found that the addition of periodic antimicrobial instillation significantly reduced the microbial bioburden. No randomised controlled trials have yet addressed the use of NPWT with periodic instillation of antimicrobial solutions. The results from infiltration-assisted NPWT are shown in **table 4**.

Stinner et al found that the introduction of a silver-impregnated interface reduced growth of both *pseudomonas* and *staphylococcus aureus* <sup>11</sup>. By contrast Boone et al found that silver-impregnated polyurethane foam did not influence the bacterial bioburden of the wound bed <sup>8</sup>

## Discussion

We have found a lack of consensus in the literature regarding the influence of NPWT on the bacterial bioburden of the wound bed. Seven studies concluded that NPWT significantly reduced the bacterial bioburden of the wound bed relative to control dressings on at least one time point over the experimental period <sup>1,4,10,16,18,24,25</sup>. By contrast 8 studies concluded that NPWT did not influence the bacterial bioburden independently. Five studies suggested that the influence of NPWT on was bacterial species-dependent, suppressing growth of pseudomonas and/or other non-fermentive Gram-negative bacilli such as acinetobacter <sup>11-14,22</sup>. The results of this systematic review call into question the findings of the seminal paper by Morykwas et al <sup>1</sup>, which is often quoted as justification for using NPWT on contaminated wounds in the expectation that this therapy will cleanse the wound, facilitating reconstruction by minimising the extent of debridement and/or the need to remove indwelling prostheses <sup>31,32</sup>. Our results instead suggest that the relationship between NPWT and bacterial growth kinetics is more complex and is probably species-dependent. The lack of a definitive conclusion on this point reflects the heterogeneous nature of the experimental protocols, as half of the clinical studies included were not designed to evaluate species-selectivity. Moreover, studies reporting reduced deep infection rate following open fracture treated with NPWT <sup>33</sup> and infection-free prosthetic preservation with NPWT <sup>31</sup> both describe radical debridement as part of the protocol, so the contribution of NPWT to the outcomes is unclear. We also found that the baseline flora was very different for wounds of different sites and aetiologies. For example, combat extremity trauma exhibited a very different baseline flora from chronic cutaneous wounds and intra-abdominal wounds. While this is not surprising, it must be factored into any evaluation of

the capacity of NPWT to exert a dynamic effect on wound flora. It is for this reason that experimental wounds are useful and the data from these studies supports the conclusion that NPWT selectively suppresses *pseudomonas* species. This is based on 3 experimental studies <sup>11–13</sup> and 2 clinical studies <sup>14,22</sup>. Interestingly, Anagnostakos & Mosser found that the bacterial species identified on NPWT foam correlated poorly with the species cultures from tissue specimens harvested at the wound bed <sup>35</sup>. Whether NPWT suppresses anything else remains conjectural.

Wound healing was found not to correlate with overall bacterial bioburden. This calls into question the importance of the traditional infection threshold of  $10^5$  CFU/g of tissue <sup>36</sup> and supports recent work demonstrating that, in some circumstances, colonisation in excess of  $10^5$  CFU/g may not adversely affect healing <sup>37,38</sup>. The observation is species-dependent, with impaired healing at lower individual bacterial biodensity in the presence of high virulence or bacterial synergism <sup>37,38</sup> hence the importance of the particular floral combination in each wound and, by extension, the influence of NPWT on that combination.

The implications of this study are twofold. Firstly, the findings suggest that NPWT should not be used in the expectation that it can clear the wound of bacterial contamination thereby avoiding the difficult reconstructive decisions. Rather, it should be used as an adjunct to definitive surgical management <sup>39</sup>. Secondly, the acknowledgement that bacteria proliferate under NPWT and on polyurethane foam emphasises the need for regular dressing changes combined with debridement and/or irrigation <sup>34,35</sup> when treating chronic wounds. The efficacy of antibacterial foams and interface materials has been reported in small clinical series <sup>40,41</sup> and demonstrated in an *in-vitro* model <sup>42</sup>. However, there is no clear consensus in the literature to define how these materials influence bacterial bioburden

in various clinical settings <sup>8,11</sup>. Similarly, the use of NPWT devices that permit instillation of saline or antibacterial solutions has shown promise in clinical series <sup>43,44</sup>. Based on the limited data available, NPWT with instillation (NPWTi) probably does influence bacterial bioburden and this effect is probably species-dependent. The differences in bacterial growth with gauze or polyurethane foam and the influence of an interface material remain unclear.

There were a number of limitations to this study. Firstly, it is worth stressing that from a clinical point of view our interest lies in infection-free wound healing and/or wound bed preparation prior to definitive surgical closure. The microbiological environment of the wound bed and the extent to which NPWT influences this is of interest only insofar as it may be used to achieve this end. The microbiological profile of wounds is relevant because in practice NPWT is used to temporise and to manage difficult wounds in circumstances where infection of the wound bed might upgrade the reconstructive requirements. As the presence of bacterial pathogens promotes inflammatory cell recruitment and thus expression of pro-inflammatory cytokines, NPWT may in part act by reducing the inflammatory cell infiltrate and tip the balance towards an anabolic environment <sup>3</sup>. Hence the relationship between bacterial bioburden and wound healing under NPWT is a complex.

Secondly, the heterogeneity of the included studies precluded meaningful meta-analysis and renders this review descriptive in nature. Thirdly, it is clear that when evaluating wounds treated with NPWT the growth kinetics of one species must be evaluated in the context of the others seeking to populate the same niche. This adds a potentially confounding variable into our analyses which is difficult to control. Interestingly two studies using *staphylococcus aureus* as a control species to evaluate growth kinetics of *pseudomonas aeruginosa* reported that the tissue density of *staph. aureus* rose in



proportion to the fall in tissue density of *pseudomonas aeruginosa*. This adds to the evidence that NPWT has a selective action on bacterial growth. In clinical practice, were the Gram-positive cocci at the wound bed highly virulent, such as a Panton-Valentine Leukocidin (PVL) containing *staph. aureus*, this may result in clinical deterioration. Indeed, positivity for PVL-*staph. aureus* at the wound bed may be a contraindication to the use of NPWT on the basis of these results. As to why NPWT negatively impacts on *pseudomonas* species, proliferation may be impaired either by an immune-mediated effect, an unfavourable change in the bacterial microenvironment, including an effect on the biofilm as reported by Li et al <sup>25</sup> or by a direct action on bacterial metabolism. Moreover, it is uncertain how the wider change in the microenvironment under NPWT, such as local tissue oxygenation and pH alters bacterial behaviour <sup>45</sup>. There are, at present, insufficient data to establish the extent to which the observed effect of NPWT on *pseudomonas* is species or strain-specific.

We have yet to fully appreciate how NPWT influences commensals, pathogens and nosocomial contaminant species at the wound bed. Further work must look to delineating the bacterial species-specific effects of NPWT how to manipulate the microbiological profile of the wound using NPWT to enhance healing. Arguably, such work requires more focus on the experimental models as here we have end to end control over the wound environment and the larger models (such as porcine wound models) are likely to replicate the human wound environment. More work is also needed to evaluate the influence of NPWT on bacterial growth within ischaemic wounds.

In summary, the common assertion that NPWT reduces the bacterial growth within the subjacent wound is an over-simplification. Instead, NPWT may have a role in suppressing proliferation of selective bacterial species such as NFGNB including *Pseudomonas*

*aeruginosa* but, in doing so, there is evidence that the depopulated niche is exploited by Gram-positive cocci. Future work should seek to define the growth kinetics of common wound pathogens under NPWT and NPWT should not be used as a substitute for adequate surgical debridement and, where indicated, prompt definitive closure.

**Conflict of Interest Statement:** None

## Bibliography

- 1 Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. *Ann. Plast. Surg.* 1997. p. 553–562.
- 2 Argenta LC, Morykwas MJ. Vacuum-assited closure: A new method for wound control and treatment: Clinical experience. *Ann Plast Surg.* 1997; **38**: 563–577.
- 3 Glass GE, Murphy GF, Esmaeili a, Lai L-M, Nanchahal J. Systematic review of molecular mechanism of action of negative-pressure wound therapy. *Br J Surg* 2014; **101**: 1627–1636.
- 4 Liu D, Zhang L, Li T, Wang G, Du H, Hou H, *et al.* Negative-Pressure Wound Therapy Enhances Local Inflammatory Responses in Acute Infected Soft-Tissue Wound. *Cell Biochem Biophys.* 2014; **70**: 539–547.
- 5 Moher D, Liberati A, Tetzlaff J, Altman DG, Grp P. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLOS Med.* 2009; **6**: e1000097.
- 6 Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, *et al.* GRADE guidelines: 1. Introduction - GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol.* 2011; **64**: 383–394.
- 7 Guyatt GH, Oxman AD, Sultan S, Glasziou P, Akl EA, Alonso-Coello P, *et al.* GRADE guidelines: 9. Rating up the quality of evidence. *J Clin Epidemiol.* 2011; **64**: 1311–1316.
- 8 Boone D, Braitman E, Gentics C, Afthinos J, Latif J, Sordillo E, *et al.* Bacterial burden and wound outcomes as influenced by negative pressure wound therapy. *Wounds.* 2010; **22**: 32–37.
- 9 Assadian O, Assadian A, Stadler M, Diab-Elschahawi M, Kramer A. Bacterial growth kinetic without the influence of the immune system using vacuum-assisted closure dressing with and without negative pressure in an in vitro wound model. *Int Wound J* 2010; **7**: 283–289.

- 10 Zhou M, Yu A, Wu G, Xia C, Hu X, Qi B. Role of different negative pressure values in the process of infected wounds treated by vacuum-assisted closure: An experimental study. *Int Wound J*. 2013; **10**: 508–515.
- 11 Stinner DJ, Waterman SM, Masini BD, Wenke JC. Silver dressings augment the ability of negative pressure wound therapy to reduce bacteria in a contaminated open fracture model. *J Trauma* 2011; **71**: S147-50.
- 12 Liu Y, Zhou Q, Wang Y, Liu Z, Dong M, Wang Y, *et al*. Negative pressure wound therapy decreases mortality in a murine model of burn-wound sepsis involving pseudomonas aeruginosa infection. *PLoS One*. 2014; **9**: 1–7.
- 13 Lalliss SJ, Stinner DJ, Waterman SM, Branstetter JG, Masini BD, Wenke JC. Negative pressure wound therapy reduces pseudomonas wound contamination more than Staphylococcus aureus. *J Orthop Trauma*. 2010; **24**: 598–602.
- 14 Moues C, Vos M, Bemd G Van Den. Young Investigator Award Article: Bacterial load in relation to vacuum-assisted closure wound therapy: A prospective randomized trial. *Wound Repair and Regeneration* 2004; 11–17.
- 15 Braakenburg A, Obdeijn MC, Feitz R, van Rooij I a LM, van Griethuysen AJ, Klinkenbijn JHG. The clinical efficacy and cost effectiveness of the vacuum-assisted closure technique in the management of acute and chronic wounds: a randomized controlled trial. *Plast Reconstr Surg* 2006; **118**: 390-7-400.
- 16 Tuncel U, Erkorkmaz U, Turan A. Clinical evaluation of gauze-based negative pressure wound therapy in challenging wounds. *Int Wound J*. 2013; **10**: 152–158.
- 17 Monsen C, Wann-Hansson C, Wictorsson C, Acosta S. Vacuum-assisted wound closure versus alginate for the treatment of deep perivascular wound infections in the groin after vascular surgery. *J Vasc Surg* 2014; **59**: 145–151.

- 18 Tan Y, Wang X, Li H, Zheng Q, Li J, Feng G, *et al.* The clinical efficacy of the vacuum-assisted closure therapy in the management of adult osteomyelitis. *Arch Orthop Trauma Surg.* 2011; **131**: 255–259.
- 19 Weed T, Ratliff C, Drake DB. Quantifying Bacterial Bioburden During Negative Pressure Wound Therapy. *Ann Plast Surg* 2004; **52**: 276–279.
- 20 Isago T, Nozaki M, Kikuchi Y, Honda T, Nakazawa H. Negative-pressure dressings in the treatment of pressure ulcers. *J Dermatol.* 2003; **30**: 299–305.
- 21 Khashram M, Huggan P, Ikram R, Chambers S, Roake J, Lewis D. Effect of TNP on the microbiology of venous leg ulcers: a pilot study. *J Wound Care.* 2009; **18**: 164–167.
- 22 Sheppard FR, Keiser P, Craft DW, Gage F, Robson M, Brown TS, *et al.* The majority of US combat casualty soft-tissue wounds are not infected or colonized upon arrival or during treatment at a continental US military medical facility. *Am J Surg* 2010; **200**: 489–495.
- 23 Pliakos I, Michalopoulos N, Papavramidis TS, Arampatzi S, Diza-Mataftsi E, Papavramidis S. The effect of vacuum-assisted closure in bacterial clearance of the infected abdomen. *Surg Infect* 2014; **15**: 18–23.
- 24 Li J, Topaz M, Tan H, Li Y, Li W, Xun W, *et al.* Treatment of infected soft tissue blast injury in swine by regulated negative pressure wound therapy. *Ann Surg* 2013; **257**: 335–344.
- 25 Li T, Zhang L, Han L, Wang G, Yin P, Li Z, *et al.* Early application of negative pressure wound therapy to acute wounds contaminated with *Staphylococcus aureus*: An effective approach to preventing biofilm formation. *Exp Ther Med.* 2016; **11**: 769–776.
- 26 Phillips PL, Yang Q, Schultz GS. The effect of negative pressure wound therapy with periodic instillation using antimicrobial solutions on *Pseudomonas aeruginosa* biofilm on porcine skin explants. *Int Wound J.* 2013; **10**: 48–55.
- 27 Davis K, Bills J, Barker J, Kim P, Lavery L. Simultaneous irrigation and negative pressure wound

- therapy enhances wound healing and reduces wound bioburden in a porcine model. *Wound Repair Regen.* 2013; **21**: 869–875.
- 28 Daeschlein G, Napp M, Lutze S, von Podewils S, Jukema G, Fleischmann W, *et al.* Comparison of the effect of negative pressure wound therapy with and without installation of polyhexanide on the bacterial kinetic in chronic wounds. *Wound Med* 2016; **13**: 5–11.
  - 29 Goss SG, Schwartz JA, Facchin F, Avdagic E, Gendics C, Lantis JC. Negative pressure wound therapy with instillation (NPWTi) better reduces post-debridement bioburden in chronically infected lower extremity wounds than NPWT alone. *J Am Coll Clin Wound Spec* 2012; **4**: 74–80.
  - 30 Moues CM, van den Bemd GJCM, Heule F, Hovius SER. Comparing conventional gauze therapy to vacuum-assisted closure wound therapy: A prospective randomised trial. *J Plast Reconstr Aesthetic Surg.* 2007; **60**: 672–681.
  - 31 Mayer D, Hasse B, Koelliker J, Enzler M, Veith FJ, Rancic Z, *et al.* Long-term results of vascular graft and artery preserving treatment with negative pressure wound therapy in Szilagyi grade III infections justify a paradigm shift. *Ann Surg* 2011; **254**: 754–59
  - 32 Tocco MP, Ballardini M, Masala M, Perozzi A. Post-sternotomy chronic osteomyelitis: Is sternal resection always necessary? *Eur J Cardio-thoracic Surg.* 2013; **43**: 715–721.
  - 33 Stannard JP, Volgas DA, Stewart R, McGwin G, Alonso JE. Negative pressure wound therapy after severe open fractures: a prospective randomized study. *J Orthop Trauma* 2009; **23**: 552–557.
  - 34 Yusuf E, Jordan X, Clauss M, Borens O, Mäder M, Trampuz A. High bacterial load in negative pressure wound therapy (NPWT) foams used in the treatment of chronic wounds. *Wound Repair Regen* 2011; **21**: 677–681.
  - 35 Anagnostakos K, Mosser P. bacterial identification on NPWT foams: clinical relevance or

- contamination? *J Wound Care*. 2012; **21**: 226–239.
- 36 Robson M, Heggers J. bacterial quantification of open wounds. *Mil Med*. 1969; **134**: 19–24.
  - 37 Bowler PG. The 10(5) bacterial growth guideline: reassessing its clinical relevance in wound healing. *Ostomy Wound Manag*. 2003; **49**: 44–53.
  - 38 Edwards R, Harding KG. Bacteria and wound healing Colonisation Contamination. *Curr Opin Infect Dis*. 2004; **17**: 91–96.
  - 39 Baillot R, Cloutier D, Montalin L, Côté L, Lellouche F, Houde C, *et al*. Impact of deep sternal wound infection management with vacuum-assisted closure therapy followed by sternal osteosynthesis: a 15-year review of 23 499 sternotomies. *Eur J Cardio-thoracic Surg*. 2010; **37**: 880–887.
  - 40 Payne JL, Ambrosio AM. Evaluation of an antimicrobial silver foam dressing for use with V.A.C.?? therapy: Morphological, mechanical, and antimicrobial properties. *J Biomed Mater Res - Part B Appl Biomater*. 2009; **89**: 217–222.
  - 41 Gerry R, Kwei S, Bayer L, Breuing KH. Silver-impregnated vacuum-assisted closure in the treatment of recalcitrant venous stasis ulcers. *Ann Plast Surg* 2007; **59**: 58–62.
  - 42 Ngo QD, Vickery K, Deva AK. The effect of topical negative pressure on wound biofilms using an in vitro wound model. *Wound Repair Regen*. 2012; **20**: 83–90.
  - 43 Gabriel A, Shores J, Heinrich C, Baqai W, Kalina S, Sogioka N, *et al*. Negative pressure wound therapy with instillation: a pilot study describing a new method for treating infected wounds. *Int Wound J* 2008 Jun; **5**: 399–413.
  - 44 Brinkert D, Ali M, Naud M, Maire N, Trial C, T??ot L. Negative pressure wound therapy with saline instillation: 131 patient case series. *Int Wound J*. 2013; **10**: 56–60.
  - 45 Schaible B, Taylor CT, Schaffer K. Hypoxia increases antibiotic resistance in *Pseudomonas aeruginosa* through altering the composition of multidrug efflux pumps. *Antimicrob Agents*

*Chemother.* 2012; **56**: 2114–2118.



**Figure legends:**

**Figure 1** Literature search and study selection

**Table 1** Results summary from experimental studies

**Table 2** Results summary from randomised controlled trials

**Table 3** Results summary from clinical studies

**Table 4** Results summary from studies evaluating infiltration NPWT (NPWTi)

**Supplemental table 1** Search terms

**Supplemental table 2** GRADE evidence profile

**Supplemental table 3** Characteristics of included studies

**Table 1** Results summary from experimental studies

Reference	Features	Findings	Notes
Morykwas <sup>1</sup>	Porcine wound model inoculated with Staph. aureus (3) and Staph epidermis (2)	CFU/g significantly reduced between day 4 and 5 with TNP only. No difference between bacterial strains	High inoculum ( $10^8$ organisms) Only significant difference was between day 4 and 5 and error bars indicate variation atypically large compared with rest of experiment. Significance reliant on rise in CFU/g in control which was against the trend for the remainder of the experimental period
Boone <sup>8</sup>	Porcine wound model  Comparison between NPWT, silver impregnated gauze + NPWT in management of wounds inoculated with multiple organisms over 7 days	Overall bacterial bioburden increased over duration of study, peaking at day 4  No significant difference in overall bacterial bioburden between NPWT, silver impregnated foam+NPWT or control dressings Most wounds cultured additional species by day 7, including Proteus, E. coli and $\beta$ -hemolytic Strep	Low inoculum ( $10^7$ organisms) but longer incubation time (72hrs) Different NPWT regimen (intermittent)
Assadian <sup>9</sup>	Non-viable (porcine) tissue model inoculated with MRSA  Comparison of NPWT vs. NPWT dressings only	Bacterial growth kinetics similar with or without NPWT (increase in bacterial count over 48 hours then plateau)	Low inoculum ( $10^6$ organisms) Controlled for blood flow and immune system Not representative of clinical practice
Zhou <sup>10</sup>	Porcine wound model inoculated with Staph aureus  Comparison of NPWT @ -75, -	At day 3, 5, and 7 NPWT at all pressures demonstrated significant reduction of bacterial count versus either NPWT dressings only or gauze only.	High inoculum ( $>10^8$ organisms) Bacterial count fell over 7 days in all conditions

	150, -225, -300mmHg with NPWT dressings only and gauze only		
Stinner <sup>11</sup>	Goat open tibial fracture model  Comparison of NPWT +/- Acticoat as interface	Addition of Acticoat reduced pre-debridement bacterial counts for both Pseudomonas and Staph aureus at all time-points compared with NPWT alone NPWT alone reduced Pseudomonas but increased Staph aureus over 6 days	High inoculum (>10 <sup>8</sup> organisms) Results expressed as percentage of results 6hr post inoculum prior to first debridement
Liu <sup>12</sup>	Murine burn model  Comparison of NPWT vs. WTD dressings in managing scald infected with pseudomonas	NPWT significantly reduced Pseudomonas compared with wet to dry (WTD) dressings as measured by both tissue culture and bioluminescence up to day 7	High inoculum (50µl 10 <sup>9</sup> organisms) Microbiology cultures blinded High quality study design & analysis
Lallis <sup>13</sup>	Goat open tibial fracture model  Comparison of NPWT with WTD dressings	NPWT (PU foam or gauze) significantly reduced pre and post debridement Pseudomonas compared with WTD dressings at all time-points NPWT (gauze) made no significant difference to pre and post debridement Staph aureus compared with WTD dressings at any time-point (overall 6 day increase with both dressings) NPWT reduced Pseudomonas but increased staph aureus over 6 days	High inoculum (>10 <sup>8</sup> organisms) Results expressed as percentage of results 6hr post inoculum prior to first debridement  WTD dressing changes 2x daily
Li <sup>24</sup>	Porcine blast injury model  Comparison of NPWT @ -5 to -35 kPa vs. WTD dressings in management of blast left for 48hrs	NPWT significantly reduced both aerobic and anaerobic bacterial counts from day 1 post treatment at -10 to -35kPa (-75 to -263mmHg) at all time points (to day 20).  NPWT increased relative % of Staph epidermidis & group A Strep beyond 14 days	No inoculum. Blast left for 48hrs prior to debridement and dressing.  Influence on Pseudomonas spp. unclear as not present at start
Liu <sup>4</sup>	Rabbit wound model  Comparison of NPWT vs. WTD dressings in full thickness wounds inoculated with Staph. aureus	NPWT significantly reduced Staph. aureus compared with WTD dressings as measured by tissue culture at days 2, 4 and 8	No significant difference observed at day 6

	over 8 days		
Li <sup>25</sup>	Rabbit wound model	NPWT significantly reduced Staph. aureus compared with no dressings from post op day 8 (5 days after inoculation and NPWT application)	Wounds inoculated on day 3 post wound creation and NPWT applied 6hrs later
	Comparison of NPWT vs. no dressing in full thickness ear wounds inoculated with staph. Aureus over 13 days		

**Table 2** Results summary from randomised controlled trials

Reference	Features	Findings	Notes
Moues <sup>14</sup>	Comparison of NPWT with wet to dry dressings in management of acute and chronic wounds	No significant change in overall bacterial bioburden over duration of study for either NPWT or control. Significant reduction in non-fermentive Gram neg. bacilli with NPWT (vs. control) Significant increase in Staph aureus with NPWT (vs. control)	Better wound healing seen with NPWT- did not correlate with bacterial load Microbiology cultures blinded NPWT did not influence Enterobacteriae or anaerobes
Braakenburg <sup>15</sup>	Comparison of NPWT with various other dressings in management of acute and chronic wounds	Over study period, neither modality significantly reduced the number of positive swabs from the wound bed (88% to 84%; NPWT vs. 47% to 58%; alginate)	No bacterial sub analysis
Tuncel <sup>16</sup>	Comparison of gauze-based NPWT with polyhexanide-soaked gauze dressings in management of sub-acute and chronic wounds	22 of 25 (NPWT) vs. 16 of 25 (conventional) exhibited negative wound cultures at study endpoint (P<0.05)	Dynamic change in bacterial bioburden unclear No sub analysis of which bacteria were selectively reduced by NPWT
Monsen <sup>17</sup>	Comparison of NPWT with alginate dressing in management of perivascular groin infections	Over 21 days, neither modality significantly reduced the number of positive swabs from the wound bed (9 of 10 to 8 of 10; NPWT vs. 8 of 10 to 6 of 10; alginate)	NPWT resulted in better healing but not attributable to changes in bacterial bioburden

**Table 3** Results summary from clinical studies

Reference	Features	Findings	Notes
Tan <sup>18</sup>	?non-randomized comparison of NPWT with conventional dressings in heterogeneous osteomyelitis cohort	29 of 35 (NPWT) vs. 15 of 33 (conventional) exhibited negative wound cultures at study endpoint (P<0.05)	Uncertain how many samples were required to confirm negative bacterial bioburden  Some implants retained acutely. Long term salvage unclear
Weed <sup>19</sup>	Analysis of dynamics of bacterial bioburden of wound under NPWT	No significant change in bacterial bioburden identified from beginning to end of NPWT therapy	Very heterogeneous population (in-patient & out-patient, acute & chronic wounds) Accuracy of swab sampling method to quantify bacterial load No species analysis Comparison of only first and last culture results
Isago <sup>20</sup>	Analysis of dynamics of bacterial bioburden of pressure ulcers under NPWT	Bacterial bioburden stable in 7 of 10 cases and minimized in 3 of 10 Bacterial species minimized were Pseudomonas in 1 case and Staph aureus in 3 cases (incl. MRSA in 2)	No prior debridement or antibiotics
Khashram <sup>21</sup>	Analysis of dynamics of bacterial bioburden of venous ulcers under NPWT	Median log <sup>10</sup> CFU/cm <sup>2</sup> increased over duration of study No change in species observed	Short duration 72 hours between dressing changes
Sheppard <sup>22</sup>	Analysis of dynamics of bacterial bioburden of combat extremity trauma under NPWT	167 biopsies negative; 75 biopsies positive. Relative % of positive biopsies increased over time under NPWT  Relative % of biopsied positive for pseudomonas decreased over time	No evidence that NPWT reduced Gram negative bioburden overall but did reduce Pseudomonas bioburden  Only bioburden of >10 <sup>5</sup> organisms included as thought to be more clinically significant

Pliakos <sup>23</sup>	Analysis of dynamics of bacterial bioburden of peritoneum after laparotomy + NPWT following abdominal sepsis	Over study period, number of positive bacterial cultures from peritoneal aspirate increased from 19 of 39 to 30 of 39	No control intervention so role of NPWT in increasing positive bacterial cultures uncertain. Influence on pseudomonas uncertain
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**Table 4** Results summary from studies evaluating instillation NPWT

Reference	Features	Findings	Notes
Phillips <sup>26</sup> (experimental)	Porcine skin explant model  Comparison of NPWTi (eight instillation solutions) with NPWT and untreated control	NPWTi with most antiseptic solutions significantly reduced bioburden of pseudomonas. Most effective were 10% Povidone iodine, 0.2% pDADMAC and Prontosan. NPWT alone made no significant reduction in bioburden	Inoculum of 10 <sup>6</sup> CFU <i>Pseudomonas</i> for 3.5 days Analysis after 24 hrs. therapy
Davis <sup>27</sup> (experimental)	Pig skin wound model, 6 wounds per pig, 6 pigs  Comparison of Control with NPWT and NPWTi (0.9% saline or 0.01% PHMB, high or low flow)	NPWT and NPWTi significantly reduced <i>Pseudomonas</i> bioburden vs. control at 21 days.  No difference in wound healing between NPWT and NPWTi, although both better than control.	Inoculum of 500 CFU <i>Pseudomonas</i> for 3 days  Quantum™ pump and black foam (ITI)
Daeschlein <sup>28</sup> (clinical)	Non-randomized comparison of NPWT with NPWTi in chronic wound cohort.	Over study period (2-7 days per wound), number of CFU per wound swab did not change significantly in either group  Bacterial flora appeared unrelated to clinical outcome (successful graft take).	Significant baseline differences in age, BMI, comorbidities and wound size between groups.
Goss <sup>29</sup> (clinical)	Sequential allocation of chronically infected lower limb wounds to NPWT or NPWTi	Over 7 days, wounds treated with NPWTi (quarter strength Dakin's solution) reduced bioburden by 10.6x10 <sup>6</sup> per gram of tissue, whilst those treated with conventional NPWT increased bioburden by 28.7x10 <sup>6</sup> per gram.	